PERFORMANCE ANALYSIS OF MEDICINAL *Kaempferia* SPECIES

By

AKOIJAM RANJITA DEVI

(2016-22-004)

THESIS

Department of Plantation Crops and Spices

COLLEGE OF HORTICULTURE

KERALA AGRICULTURAL UNIVERSITY

VELLANIKKARA, THRISSUR - 680 656

2019

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Submitted in partial fulfillment of the requirement for the degree of

Doctor of Philosophy in Horticulture

Faculty of Agriculture

Kerala Agricultural University

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COLLEGE OF HORTICULTURE

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VELLANIKKARA, THRISSUR - 680 656

KERALA, INDIA

2019

DECLARATION

I, **Akoijam Ranjita Devi** (2016-22-004) hereby declare that, this thesis entitled **"Performance analysis of medicinal *Kaempferia* species"** is a bonafide record of research done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title of any other University or Society.

Place: Vellanikkara **Akoijam Ranjita Devi**

Date: 2016-22-004

CERTIFICATE

Certified that this thesis entitled **"Performance analysis of medicinal *Kaempferia* species"** is a record of research work done independently by **Ms. Akoijam Ranjita Devi (2016-22-004)** under my guidance and supervision and that, it has not previously formed the basis for the award of any degree, diploma, fellowship or associate ship to her.

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CERTIFICATE

We, the undersigned members of the advisory committee of **Ms. Akoijam Ranjita Devi (2016-22-004),** a candidate for the degree of **Doctor of Philosophy in Horticulture** with major field in Plantation Crops and Spices, agree that the thesis entitled **"Performance analysis of medicinal *Kaempferia* species"** may be submitted by Ms. Akoijam Ranjita Devi (2016-22-004), in partial fulfilment of the requirement for the degree.

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EXTERNAL EXAMINER

Dedicated to

my beloved parents and my guide Dr.

N. Mini Raj

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Date:

Place: KAU, Vellanikkara ***Akoijam Ranjita Devi***

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Introduction

1. INTRODUCTION

The natural products continue to play a highly significant role in drug discovery and development process (Newman and Cragg, 2012). The plants are abundant natural source of potential new medicines. The herbal industry is picking up at a fast pace worldwide. Herbs and botanicals now appear in more products and have more medicinal applications than ever before. Almost 25-40 per cent of the active components of the allopathic medicine have their origins from plants and the clues to discover them came from folklore medicine of various cultures. But majority of the herbal medicines are not been well- researched and scientifically tested, with the exception of a few that have been adequately analysed, standardised and tested for efficacy and safety (Canter and Ernst., 2004; Loya *et al*., 2009; Cohen and Ernst, 2010). Moreover, only a small fraction of the world's biodiversity has been explored for bioactivity to date.

The medicinal *Kaempferia* species belong to the family Zingiberaceae. The genus contains approximately 50 species distributed from India to Southern China and Malaysia which are source of valuable bioactive compounds. *K. galanga*, *K. rotunda* and *K. scaposa* are the species found in South India. Amidst these, *K. rotunda* and *K. galanga* are mostly used for medicinal purpose in India.

Kaempferia galanga, popularly known as aromatic ginger or *Kacholam*, originated in India, is a highly priced medicinal plant, commercially cultivated in India, South East Asia and China. It is a glabrous aromatic herb which forms a component of over 59 Ayurvedic medicines (Sivarajan and Balachandran, 1994) and is extensively used in pharmaceutical industries for preparation of ayurvedic drugs, perfumery, cosmetics and as spice ingredients (Rahman *et al.*, 2004). It has antinociceptive activity (Ridtitid *et al*., 2008), antidaibetic (Chowdhury *et al*., 2014), antioxidant and antimicrobial (Rao and Kaladhar, 2014), sedative activity (Ali *et al*. 2015), and anti-inflammatory property (Jagadish *et al*., 2016).

Kaempferia rotunda or Indian crocus/*Chenghazhineer kizhangu/Leipaklei* is another fragrant aromatic herb, distributed throughout India. This plant is also

considered as an important medicinal plant in the ancient system of traditional medicine in India and Indonesia against abdominal pain, wounds, diarrhoea and colic disorder (Atun and Arianingrum, 2015). *Kaempferia rotunda* is also used to improve complexion and cures burning sensations (Sereena *et al*., 2011). The main bioactive constituent is cretopoxide which is useful in inhibition of tumors (Kupchun *et al.*, 1968). Various other medicinal properties such as anthelmintic activity (Agrawal *et al*., 2011), antiviral activity (Aznam *et al*., 2012), antihyperglycemic activity (Sultana *et al*., 2012), anticancer property (Kabir *et al*., 2011; Kabir and Reza, 2014; Atun and Arianingrum, 2015; Ahmed *et al*., 2017) are reported in this plant. The plant is widely used various Ayurvedic formulations including the much reputed health tonic *Chyavanaprash.*

Kaempferia parviflora, popularly known as black ginger or Thai ginseng, is another plant under this genus which has potential for great exploitation on commercial basis. The plant is indigenous to the north-eastern part of Thailand. Rhizomes of *K. parviflora* have been used as traditional medicine for rectifying male impotence, body pains and gastrointestinal disorders among local people in the Northeast of Thailand (Yenjai *et al*., 2004). The people of Thailand also use this plant for seasoning their traditional foods and as a traditional medicine (Alveno, 2012). It is also well-known as an energy enhancer with exceptional tonic effect. The fresh or dried rhizomes, dried powder in tea bag and wine are various products used by the inhabitants of Thailand. The rhizome is reported to have antimicrobial, aphrodisiac, anti-gastric ulcer, antidepressant, anticholinesterase, anti-obesity, vasodilation and antioxidant effect. Traditional medicines using *K. parviflora* are permitted by Thai Food and Drug Association which include capsules, pills, tablets, powders and essence tincture (Wattanasri, 2016).Various *in vivo* experiments in the test animals using *K. parviflora* extract showed reduction in obesity, diabetes type II, cardiovascular disease and inflammatory activity (Sae-wong *et al*., 2009; Yorsin *et al*., 2014). In addition to this, the consumption of rhizome extracted with alcohol strengthened the body in general (Maneenon *et al*., 2015). It also acts as modulator of multidrug resistance in cancer cells (Patanasethanont *et al*., 2007). In India it has

been reported to be grown in the tropical evergreen forest of Imphal east district of Manipur (Devi *et al.*, 2016).

Even though the *Kaempferia* species are used in number of traditional medicines, research findings are mostly confined to *K. galanga* which is an already exploited species. Research data on *K. rotunda* and *K. parviflora* is scanty. Systematic crop improvement research has been done in *K. galanga* however, not in other species *viz*. *K. rotunda* and *K. parviflora*. Basic understanding on the growth and development of these species is essential for their domestication and commercial cultivation. Biochemical and pharmacological studies would support their utilisation as a medicinal drug. In this context, the present study is proposed to evaluate the medicinal *Kaempferia* species in terms of growth and development, morphology, anatomy, floral biology, yield, medicinal properties and pharmacological aspects.

Review of literature

2. REVIEW OF LITERATURE

This chapter contains a comprehensive review of research work done on *Kaempferia* genus with emphasis on *K. galanga*, *K. rotunda* and *K. parviflora*. Wherever literature is not available, studies on other Zingiberaceae plants are also included.

2.1 *Kaempferia* GENUS

The genus *Kaempferia* L. is a small herb belonging to the family Zingiberaceae. They are commonly distributed in the tropics (Omanakumari and Mathew, 1985). Around 22 species are reported from Indian sub-continent (Hooker, 1984). The reported chromosome number of *Kaempferia rotunda* L. is 2n=54 (Raghavan and Venkatasubban, 1943), 2n=33 (Chakraborti, 1948) and 2n=44 (Omanakumari and Mathew, 1985). *Kaempferia galanga* is reported to have varying chromosome numbers *viz*. 2n=54 (Raghavan and Venkatasubban, 1943; Omanakumari and Mathew, 1985), 2n=44 (Raghavan and Arora, 1958), 2n=22 (Sharma and Bhattacharyya, 1959). The chromosome number of *Kaempferia parviflora* is 2n=24 as reported by Nopporncharoenkul *et al*., (2017).

Botanical classification of three *Kaempferia* species is given below:

Kingdom

Plantae

↓

Sub-kingdom

Phanerogamae

↓

Division

Spermatophyta

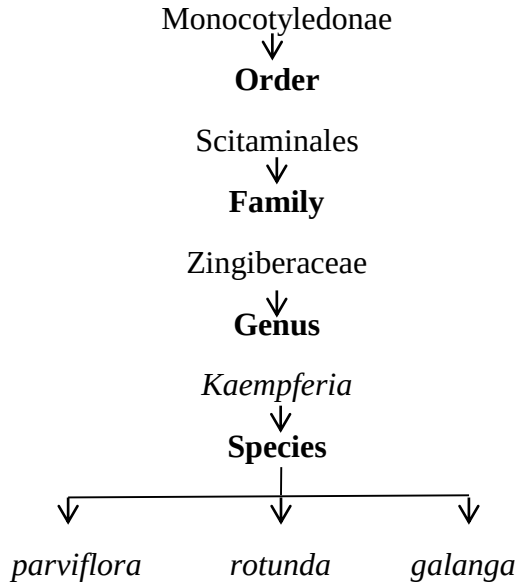
↓

Subdivision

Angiospermae

↓

Class



Kaempferia parviflora is a herb, 30-40 cm tall. Its leaves are one to several; blades ovate or oblong, slightly unequal sided, apex acute, base subcordate, adaxial surface yellow green, abaxial surface green, petiole 17 x 0.5 cm, leaf scales 7 cm long, margin undulated and red tinted. The rhizome is subglobose with several succulent roots in a fascicle. Its interior flesh is purple with an exterior of brownish skin. Its inflorescence is enclosed by two innermost leaf sheaths (Labrooy *et al*., 2016[b]). Rhizome of *K. parviflora* is fleshy and cross section of rhizome is orbicular or ellipse and have a circle line in the center. The flesh color is violet to blackish purple (Evi, 2012). The purplish color of the rhizome leads to the name Kra-chai-dahm in Thailand (Putiyanan *et al*., 2008).

Kaempferia rotunda is an aromatic herb with tuberous root-stalk and very short stem. The leaves are simple, few, erect, oblong, or ovate-lanceolate, acuminate, 30 cm long, 10 cm wide, variegated green above and tinged with purple below. Flowers are fragrant, white, tip purple or lilac arranged in crowded spikes opening successively. The plant produces a sub-globose tuberous rhizome from which many roots bearing small oblong or round tubers arise (Warrier *et al*., 1995).

Kaempferia galanga is an aromatic rhizomatous annual herb which spreads horizontally on the ground. It is a plant with tuberous root. The rhizome is 2 to 3

cm long, 1 to 2 cm in girth, crowded and strongly aromatic. It possesses numerous roots, bearing ovoid to spherical 1.5 to 2 cm long, 0.5 to 1 cm thick, white tubers. It has leafy shoot, stemless, almost horizontal near the ground. *K. galanga* possess few leaves, 2 to 3 in number. Its lamina is 10 to 15 cm long, 6 to 10 cm broad, ovate to orbicular, with rounded or subcordate base and broadly pointed tip. It has a hyaline margin, dark green upper surface, glabrous, pale green lower surface, white with violetish tinge towards the tip and more over it is densely hairy (Sabu, 2006).

Wood (1991) has studied the biogeography and evolution of *Kaempferia galanga.* The plant is believed to be of East Asian origin, most probably Burma. *Kaempferia rotunda* is native to China and the Indian subcontinent. In India, *K. parviflora* has been reported to be growing in the tropical evergreen forest of Imphal east district of Manipur (Devi *et al*., 2016). Other species of *Kaempferia* like *Kaempferia marginata* is found in its natural habitat at Manipur. It has been used as traditional medicine in China, Thailand and in Manipur (Kishor, 2012). *Kaempferia scaposa* is an erect perennial herb with fleshy sweet scanted rhizome, widely used in several indigenous medicinal formulations (Jagtap, 2015). It is distributed widely in peninsular and extrapeninsular parts of India. *Kaempferia elegans* is an ornamental foliage plant used for landscaping features.

2.2 MEDICINAL PROPERTIES

2.2.1 *Kaempferia parviflora*

Rhizomes of *K. parviflora* have been used as traditional medicine for rectifying male impotence, body pains and gastrointestinal disorders among local people in the Northeast of Thailand (Yenjai *et al*., 2004). In Thailand, *K. parviflora* is well-known as an energy enhancer with exceptional tonic effect. Fresh or dried rhizomes, dried powder in tea bag and wine are various products used by the inhabitants of Thailand. Rhizome is reported to have antimicrobial, aphrodisiac, anti-gastric ulcer, antidepressant, anticholinesterase activity, anti-obesity, vasodilation and antioxidant effect. Traditional medicinal preparations of *K.*

parviflora is permitted by Thai FDA which include capsule, pill, tablet, powder and essence tincture (Wattanasri, 2016).

Various *in vivo* experiments in test animals, using *K. parviflora* extract showed reduction in obesity, diabetes type II, cardiovascular disease and also anti-inflammatory activity (Yorsin *et al*., 2014; Sae wong *et al*., 2009).

The effect of *K. parviflora* in strengthening the body in general and as sexual stimulant by the consumption of rhizome extracted with alcohol was reported by Maneenon *et al*. (2015). Wattanathorn *et al*. (2012) also reported about the enhanced male sexual behaviours in aging rats by the administration of crude extract of *K. parviflora*.

Increased whole-body EE (Energy Expenditure) in healthy men by the administration of ethanolic extract of *K. parviflora* as reported by Matsushita *et al*. (2015) may be useful as an anti-obesity regimen.

Another study in male mice showed that *K. parviflora* improved physical fitness performance and muscular endurance (Toda *et al*., 2016).

K. parviflora also acts as modulator of multidrug resistance in cancer cells (Patanasethanont *et al*., 2007). Adaptogenic activities of *K. parviflora* has been reported by Pripdeevech *et al*. (2012) in mice.

Tewtrakul and Subhadhirasakul (2007) reported anti-allergic property of ethanolic extract of *K. parviflora* in RBL-2H3 cell line with IC_{50} value of 10.9 µg/ml.

The clinical trials on anti-obesity effects of *Kaempferia parviflora* extract in overweight and pre-obese Japanese subjects was conducted by Yoshino *et al*. (2018) and reported that the active KPE group showed significant reduction in abdominal fat area *i.e*. subcutaneous, visceral, and total fat and the level of triglycerides after 12 weeks when compared with the placebo group.

Thao *et al*. (2016) reported the antioxidant activity of *K. parviflora* using peroxyl radical-scavenging and reducing capacities. Compound 5 exhibited significant peroxyl radical scavenging capacity, while compound 13 showed significant reducing capacity at 10.0 µM.

2.2.2 *Kaempferia rotunda*

Kaempferia rotunda is also considered as an important medicinal plant in the ancient system of traditional medicine in India and Indonesia against abdominal pain, wounds, diarrhoea and colic disorder (Atun and Arianingrum, 2015). The main bioactive constituent is cretopoxide useful in inhibition of tumors (Kupchun *et al.,* 1968).

Kaempferia rotunda is used in several Ayurvedic preparations which include *Chyavanaprasam* used for improving health and complexion, for gastric complaints, curing burning sensation and insomnia (KAU, 2019). It is also used for preparation of *Hallakam* as reported by Joshi *et al*. (2011).

Kaempferia rotunda has been described in Ayurveda for its stomachic, antiinflammatory, antitumour, antiulcer, wound healing, emetic and vulnerary actions (Nambiar, 1993).

Kaempferia rotunda is one of the important ingredients in various Ayurvedic medicines as mentioned by Warrier (2016). *Charngeryadi ghritam*, *Mahakalyanakaghritam*, *Anutailam, Amritadi Tailam, Anutailam*, *Prapaundarikadi Tailam* and *Asokarishtam* requires *K. rotunda* as one of the ingredients.

The wound healing property of *K. rotunda* has been reported by Imam *et al*. (2013). The study on the excision wound animal model revealed that the drug extract affected the rate of wound contraction significantly at both dose levels *i.e.* at 250 mg/kg and 500 mg/kg of body weight of the rats. This finding provides an insight into the usage of *Kaempferia rotunda* Linn. leaf in traditional treatment of wounds, burns and in reducing swellings.

Agrawal *et al*. (2011) reported the anthelmintic activity of alcoholic extract *K. rotunda* at the concentration of 25, 50 and 100 mg/ml against *Pheretima posthuma* and *Ascardia galli.*

The antiviral activity was reported in hexane extract of *K. rotunda* against AI virus H5N1 (Aznam *et al*., 2012).

The methanolic extract *K. rotunda* showed antihyperglycemic activity in a study conducted in glucose loaded mice (Sultana *et al*., 2012).

The anticancer property of *K. rotunda* both *in vitro* using cell lines and *in vivo* in mice has been reported in several studies (Atun and Arianingrum, 2015; Kabir *et al*., 2011; Kabir and Reza, 2014; Ahmed *et al*., 2017).

The antimicrobial property of *K. rotunda* has been reported by many authors (Kabir *et al*., 2011; Malayahayati *et al*., 2018; Kumar *et al*. 2015; Kabir and Reza, 2014).

2.2.3 *Kaempferia galanga*

Kaempferia galanga which originated in India is a highly priced medicinal plant which is also cultivated in South East Asia and China. It forms a component of over 59 Ayurvedic medicines (Sivarajan and Balachandran, 1994) and is extensively used in pharmaceutical industries for preparation of Ayurvedic drugs, perfumery, cosmetics and as spice ingredients (Rahman *et al.*, 2004).

The officinal part of the plant is the rhizome which enters into the composition of major Ayurvedic formulations like *Valiyarasnadikashayam*, *Asanaeladi tailam* and *Dasamularishtam* (Sivarajan and Balachandran, 1994).

Kaempferia galanga is also an ingredient in preparation of *Valiya karpuradi churnam, Manjishthadi kwatham, Amritadi Tailam, Asaneladi Tailam/ Keratailam, Himasagaratailam, Mahakukkutamamsatailam, Mahamashatailam, Nagaradi Tailam* and *Eladi Churnam* (Warrier, 2016).

According to Jagadish *et al*. (2016), *K. galanga* suppressed the progression of acute and chronic inflammation when petroleum ether extract was given to adjuvant-induced chronic inflammation in rats.

Ali *et al*. (2015) reported the sedative activity of acetonic leaf extract of *K. galanga* (200 mg/ kg) using thiopental sodium induced sleeping time (358.55% effect), reduction of locomotor and exploratory activities in hole cross (95.09 % suppression) and open field (95.58 % suppression) tests in Swiss albino mice.

The antinociceptive activity was observed by Ridtitid *et al*. (2008) when mice and rats were tested in acetic acid-induced writhing, formalin, hot plate and tail-flick test models using methanolic extract of *K. galanga.*

The chloroform extract of *K. galanga* exhibited vasorelaxant effect in an examination on the smooth muscle contraction of the rat aorta (Mustafa *et al*., 1996).

The antihyperglycemic activity was noticed by Chowdhury *et al*. (2014) while using methanolic extract of *K. galanga* in carrageenan-induced paw oedema test.

According to Rao and Kaladhar (2014), *K. galanga* exhibited antioxidant and antimicrobial activity in methanol, ethylacetate, ethanol and aqueous extracts of rhizome. IC_{50} ranged from 490 µg/ml to 720 µg/ml. In tested microbes (*Bacillus subtilis, Klebsiella pneumonia, Pseudomonas aeruginosa, Aspergillus niger ,Candida albicans etc.*) zones of inhibitions ranged from 9.5 to 22 mm.

Antioxidant potential of *K. galanga* was also reported by Kaushita *et al*. (2015) in hydroethanolic extract.

2.3 GROWTH AND DEVELOPMENT IN *Kaempferia* SPECIES

2.3.1 Growth habit

Labrooy *et al*. (2018) reported two types of *Kaempferia parviflora viz*. *K. parviflora* T. and *K. parviflora* M. *K. parviflora* T. had erect growth habit, leaves were ovate in shape and yellow green colour, margin of the leaves were dark red in colour and the internal rhizome colour as dark purple. While *K. parviflora* M. had the similar features except that the leaf margin was red in colour and the inner rhizome was purple in colour. Rhizome colour of *K. parviflora* ranged from dark purple to black rhizomes and these colours lead to its name "Krachaidum" or "Black Ginger" (Wattanasri, 2016).

According to Aiyer and Kolammal (1964), *K. galanga* leaves are deep green in colour, orbicular or sub-orbicular, orbiculate-ovate or ovate cordate in shape. They have thin membraneous blade, margin are wavy. Main tubers were conical shaped, with transverse or horizontal or annular scars of scales, tubers of fairly smooth and greyish or light brown colour.

Indrayan *et al*. (2007) reported the morphological variability of two varieties of *K. galanga* (Kasthuri and Rajani). They reported that rhizome colour of Kasthuri was light brown while it was creamish brown for Rajani. Kasthuri had shining and thicker leaves when compared to Rajani. More number of leaves were observed in Kasthuri and the size of leaves ranged from 10-15cm in Kasthuri, while it was 7.5 -12.5 cm in Rajani.

Kaempferia rotunda rhizomes are very fleshy, 3 - 3.5 cm in length and 1.5 - 1.75 cm in diameter. Each tuber has the shape of club with a bulged lower portion and a stalk like cylindrical upper portion (Sereena *et al*., 2011). There are 2-4 number of leaves according to Phokham *et al*. (2013), petiole are hairy, 1-2 cm long, and leaves are elliptic to lanceolate-oblong.

2.3.2 Sprouting and plant growth

Mahender *et al*. (2015) revealed that days to first sprouting varied with the different size of rhizome in ginger. *Kaempferia parviflora* rhizome took seven days to sprout at Anantharajupet after the treatment of 750 mg per litre ethephon (Labrooy *et al*. 2016[a]).

In a study conducted by Joy *et al*. (2006), number of suckers produced for *K. rotunda* was 3.5 per plant on application of FYM at the rate of 15 t/ha.

According to Chandana (2011), in an experiment conducted at Kerala for standardization of Good Agricultural Practices (GAP) in 'kacholam' (*Kaempferia galanga* L.) for yield and quality, the maximum foliage spread was 22.88 cm (NS) at three months after planting, while it was 22.96 cm (EW) at three months after planting in the control group**.**

A study conducted on the performance of three turmeric cultivars (Duggirala, Zedory and Krishna) in Pakistan revealed that rhizomes took 44.67 to 49.33 days to sprout (Jilani *et al*., 2012).

Planting of ginger variety IIHR Mahima on 15th June at Dapoli, Maharashtra took 14.92 days for sprouting of rhizomes, while planting on 15th April took 26.04 days for sprouting (Yadav *et al*., 2013).

Asafa and Akanbi (2018) studied the effect of propagule size on the number of tillers per plant of ginger at South Western Nigeria which showed that the number of tillers produced per plant was highest at 40 g rhizome size.

Highest number of tillers (4.24) per plant were recorded from the mother rhizome size of 50-60 g in turmeric in the mid hill condition of Arunachal Pradesh (Angami *et al*., 2017).

An experiment conducted under greenhouse condition for growth and yield of turmeric showed that the number of tillers increased with increase in the size of rhizome (Hossain *et al*., 2005).

Tillers produced per plant ranged from 1-4 in *Curcuma amada* in the nine accessions studied at Tsukuba, Japan (Jatoi *et al*., 2015).

Sankari *et al*. (2016) studied performance of 38 heliconia cultivars for yield and quality in the Eastern Ghat region. The plant spread in East-West direction ranged from 0.6 m to 2.6 m, while the spread in north-south direction ranged from 0.60 m to 2.8 m.

Turmeric is a long duration crop which required about 4-5 months for the initial growth (Hemalatha and Chitra, 2018). Rao *et al*. (2005) recorded the crop duration of 45 turmeric cultivars which ranged from 255.19 days in cluster I and 185.07 days in cluster VI.

According to Rahman *et al*. (2009), harvesting of ginger extend from November to January in North eastern region of India and the crop attained maturity in 8-9 month.

2.3.3 Leaf characters

Leaf shape or leaf character is erect/ lanceolate for *K. rotunda* and erect or semi-erect/ lanceolate for *K. parviflora* (Chandrmai *et al*., 2012).

No significant difference in leaf length was observed among the different genotypes of *K. galanga* over open and shaded conditions but there were significance differences in leaf area and leaf area ratio under Kerala conditions (Latha, 1994).

A study carried out on *Kaempferia elegans* to understand the variations of plants collected from different localities of South India by Sabu *et al*. (2013)

showed that leaf length ranged from 15-17.9cm and leaf breadth ranged from 9.7-11.5cm.

Kaempferia parviflora grown under shade treatments revealed better growth and development at 50 per cent and 70 per cent shade levels in Malaysia (Abdullah *et al*., 2014). Thirty per cent shading yielded plants with highest photosynthesis rate and number of leaves. It also gave earliest shoot emergence compared with other treatments.

Parida *et al*. (2011) performed experiment on tissue cultured *Alpinia galanga* plantlets which were transplanted to the field and evaluated for phenotypic characteristics. The experiment revealed the leaf length and leaf breadth of 34.6 ± 2.03 and 4.8 ± 0.6, respectively for conventionally propagated plants, while it was 36.2 ± 0.8 and 5.2 ± 0.6, respectively for micro propagated plants.

Ten turmeric selections were evaluated under rainfed conditions for three crop seasons in the high altitude areas of Chintapalli in Andhra Pradesh by Naidu and Murthy (2013). Among the selected varieties, TU-1 exhibited the highest leaf length of 22cm and leaf breadth of 6cm.

In an experiment conducted on twelve varieties of turmeric during kharif season at Chintapalli under rainfed conditions (Kumar *et al*., 2015) the varieties differed in growth, yield and quality aspects. Leaf length was highest in the variety Roma (61.96 cm) and lowest in the variety Suranjana (36.037cm). Highest leaf breadth was recorded in Roma and lowest in NH-1.

Reddy *et al*. (2016) evaluated the effect of rhizome size and plant spacing of ginger cv. Maran. Leaf length and leaf breadth were found to be significantly highest in plants raised from 40 g seed rhizomes (23.71 cm and 2.46 cm, respectively).

Krishna *et al*. (2019) evaluated 19 genotypes of turmeric for growth and yield. Among the genotypes studied, BSR-2 recorded the highest leaf area of 13454.61 cm^2. Leaf area per plant ranged from 5362.86 to 13454.61 $cm^{2.}$

2.3.4 Rhizome characters and yield

The variability was observed in fresh as well as dry rhizome yield among the 10 types of *K. galanga* by Latha (1994) under Kerala conditions. Fresh and dry rhizome yield ranged from 48.81 to 74.93 g/plant and 10.00 to 19.81 g/plant respectively.

Prasannakumari *et al*. (1994) evaluated five local types of *K. galanga* and found that they differed significantly in fresh and dry yield of rhizome, dry recovery and oil yield.

The fresh rhizome yield of *K. galanga* ranged from 29.7 to 48.6 g/plant and dry rhizome yield from 6.5 to 14.5 g/plant among the 12 types evaluated (Prasannakumari *et al*., 1997).

The fresh rhizome yield of *K. galanga* was high when grown as an intercrop in coconut gardens under gardens of Kerala (Maheshwarappa *et al*., 2000). They also observed increased in oil and oleoresin content with increase in shade intensities.

A study conducted on *K. rotunda* for fresh rhizome yield recorded rhizome yield of 20 t/ha under rainfed conditions when it was planted at the spacing of 20 x 20 cm, mulched with 20 t/ha of leaves and applied with organic manure at 15-30 t/ha (Joy *et al*., 2006).

Rhizome yield in *K. galanga* was significantly higher in locations with 50 per cent shade intensity (Gangadharan and Menon, 2003). They also reported that levels of shade did not significantly affect the vegetative growth.

A study conducted by Chaudhary *et al*. (2006) using five varieties of turmeric *viz*. Krishna, Suvarna, Rajendra Sonia, Suguna and Sudarshana showed that the variety Krishna recorded highest fresh rhizome weight (405.60 q/ha) and cured rhizome yield (65.80 q/ha). They also reported highest length and breadth of rhizome in the variety Krishna.

According to Mohan *et al*. (2017), among the five genotypes of turmeric evaluated for yield under Karnataka conditions, IISR Pratibha recorded the highest yield in terms of fresh and dry rhizome yield as well the maximum length and girth of rhizome.

On evaluation of 15 bold rhizome accessions of ginger for yield and quality, by Sasikumar *et al*. (2003), accessions 35 and 107 were selected and released for cultivation as IISR Rejatha and IISR Mahima, respectively.

Significant variations were observed on account of growth, yield and quality parameters in 25 genotypes of *Curcuma longa*, *Curcuma caesia* and *Curcuma aromatica*. Among the genotypes studied, *C. caesia* showed highest growth and yield (Lynrah and Chakrabarthy, 2000).

Amzad-Hossain (2010) concluded that the turmeric should be harvested in January for higher dry yield in Okinawa. The maximum dry yield was obtained when shoots were withered completely.

Singh *et al*. (2013) reported from Mizoram that planting of turmeric during last week of April gave better performance of plant growth, higher rhizome yield and higher quality aspects. They also reported significant reduction in yield and quality on account of delayed planting.

The variation in yield and quality profile was observed in *Curcuma aromatica* and *Curcuma amada* over the three growth stages under Kerala conditions (Sajitha and Sasikumar, 2014).

The phytoconstituents of turmeric like oil, oleoresin and curcumin varied in the rhizomes collected from different locations. It was attributed to the influence of different agro climatic zones from which the genotypes were collected (Alam and Naik, 2009).

2.3.5 Root tubers

Fleshy tubers which are white or watery pearl colour have been reported to be present in many genera of the tribes Globbeae and Zinziberaea including *Kaempferia* species by Sabu (2006). Sereena *et al*. (2011) have observed the presence of club shaped tubers in *K. galanga*. Rhizome size and the number of storage roots *i.e*. root tubers affected the sprouting, growth, yield and quality of *Cucurma alismatifolia* indicating an important role for storage food reserves such as the carbohydrates and proteins in these organs (Anuwong *et al*., 2014).

Seema (2015) described the root tubers in various *Curcuma* species. *Curcuma amada* has sessile tubers, thick and 5-10 × 2- 3 cm in size, cylindrical or ellipsoid in shape and branched while roots are fleshy and root tubers are absent. She also opined that *Curcuma zedoaria* has thick sessile tubers of 3 cm and are branched and pearl white inside, while *C. aromatica* had many sessile tubers which are yellow. *Curcuma caesia* has sessile tubers which are branched and condensed. The roots are fleshy with many root tubers which are ovate-oblong, watery-pearl in colour. *Curcuma haritha* has branched sessile tubers. In *Curcuma ecalcarata*, root tubers are fusiform and white inside. In *Curcuma oligantha*, root tubers are large, 4-5 × 2-3 cm in size, ovate or fusiform and white in colour inside. In *Curcuma raktakanta*, roots are many and fleshy. Root tubers are 5 × 2.5 cm in size, fusiform and yellow in colour inside with a white peripheral region. In *Curcuma aeruginosa*, the roots are fleshy with many root tubers which are ovate-oblong and creamy.

The tuberous roots are modified form of contractile roots where terminal part is swollen and form an egg shape in *C. alismatifolia* (Ruamrunsgri, 2015). He observed that at least four storage roots attached to rhizome are required for

exporting *C. alismatifolia* which stored carbohydrate that would be utilized by the plant for proper growth and development in the next season.

2.4 FLORAL BIOLOGY

Floral morphology of *K. galanga* has been described by many workers (Kirtikar and Basu, 1935 and Sabu, 2006). Plant will start flowering in June and flowering will be ended by September. The flowers are produced directly from the rhizome and open in sequence. The peak flowering stage was attained during July-August (Rajagopalan, 1983). According to Sabu (2006), inflorescence is sessile with 6-12 or more flowers. One flower opens at a time. Bracts are glabrous ovate-acuminate white with light green tip and bifarious with outer larger one and smaller inner ones, 3-4 × 1 cm long. Calyx is equal or shorter than bracts and glabrous, three cm long. Corolla tube is 4.5 cm long with lobes of 2.5-3.0 cm long and linear. Labellum is slightly broader with 2.3 × 2.5 cm, divide 2/3rd to the base, and each lobe splits shortly into two unequal halves. Lateral staminoids are obovate and white in colour. Anthers are white and stigma is globular with lateral slit. The ovary is tricarpellary with many ovules on axile placentation.

Kaempferia parviflora is a perennial herb; rhizomes black, strongly branched, root fibres slender; leaves thin, ovate, light shiny green, 13.5-23.2 cm × 9.3-13.5 cm, apex acute, glabrous; petiole long, 9 cm-10 cm. Inflorescence pedunculate, 5.1-5.4 cm, flowers white, purple tinged darker in the middle of lip; eight flowered inflorescence in which, only one flower open in a day. They are sweet scented with lanceolate bracts, slightly greenish, 1.4-1.7 × 33.5 cm; calyx white 2.4 × 0.7 cm, corolla lobes linear white 1.5 cm; lateral staminodes linear, 1.1 × 0.3 cm, white; labellum white and purple tinged at the base, lip lilac obovate, cuneate, bifid, upto 1/4th its length, 1.2 × 0.8 cm; anther sessile, 0.4 × 0.1 cm, crested; stigma 4.2 cm; ovary velutinous, trilocular, elliptic (Devi *et al*., 2016).

K. rotunda is found in various parts of India and adjoining regions mostly in wild. The plant is raised in small herbal nurseries for application in medicinal preparations. Sanskrit name *bhumi champa* means blooming from within earth. The

indigo-coloured flower shoots arise from within the soil. In fact, the flower emerges much in advance of the whitish leafy shoot. The flower and leaf are never seen at the same time (Nair, 2004).

According to Kirtikar and Basu (1935), flowers are 1cm long, borne in radicle scapes, spreading linear petals. Staminods are white in colour, acute shape with 3.8-5.0 cm length, lilac or reddish colour lip, bifid and lanceolate anther lobes.

2.5 VIVIPARY

Vivipary in flowering plants is defined as the precocious and continuous growth of the offspring when still attached to the maternal plant. There are two main types of vivipary, true vivipary (sexually produced offspring) and pseudovivipary (asexually produced offspring). The ones with true vivipary tend to inhabit shallow marine habitats while pseudoviviparous species are mostly found in alpine, artic or arid habitats (Elmqvist and Cox, 1996). Vivipary is noticed in several members of Zingiberaceae family.

Bhadra *et al*. (2013) reported the phenomenon of vivipary in *Hedychium eltum* in West Bengal, India during monsoon season (July-August). They reported that an average of around 60 seedlings were obtained from three viviparous inflorescence and three seedlings were obtained per capsule.

Ashokan and Gowda (2018) reported vivipary in four different *Hedychium* species namely *H. marginatum*, *H. speciosum* var. gardnerianum, *H. thyrsiforme*, and *H. urophyllum* during a three year study in north eastern India. They further reported that the type of vivipary could be facultative vivipary since the fruits of *Hedychium* dehisced before seed maturation. A true vivipary require the embryo to penetrate the fruit pericarp during germination.

Seeds of *Alpinia mutica* showed vivipary (Aswani and Sabu, 2015). The embryo grow first through the seed coat and then out of the fruit wall while still

attached to the parent plant and they found that 71 per cent of seed germinated in the moist soil.

Vivipary was noticed in the orchid *Epiphyllum phyllanthus* by Cota-Sa ´nchez and Abreu (2007) and reported that it is an intrinsic reproductive mechanism supporting the germination and dispersal of the fittest offspring regardless of substrate and environmental conditions.

Viviparous germination occurred in members of Rhizophoraceae *viz*. *Aegialitis rotundifolia*, *Aegiceras corniculatum*, *Avicennia* spp. and *Nypa fruticans*. Germination occured following an incipiently viviparous type where the hypocotyl pierces out of the seed coat but not from the exocarp at the time of dispersal in Sunderbans, India. It was defined as cryptovivipary by Das and Ghose (2003).

Anand and Mathur (2012) documented the occurrence of cryptovivipary in *Tagetes erecta* L. It was observed in the dormant twig and dried flower in local gardens of Jammu and Kashmir during heavy monsoon (late June to early August).

Embryos of viviparous mangrove species do not have a dormancy period. The dormancy, under natural conditions, is defined as the lack of germination due to certain characteristics of the seed (Baskin and Baskin, 2000). When there is no dormancy at all and seeds even germinate before abscission, this is called true vivipary. In viviparous plants the offspring grows continuously while still attached to the mother tree (Goebel, 1905).

The seeds that do not tolerate desiccation are called recalcitrant seeds (Tweddle *et al*., 2003). Recalcitrance of seeds, as in viviparous plant species, has ecological advantages. The absence of a dormancy period during which metabolism is extremely low enables seeds to continue building up reserves while it is attached to the parent plant (Juncosa, 1982; Farnsworth, 2000).

In plants exhibiting true vivipary, the seed germinates while it is still attached to the mother tree. Germination is generally defined as the moment at

which the embryo protrudes the surrounding structures, in most cases with the radicle first, so that the embryo becomes visible (Nonogaki *et al*., 2010). In some species, the embryo only grows through the seed coat and not through the surrounding fruit while it is still attached to the parent tree, this is called cryptovivipary (Tomlinson, 1999).

Farnsworth (2000) observed that all viviparous species share certain hormonal characteristics and all grow in wet habitats. Therefore, he hypothesised that the lack of dormancy, and thus recalcitrance and vivipary are common in wetland habitats like the one of mangroves.

2.6 ANATOMY

The anatomical studies can be used as a taxonomic tool because of their high structural diversity (Wilkinson, 1979) which has been used successfully to assess taxonomic status and help in the identification of different species. The main aim of anatomical characterization is to relate the structural analysis and to support the taxonomic classification of the plants in which the characters are demonstrated (Metcalfe and Chalk, 1979). Gilani *et al*. (2002) reported the use of anatomical studies along with morphological parameters for resolving taxonomic problems in monocots.

Stomatal index (SI) is the proportion of stomata relative to the total epidermal cell number and is a useful morphological parameter for species identification since environmental factors do not influence stomatal index. Stomatal index was calculated according to the formula given by Salisbury (1927); SI= S/ S + E x 100, where S= no. of stomata per unit area, E= no. of epidermal cell per unit area.

A study conducted on morpho-anatomical features of *K. galanga* by Roy *et al.* (2013) revealed that rhizomes are bulbous with close aggregation and the anatomical structure consist of 1- cell-layered epidermis, then 1-2 celled-layered hypodermis or the absence of hypodermis, followed by the cork cambium layer of

few cell-layered thick; then the parenchymatous cortical zone of massive cell layers, then endodermis encircling the ground tissue with the numerous vascular bundles. The adaxial epidermis is papillate in *Kaempferia rotunda.* It has soft or delicate hair which is one- or more-celled, simple, blunt or pointed hair.

Uma and Muthukumar (2014) reported that epidermis is uniseriate, bearing unilocular unicellular hairs. The exodermis, two (*K. galanga and K. rotunda*) layered with cells and contain suberized walls. The cortex is 15 cells wide, parenchymatous, with intercellular spaces that are linear in the outer cortical layers in *K. galanga* and triangular in the inner cortical layers in *K. rotunda*. The endodermis is uniseriate with thin-walled cells (*K. galanga*) or cells with suberized 'U'-shaped thickened walls (*K. elegans* and *K. rotunda*). The pericycle is uniseriate with thin-walled cells. Vascular cylinder is solitary, 8-12 arches, exarch, vascular bundles radially arranged with the xylem distributed in two rows between islands of phloem.

The transverse sections of the rhizome of *Alpinia galanga* showed that the epidermis composed of a single row of narrow tangentially elongated cells, fibro vascular bundles are scattered throughout the cortex and stele without any definite orientation and are collateral endarch and closed type, starch grains showed variation in size and shape (Chitra and Thoppil, 2008).

Transverse sections of roots of *Curcuma alismatifolia* showed that the epidermis was single layered and irregularly polygonal, cortex was composed of large thin-walled parenchyma cells and multi-layered, endodermis was not well defined, vascular bundles were radial and exarch; stomata were mostly tetracytic and found in both leaf surfaces, abaxial leaf surface had a higher stomatal density and stomatal index than the adaxial (Anuwong *et al*., 2014).

The transverse section of the rhizome of ginger included different types of tissues which are epidermis, cork, cortex and vascular bundles. The cortex was formed of parenchyma cells which often contain starch and oils in large amounts (Eltahir *et al*., 2018).

Sereena *et al*. (2011) reported that *Kaempferia rotunda* showed correct demarcation between the outer and inner zone by endodermis like layer termed as endodermoidal layer, starch grains present were oval in shape.

Salasiah and Meekiong (2018) studied six genera of tribe Alpinieae and found that epidermal cells of the adaxial surface were hexagonal or more or less polygonal in shapes in all selected species with straight anticlinal walls at both surfaces. Different sizes of epidermal cells are also shown in all species. The type of stomata observed was of tetracytic form in all species. Most of species possessed simple and unicellular trichomes.

The transverse section of root tubers of *Curcuma* species exhibited large parenchymatous cortex and pith filled with starch. Starch grains were larger in size compared to those in the rhizome, vascular region was found as a continuous ring within the endodermis. Endodermis was found without any thickening and was made of rectangular shaped cells (Seema, 2015).

In *K. parviflora* rhizome, Zulfa (2012) observed that there were secretory cell, identified as oil cell that contained oil component, reddish cell-like structures were also found in the rhizomes and was identified as vacuola that contained flavonoids.

2.7 PHYTOCHEMISTRY: PRIMARY AND SECONDARY METABOLITES

Medicinal plants are rich in secondary metabolites which include flavonoids, alkaloids, saponins and related active metabolites which are of great medicinal value and have been widely used in the drug and pharmaceutical industry. These secondary metabolites are stated to have many biological and therapeutic properties (Dash *et al*., 2017).

Total carbohydrate, starch, sugar, amino and flavone content were analyzed in *Kaempferia galanga* by Kalaiselvi and Ponmozhi (2011). The content of total carbohydrate was 1.9–0.02 mg/g, starch was 1.7–0.01 mg/g, reducing sugars was

0.2–0.17 mg/g, non-reducing sugars was 0.2–0.32 mg/g, amino acids was 3.1–0.18 mg/g, flavone and flavanol was 30.8–0.73 mg/g and flavonone and dihydroflavanol was 8.2–0.25 mg/g.

Diversity of flavonoid content in *K. parviflora* from 12 different origins of Thailand was studied by Sutthanut *et al*. (2007). Total flavonoid content in the samples ranged from 23.86 to 60.98 mg/g.

Rhizomes from eight-month old plants *K. parviflora* gave the highest yield of flavonoids (Ab-Rahman *et al*., 2018). Optimal water extraction of this compound was attained at 90˚C for an extraction duration of 120 minutes. An average of 81 µg QCE/g dry weight of total flavonoids were obtained under these conditions.

Chaiyasut *et al*. (2018) evaluated *Lactobacillus paracasei* HII01 mediated fermented *K. parviflora* Wall. rhizome juice. The reducing sugar content of the samples was reduced while fermentation period was increased. The samples F1, F2, F3, and F4, showed a reduction in sugar content from 87.32 to 0.86, 68.25 to 1.5, 70.12 to 0.93, and 63.21 to 2.2 mg glucose equivalent per ml sample, respectively.

Kaempferia rotunda showed the presence of flavonoids, tritepenoids and steroids in the phytochemical screening of leaves and rhizome extracts (Desmiaty *et al*., 2018). Preliminary phytochemical analysis of hexane, methanol, ethanol and aqueous extract of *K. rotunda* have shown the occurrence of steroids, alkaloids, flavonoids, terpenoids and saponins (Kumar *et al*., 2015).

The extracts of *Kaempferia galanga* rhizome revealed the presence of resins, sterols and triterpenoids in petroleum ether extract; sterols, flavonoids, triterpenoids, and resins in chloroform extract; steroids, alkaloids, triterpenoids, flavonoids, and proteins in methanolic extract; water extract showed the occurrence of proteins, saponins and carbohydrates (Rajendra *et al*., 2011). Preliminary screening of *Kaempferia scaposa* by Jagtap (2015) indicated that rhizomes are rich in primary and secondary metabolites such as carbohydrates, vitamin C and E, alkaloids, flavonoids, glycosides, phenols, saponins and minerals.

The peak accumulation of phenolics, total protein and difurocumenonol were observed in 180 days old rhizome of mango ginger. Policegoudra *et al*. (2011) standardized 180 days as the optimum maturity standard for harvest of mango ginger rhizome.

According to a study conducted by Moreschi *et al*. (2006) at Brazil, per cent content of starch in dried ginger and dried turmeric were 25 ± 3 and 29.2 ± 0.6 respectively and the per cent content of total sugar were 1.15 ± 0.03 and 1.3 ± 0.2 respectively for the dried ginger and dried turmeric.

2.7.1 Volatile oil

The content of essential oil varied in *K. galanga* rhizome with varying time of collection. Indrayan *et al*. (2007) reported that the yields and colours of oils from the rhizome of two varieties of *K. galanga* rhizomes (Kasthuri and Rajani) varied slightly. Yield of essential oil was 1.88 per cent for Kasthuri and 1.76 per cent for Rajani. It was light brown colour for Kasthuri and pale yellow colour for Rajani. The oil and oleoresin content in *K. galanga* were slightly higher for pure crop when compared with intercrop (Kurian *et al*., 2000).

Sereena *et al*. (2011) identified 13 compounds from the essential oil of fresh sample of *K. rotunda* by GCMS analysis. Among the compounds, bornyl acetate and benzyl benzoate were the major constituents.

Indrayan *et al*. (2007) identified 58 compounds in the variety Kasthuri and 56 compound in the variety Rajani of *K. galanga* rhizome oil using GCMS analysis and observed that 45 compounds are common in both the varieties but 13 compounds present in Kasthuri oil are not present on Rajani oil and 11 compounds present in Rajani oil were absent in Kasthuri oil.

Volatile oil of the dried rhizomes of *K. galanga* contain major chemical constituents like ethyl-p cinnamate (31.77 %), methyl cinnamate (23.23 %),

carvone (11.13 %), eucalyptol (9.59 %) and pentadecane (6.41 %) (Tewtrakul *et al*., 2005).

Suphrom *et al*. (2017) detected groups of volatile substances in *K. rotunda viz*. monoterpenes, sesquiterpenes, diterpenes, long chain hydrocarbons, ester of fatty acids, benzyl derivatives, cyclohexane diepoxide and phytosterols. The main constituents found EtOH extracts were benzyl benzoate and crotepoxide.

Volatile oils of *K. parviflora* rhizomes was reported to have 20 compounds (Pitakpawasutthi *et al*., 2018). Major components observed were α-copaene (11.68 %), dauca-5, 8-diene (11.17 %), camphene (8.73 %), β-pinene (7.18 %), borneol (7.05 %), and linalool (6.58 %). Bhuiyan *et al*. (2008) reported the chemical constituents of *K. galanga* leaf and rhizome oils analyzed by gas chromatography mass spectroscopy (GC-MS). One hundred and eight components were identified in the leaf oil. The major components were linoleoyl chloride (21.42 %), caryophyllene oxide (11.75 %), cubenol (9.66 %) and caryophyllene (5.60 %). Eighty one components were identified in rhizome oil with the main components being 2-propenoic acid, 3-(4-methoxyphenyl),-ethyl ester (63.36 %), ethyl cinnamate (6.31 %), 4-cyclooctene -1-methanol (4.61 %), caryophyllene oxide (4.37 %) and limonene (3.22 %).

2.7.2 Components of leaf and rhizome extracts

The leaf and rhizome extracts of *K. parishii* were analyzed by GC-MS for determining their chemical constituents by Sahoo *et al*. (2016) and it revealed the presence of phytol (72.55±0.5 %), hexadecanoic acid methyl ester (4.94±0.2 %), hexahydro farnesyl acetone (3.78±0.2 %) and dibutyl phthalate (3.31±0.2 %) in leaves; totarol (74.96±0.86 %), cembrene (2.83±0.2 %) and borneol (1.23±0.15 %) in rhizome.

Many chemical constituents were reported in *K. parviflora* such as flavonoids, phenolic glycosides, glyceroglycolipids and sphingoglycolipids. Recently, new glycosides in *K. parviflora* were isolated including kaempferiaosides

A-F (Chaipech *et al*., 2012). The main constituents of *K. parviflora* extract contained 5,7-dimethoxyflavone, 5,7,4'-trimethoxyflavone and polymethoxyflavones which have high content in this plant (Sutthanut *et al*., 2007). Chemical components of hexane, dicholomethane and ethanolic extracts of *K. rotunda* Linn. rhizome were analysed by Suphrom *et al*. (2017). The three extracts contained different quantities of chemical components *i.e*. sesquiterpenes (selinene, ylangene, gurjunene, amorphene, curcumene, eudesmol), monoterpenes (camphene, α-pinene, linalool, 1,8-cineole, camphor, α-terpineol, borneol, 1-bornyl acetate), ethyl ester of fatty acid (ethyl palmitate, ethyl linoleate, ethyl stearate) and benzyl derivatives according to their different polarities.

Methanol extract of *K. galanga* rhizome GC-MS analysis made by Ali *et al*. (2018) showed that the major constituents were palmitic acid (35.17 %), 2-Propenoic acid, 3-(4-methoxyphenyl)-, ethyl ester (10.18 %), octadecanoic acid (10.10 %), sandaracopimaradiene (8.20 %), oleic acid (22.15 %), 2-[2-(4-nonylphenoxy) ethoxy]ethanol (3.57 %), glycidyl stearate (7.27 %) and phthalic acid, 6-ethyloct-3-yl2-ethylhexyl ester (3.37 %).

2.8 PHARMACOLOGY

The diversity of medicinal plants make a remarkable opportunity for the development of new medicinal drugs in India. Pharmacological studies are of utmost importance to know the bioactive compounds, part of the plant to be used, the best extraction methods, activeness against which microorganisms and the level of relative toxicity of plant extract against human cell and their doses.

2.8.1 Toxicology studies

The toxicological study of *K. parviflora* on male rats revealed to have no significant difference of blood cell count. All dosages (60, 120, and 240 mg/kg BW, and the controls) had no effect on kidney and liver function (Sudwan *et al*., 2006).

Effect of oral administration of dichloromethane extract of *K. galanga* (100 mg/kg) and ethyl-p-methoxycinnamate (120 and 160 mg/kg) on body and organ weights were observed by Sirisangtragul and Sripanidkulchai (2011). There was no mortality and any detectable changes in behavioural activities during the treatment period in any group. There were no changes in body, liver and kidney weights and the internal organs of all animals showed no signs of abnormality. They concluded that the given doses of *K. galanga* did not produce toxicity.

Acute oral toxicity studies of the ethanolic extract of *Kaempferia rotunda* rhizome and rhizome fractions were performed on Wistar albino rats at a single test dose of 2000 mg/kg p.o. (Sini *et al*., 2014). There were no mortalities or toxic signs observed in any of the treated animals.

2.8.2 *In vivo* immunomodulatory studies

Horigome *et al*. (2014) identified four flavones from *K. parviflora* and investigated their anti-inflammatory effects in rat basophilic leukaemia cells. They found that 5, 7-dimethoxyflavone was potent in inhibiting antigen-induced degranulation revealing the anti-inflammatory property.

Flavonoids isolated from *Phyllanthus niruri* was subjected to *in vitro* immunomodulatory study using MTT assay by Jose *et al*. (2014) and they found that it had stimulatory effect on isolated normal lymphocytes.

Kim *et al*. (2018) observed the immunomodulatory effect of Kuseonwangdogo-based mixed herbal formula aqueous extract on cyclophosphamide induced immunosuppressed mouse model. They revealed that there were noticeable decrease in body weight and organ weight in cyclophosphamide treated group while the cyclophosphamide induced myelosuppressed signs were marked and dose dependently inhibited by oral administration of Kuseonwangdogo-based mixed herbal formula aqueous extract.

Morris *et al*. (2011) reported the increase in bone marrow cellularity, white blood cell count, increase in delayed type hypersensitivity when *Pluerotus* sp. powder was examined for immunomodulatory property on cyclophosphamide treated mice.

Shruthi *et al*. (2018) evaluated the immunomodulatory property of Galic acid in Swiss albino mice, using Haemagglutination antibody titre, white blood cells and red blood cell counts, platelet counts and haemoglobin levels. Results revealed that galic acid was found to exert effect on immune system.

Administration of methanolic extract and ethyl acetate fraction of *Gmelia arborea* to Wistar rats significantly increased HA titre, DTH response and increase in total white blood cell count (Shukla *et al*., 2011).

Solanum xanthocarpum showed prominent immunoprotective activity by increasing the depleted level of total white blood cell count and red blood cells, haemoglobin and neutrophil adhesion caused by the cyclophosphamide immunosuppression in Swiss albino mice (Sultana *et al*. 2011).

Administration of Swiss albino rats at the dose of 1mg/kg, 2 mg/kg, 4 mg/kg and 32 mg/kg for a period of 14 days showed that the animals treated with 1mg/kg enhanced the level of total leukocyte count, neutrophil percentage, haemoglobin, haemagglutination antibody titre and delayed hypersensitivity reaction (Sumalatha *et al*., 2012).

2.8.3 *In vitro* studies

2.8.3.1 Antimicrobial properties

A lectin isolated from *K. rotunda* tuberous rhizome was identified to have antimicrobial property when tested against eight bacteria *i.e*. *Bacillus cereus*, *Bacillus subtilis*, *B. megaterium*, *Sarcina lutea*, *Shigella shiga*, *S. dysenteriae*, *S. sonnei* and *Klebsiella* sp (Kabir *et al*., 2011).

Ethyle acetate, n-hexane and ethanol extract of *K. rotunda* were investigated for antibacterial property and found that ethyl acetate extract exhibited the maximum zone of inhibition against *Staphylococcus aureus* (Malayahayati *et al*., 2018).

Antibacterial activity of *K. rotunda* was observed against six bacteria strains using different solvent extracts by Kumar *et al*. (2015) and reported that ethyl acetate extract was potent against *L. acidophilus, S. pneumonia* and *S. pyogenes*.

Kaempferia rotunda rhizome lectin inhibited the growth of *S. aureus* and *E. coli* partially when a study was conducted by Kabir and Reza (2014) for antimicrobial property.

According to Abubakar *et al*. (2015), aqueous and ethanolic extract from leaves and stem of *Sesbania grandifolia* showed varying antimicrobial activities against *S. aureus, E. coli, S. enterica* and *P. aeruginosa*. Zone of inhibition using aqueous and ethanolic leaves extract ranged from 9.0±00 mm to 24.0±0.21 mm against *S. aureus*. Ethanolic stem extract were found to be active against *S. enterica*.

Tangerine seeds were reported to have antimicrobial property when the ethanolic and petroleum ether extract of tangerine seeds were tested against *Staphylococus aureus, E. coli* and *Klebsiella pneumonia* (Agu *et al*., 2013).

2.8.3.2 Antioxidant properties

Antioxidant property of methanolic extract of *K. rotunda* was explored by Mohanty *et al*. (2008). The property was accessed by lipid peroxidation markers and concluded that antioxidant property was inversely proportionate to the doses used.

The rhizome and leaves of *K. rotunda* and *C. zedoaria* were assessed for antioxidant property using DPPH method. Half inhibitory concentration (IC_{50}) for *K. rotunda* leaves and rhizome were 126.99 and 193.71 respectively while for the

leaves and rhizome of *C. zedoaria* IC_{50} were 45.75 and 72.61, respectively (Desmiaty *et al*., 2018).

Malayahayati *et al*. (2018) reported the antioxidant property of *K. rotunda* using DPPH method. They reported that IC_{50} was highest in ethanolic fraction followed by ethyl acetate fraction and n-hexane fraction.

Thao *et al*. (2016) reported the antioxidant activity of *K. parviflora* using peroxyl radical-scavenging and reducing capacities. Compound 5 exhibited significant peroxyl radical scavenging capacity, while compound 13 showed significant reducing capacity at 10.0 µM.

The chloroform and methanol extracts of the rhizomes of *Kaempferia angustifolia* showed strong free radical scavenging activities against 1,1-diphenyl-2-picrylhydrazyl (DPPH) with values of 615.92 mg Trolox equivalent (TE)/g each (Yeap *et al*., 2017).

2.8.3.3 Anticancer properties

Cytotoxic properties of *K. rotunda* were tested against human breast cancer T47D cell line and showed that chloroform extract had IC_{50} of 41.72 µg/ ml (Atun and Arianingrum, 2015).

Kaempferia rotunda showed antiproliferative activity against Ehrlish ascites carcinoma cells when an experiment was conducted *in vivo* in mice (Kabir *et al*., 2011). The per cent inhibition obtained were 51 per cent for the mice administered with 1.25 mg/kg/day and 67 per cent for the mice administered with 2.5 mg/kg/day.

Effect of *K. rotunda* lectin using MTT assay was investigated by (Kabir and Reza, 2014) and observed that the lectin inhibited 6.2-50.5 per cent cell growth at the range of 7.5-120 µg/ml. Thus they concluded that the lectin induced apoptotic cell death in Ehrlich ascites carcinoma (EAC) cells.

Antiproliferative activity was observed against Ehrlich ascites carcinoma (EAC) cells by Ahmed *et al*. (2017) from a lectin isolated from the tuberous rhizome of *K. rotunda*. A 59 per cent cell growth inhibition was observed against the cells at 160 µg/ml.

Banjerdpongchai *et al*. (2008) demonstrated the apoptotic effects of ethanolic *K. parviflora* rhizome extract on HL-60 cells *in vitro*. The suppression of HL-60 cell growth and decreased cell viability was in a dose- and time-dependent manner.

Materials and methods

3. MATERIALS AND METHODS

The present investigation entitled "Performance analysis of medicinal *Kaempferia* species" was carried out at the Department of Plantation crops and Spices, College of Horticulture, Kerala Agricultural University, Thrissur during 2016-18. The experimental plot falls in central Kerala at 10° 31'N latitude and 76°13' E longitude, with an altitude of 22.25 m above mean sea level. The pharmacological studies were carried out at the Department of Pharmacology and Toxicology, College of Veterinary and animal Sciences, Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur.

3.1 EXPERIMENTAL MATERIAL

A total of 18 genotypes belonging to three different species of *Kaempferia* as given below formed the material for the study

1. Thirteen collections of *Kaempferia rotunda*
2. Three collections of *Kaempferia parviflora*
3. Two collections of *Kaempferia galanga*

Details of genotypes used in the study is furnished in table 1.

Table 1: List of *Kaempferia* genotypes

Sl. No.	Code	Description	Source of collection
1.	KCP-1	Kerala Collection Parviflora-1	Botanical Survey of India, Shillong
2.	KCP-2	Kerala Collection Parviflora-2	Indian Institute of Spice Research, Calicut
3.	BSI-1	Botanical Survey of India-1	Thailand
4.	KCG	Kerala Collection Galanga	Thrissur, Kerala
5.	ArPCG-1	Arunachal Pradesh Collection Galanga	Pasighat, Arunachal Pradesh

Table 1 contd.

Sl. No.	Code	Description	Source of collection
6.	MCR-1	Manipur Collection Rotunda-1	Imphal West district, Manipur
7.	MCR-2	Manipur Collection Rotunda-2	Imphal East district, Manipur
8.	MCR-3	Manipur Collection Rotunda-3	Imphal West district, Manipur
9.	MCR-4	Manipur Collection Rotunda-4	Imphal East district, Manipur
10.	MCR-5	Manipur Collection Rotunda-5	Bishnupur district, Manipur
11.	MCR-6	Manipur Collection Rotunda-6	Thoubal district, Manipur
12.	MCR-7	Manipur Collection Rotunda-7	Imphal East district, Manipur
13.	KCR-1	Kerala Collection Rotunda-1	All India Co-ordinated Rearch Project on Medicinal and Aromatic Plants, Kerala Agricultural University, Thrissur, Kerala
14.	KCR-2	Kerala Collection Rotunda-2	Model Nursery on Spices, Kerala Agricultural University, Thrissur, Kerala
15.	KCR-3	Kerala Collection Rotunda-3	Herbal Garden, Kerala Agricultural University, Thrissur, Kerala
16.	KCR-4	Kerala Collection Rotunda-4	Kerala Forest Institute, Peechi, Kerala
17.	KCR-5	Kerala Collection Rotunda-5	Malapuram district, Kerala
18.	KCR-6	Kerala Collection Rotunda-6	Aromatic and Medicinal Plant Research Station, Odakkali

Plate 1: Experimental plot of medicinal *Kaempferia* species

3.2 DESIGN AND LAYOUT OF THE EXPERIMENT

The experiment was carried out in Completely Randomized Design with three replications. Recommended package of practices (KAU, 2016) for *Kaempferia galanga* was followed in all the three species. Two crops were raised during May to December, 2017 and May to December, 2018 in the shade net house with 50 per cent shade.

3.2.1 Pot culture experiment

Just sprouted rhizomes of 25-35 g were used as planting material. They were planted at the depth of 3-4 cm in grow bags (25×40 cm) filled with mixture of soil, sand and FYM in equal proportions (Plate 1). Immediately after planting the soil surface was mulched thickly using *Glyricidia* leaves and a light irrigation was given. Mulching was repeated two months after planting. Irrigation was given on alternate days during rainless periods.

The number of grow bags in each of the species is given below:

Kaempferia rotunda : 25

Kaempferia parviflora : 50

Kaempferia galanga : 30

From each genotype, 10 plants were tagged for recording observations.

3.2.2 Meteorological data

Weather data of the experimental site was collected from Department of Agricultural Metrology, College of Horticulture, Vellanikkara and is attached as appendix I.

3.2.3 Intercultural operations

Necessary intercultural operations were carried out during the cropping period for proper growth and development of the plants. The plants were irrigated during the rainless periods. Other intercultural operations like weeding and plant protection measures were adopted as and when required. Recommended dose of organic manure and other cultural package of practices were adopted for better crop growth as per ADHOC organic POP recommendations of KAU (Alexander *et al*., 2009).

3.2.4. Harvesting

Drying up of leaves is the sign of maturity at 7-8 months. The rhizomes were dug out carefully, leaves removed and the rhizomes were cleaned of adhering soil and debris.

3.3. MORPHOLOGICAL EVALUATION

3.3.1 Qualitative parameters

3.1.1.1 Rhizome characters

Rhizome colour and shape were recorded according to Royal Horticulture Society (RHS) colour chart. Presence or absence of root tuber was noted in all the genotypes.

3.1.1.2 Leaf characters

Mature leaf tip and leaf margin shape were recorded according to Manual of leaf Architechture (Ash, 1999). Mature leaf colour was recorded according to RHS colour chart.

3.3.2 Growth parameters

3.3.2.1 Plant growth

Days to sprout

Number of days taken for sprouting of rhizomes in each treatment was counted upto five weeks after planting, at weekly intervals.

Number of tillers

The number of aerial shoots arising around a single plant was recorded at monthly interval starting from two months after planting.

Plant spread (N-S & E-W)

Plant spread was measured at the widest part of the plant as the distance between the leaf tips of farthest leaves and expressed in centimetre.

Growth habit

General nature of growth of the three species were observed and recorded.

Crop duration

The number of days taken from planting to harvest for each accession was recorded as the maturity period. Harvesting was done when the aerial parts were completely withered.

3.3.2.2 Leaf

Number of leaves per plant

Total number of leaves produced per plant was recorded at monthly interval and the average was tabulated

Leaf length

Leaf length was measured using scale at monthly interval from one month after planting and expressed in centimetre.

Leaf breadth

Leaf breadth was measured using scale at monthly interval from one month after planting and expressed in centimetre.

Leaf area

The leaf area was measured using leaf area meter at monthly interval from one months after planting and expressed in square centimetre.

Fresh weight of leaves per plant

The leaves were separated from mature plants. The weight was recorded and expressed in g/plant.

Dry weight of leaves per plant

Leaves separated from individual plants were dried in hot air oven at 70^{o}C. The weight was expressed in g/plant.

3.3.2.3. Root and rhizome

Fresh weight of roots

The roots were separated from harvested rhizome at 8 MAP. They were cleaned and weight was recorded and expressed in g/plant.

Dry weight of roots

Roots separated from individual plants, after cleaning were dried in hot air oven at 70^{o}C. The weight was expressed in g/plant.

Length of rhizomes

The length of rhizomes was measured at 8 MAP and expressed in centimetre.

Girth of rhizomes

The thickness of rhizomes was measured using vernier calliper at 8 MAP and expressed in millimetre.

Dimensions of the root tubers

The length and breadth of root tubers were measured at 8 MAP using vernier calliper and expressed in centimetre.

3.3.2.4 Yield

Fresh yield of rhizomes

Rhizome yield of individual plant was recorded after harvesting, cleaning and removal of roots at 8 MAP and expressed in g/plant.

Dry yield of rhizomes

Harvested rhizomes were cleaned and washed thoroughly. Thereafter it was weighed and kept for drying in the hot air oven at 70^{o}C. The dry weight of rhizome was expressed in g/plant.

Drying percentage

Dry weight of rhizome per plant was calculated on initial weight basis and expressed in percentage.

$$\text{Drying percentage} = \frac{\text{Dry weight of rhizomes (g)}}{\text{Fresh weight of rhizome (g)}} \times 100$$

Biological yield

The yield of whole plant portion in individual treatment was recorded at 8 MAP and expressed in g/plant on dry weight basis.

Harvest index

Harvest index was calculated by using following formula

$$HI = \frac{Y_{econ}}{Y_{biol}}$$

Where, HI = Harvest index

Y econ = total dry weight of rhizome

Y biol = total dry weight of the plant

3.4 VIVIPARY

Vivipary was noticed in *K. parviflora*. As a confirmatory test for vivipary, recalcitrance of the seed was tested by germination test. Observations on number of viviparous seedlings, their growth characters and yield were recorded as was done in the main crop. Comparative evaluation of viviparous and rhizome borne plants were also carried out.

3.5 FLORAL BIOLOGY

The floral biology of three *Kaempferia* species was studied with regard to floral morphology and pollen characters.

3.5.1 Anthesis: time and duration

With the objective of understanding the exact time of opening, ten inflorescence were observed at 15 minutes interval from 12:30 a.m. onwards until the cessation of flower opening. The inflorescences tagged for determining the time of anthesis were also utilized for ascertaining the time of anther dehiscence.

3.5.2 Pollen viability, size and morphology

The viability of pollen was assessed on the basis of stainability of pollen grains in acetocarmine-glycerin mixture. The inflorescence was bagged on the previous day of opening of flowers. The pollen grains were collected from the newly opened flowers at 6 a.m. and stained in a drop of acetocarmine-glycerine mixture on a clean slide and kept aside for one hour. All the pollen grains which were well filled and stained were counted as fertile and others as sterile. Pollen grains were counted from 10 different samples. The values were expressed in percentage.

Pollen diameter was measured using image analyser. Mean as well as range of size were calculated. The morphology of the pollen grains were also studied.

3.5.3 *In vivo* pollen germination

Since the trial for pollen germination using sucrose at different concentrations failed to show any positive result of germination, pollen tube penetration and growth through the style was observed. The fluorescence technique given by Kho

and Baer (1968) was adopted for the study. Inflorescence were covered with polythene bags on the preceding day of the experiment. The next day flowers were fixed in FAA (Formalin 5 ml, acetic acid 5 ml and ethyl alcohol 90 ml) at 3, 6, 9, 12 and 24 hours after pollination. After 24 hours the fixed buds were transferred to 1N NaOH for eight hours at room temperature. They were washed thoroughly with distilled water and stained in 0.1 per cent aniline blue in 0.1 N K_2HPO_4 for a period of 18 hours. The pistil was later macerated in 90 per cent glycerol. *In vivo* pollen tube growth was then examined under fluorescent lamp of a binocular microscope.

3.5.5 Stigma receptivity, style length and number of ovules

For finding out the stigma receptivity, presence of stigmatic fluid was observed at two hours interval.

Quantitative measurements such as style length, ovary length and number of ovules were recorded using a stereo microscope.

3.6 INCIDENCE OF DISEASES AND PESTS

The incidence of pests and diseases were recorded and timely control measures were adopted. Though the incidence of shoot borer and stem rot was noticed during mid-growth stages of the crop, scoring was not required due to effective control measures.

3.7 BIOCHEMICAL PARAMETERS

The following biochemical parameters were estimated in the rhizomes of three species. Rhizomes were collected at their maturity when the top portion dried up.

3.7.1 Volatile oil

The volatile oil content in the fresh rhizomes in each of the genotype was estimated by hydro distillation of fresh rhizomes in a Clevenger's apparatus (Guenther, 1972). The rhizomes were sliced into small pieces after washing to remove soil. One hundred gram of the material was taken and added to distilled

water. The sliced materials were heated for 5-6 hours. The oil present at the upper most layers was collected in the ependroff tube. Then the distilled essential oils were dried over anhydrous sodium sulphate and stored in tight container for further use. All volatile essential oil extraction was replicated three times.

Percentage yield of oil was calculated on fresh weight basis.

$$\text{Oil yield (\%) (v/w)} = \frac{\text{Volume of essential oil (ml)}}{\text{Weight of raw rhizome taken (g)}} \times 100$$

3.7.2 Oleoresin

The oleoresin content in the dried rhizomes was estimated using solvent extraction method by Soxhlet apparatus (AOAC, 1990). The result was expressed in percentage.

$$\text{Oleoresin (\%) (w/w)} = \frac{\text{Weight of the extract (mg)}}{\text{Weight of dried rhizome taken (g)}} \times 100$$

3.7.3 Starch

The starch content was analysed using anthrone method (Hedge and Hofreiter, 1962). The rhizome sample of 0.1g was weighed and homogenized in hot 80 per cent ethanol to eliminate the sugars. The residue was retained after centrifugation. Then the residue was washed repeatedly with hot 80 per cent ethanol until the washings did not give colour with anthrone reagent. It was followed by drying over a water bath. Five millilitres of water and 6.5 ml of 52 per cent Perchloric acid was added to the residue. This was centrifuged and the supernatant saved. The extraction process was repeated using fresh Perchloric acid and then centrifuged. The supernatant was pooled and made up to 100 ml with distilled water. One ml aliquot of the sample was used for analysis. 4 ml of anthrone reagent was added, heated for 8 minutes in a boiling water bath. The tubes were cooled rapidly and the intensity of green to dark green colour read at 630 nm.

3.7.4 Total sugars

The total sugar content was estimated by anthrone method (Hedge and Hofreiter, 1962). One hundred milligram of the sample was weighed into a boiling tube. It was then hydrolysed in boiling water for 3h with 5ml of 2.5 N HCl. The sample was cooled to room temperature and then neutralized with solid sodium carbonate until effervescence ceased. The volume was made up to 100ml and then centrifuged. The supernatant was collected and 0.5 and 1 ml aliquots taken for analysis. The volume was made to 1 ml in the tubes by adding distilled water. 4 ml of anthrone reagent was added to the tubes, heated for 8 minutes in a boiling water bath. The sample tubes were cooled rapidly and the green to dark green colour read at 630 nm.

3.7.5 Total free amino acids

The total free amino acid was estimated as described by Sadasivam and Manickam (1992) Five hundred milligram of sample was weighed. It was ground with 80 per cent ethanol and filtered. A 0.1mL of the extract was mixed with 1 mL of ninhydrin solution. Diluent solvent (mixture of equal volume of water and n-propanol) was added to the above solution and the intensity of the purple colour was read against a reagent blank in a colorimeter at 570 nm. Leucine was used as standard.

3.7.6 Flavonoids

The flavonoids content was estimated as described by Sereena (2011). Five gram of sample was homogenized with 20 ml of methanol (80 %) in a pestle and mortar 2-3 times. The extracts was pooled and made up the volume to 50 ml. Took 1.0 ml of extract in tubes, added 0.3 ml of 5 per cent $NaNO_2$. Waited for 2 min and added 0.3 ml of 10 per cent $AlCl_3$. After another 2 min, added 3.4 ml of NaOH and allowed to stand at room temperature for 10 minutes. The absorbance was read at 510 nm against blank. Quercetin was used as standard.

Calculation

Total flavonoid content (mg quercetin equivalents/100g)

$$= \frac{OD_{510} \times \text{Std. value (mg/OD)} \times \text{Total Vol. of extract} \times 100}{\text{Assay volume} \times \text{Wt. of sample (g)}}$$

3.7.7 GCMSMS profile of volatile oil

The volatile oil was analysed by Triple quadruple GCMSMS (Model TSQ 8000 MSMS). TG5M5 column (30 mm × 0.25 mm, 0.25 µm film thicknesses) was used as stationary phase. The oven temperature started from 60° C to 240° C with a constant rate of 3°C/min. The carrier gas was helium with the flow rate of 1 mL/min. One microliter of the oil (1:100 in HPLC grade methanol) was injected by Finnigan Autoinjector AI3000 with split ratio of 10:1. MS was performed by electron impact positive mode at 70 electron volts. The chemical constituents were identified by matching mass spectra and retention time indices with NIST MS Search 2.0 Library. Peak area was shown in percentage.

3.7.8 Gas Chromatography Mass Spectrophotometry (GCMS) analysis

The active phytochemical principles of *K. parviflora* and *K. rotunda* were analysed using Shimadzu GC-MS (Model Number: QP2010S), Kerala Forest Research Institute, Peechi, Thrissur, Kerala. The oven temperature was maintained at 70^{o}C for two minute and then increased to 200^{o}C in 5 min. The injector temperature was 260^{o}C and total analysis time was 50 min. One microliter aliquots of extracts were injected into the chromatographic capillary column of length 30m, inner diameter 0.25 mm, film thickness 0.25µm after a clear baseline had been obtained. Major constituents were identified by using mass spectrum NIST 11 and WILEY.

3.7.9 Phytochemical Screening

The ethanol extracts of rhizome of *K. parviflora* and *K. rotunda* were tested for the presence of various active chemical constituents namely steroids, alkaloids, tannins, phenolic compounds, flavonoids, glycosides, diterpenes, triterpenes and saponins by the procedure described by Harborne, (1991).

Tests for detection of steroids

Salkowski test

About 5.0 mg of the rhizome extract of *Kaempferia* was mixed with 3.0 mL of chloroform and then shaken with 3.0 ml concentrated sulphuric acid. Development of red colour indicated the presence of steroids.

Lieberman Burchardt test

About 5.0 mg of the rhizome extract of *Kaempferia* was mixed with 3.0 ml of chloroform in a test tube. Then five drops of acetic anhydride and 1.0 ml of concentrated sulphuric acid were added to it through the sides of the test tube. Development of a reddish ring at the junction of two layers indicated the presence of steroids.

Tests for Detection of alkaloids

About 0.5 g of the rhizome extract of *Kaempferia* was mixed with 5.0 ml of ammonia and then extracted with equal volume of chloroform. To this extract, 5.0 ml dilute hydrochloric acid was added. The acid layer obtained was used for the following chemical tests for alkaloids.

Mayer's test

To 1.0 ml of acid layer, a few drops of Mayer's reagent (1.358 g of mercuric chloride dissolved in 60 ml of water and poured into a solution of five g of potassium iodide in 10 ml of water and then make up the volume to 100 ml with distilled water) were added. Development of a creamy white precipitate indicated the presence of alkaloids.

Wagner's test

A few drops of Wagner's reagent (2.0 g of iodine and 6.0 g of potassium iodide dissolved in 100 ml of water) were added to 1.0 ml of the acid extract. Development of reddish brown precipitate indicated the presence of alkaloids.

Hager's test

To 1.0 ml of the acid extract, a few drops of Hager's reagent (one gram of picric acid dissolved in 100 ml of water) were mixed. Development of yellow precipitate indicated the presence of alkaloids.

Dragendroff's Test

A few drops of Dragendroff's reagent (Stock solution (1) 0.6 g of bismuth sub nitrate was dissolved in two ml of concentrated hydrochloric acid and 10 ml of water was added. Stock solution (2) 6.0 g gram of potassium iodide was dissolved in 10 ml of water. Then both the stock solutions (1) and (2) were mixed together and then it was mixed with seven ml of concentrated hydrochloric acid and 15 ml of water. Sufficient amount of distilled water was added to the mixture to make up the volume to 400 ml) were mixed with 1.0 ml of acid extract. Development of a reddish brown precipitate indicated the presence of alkaloids.

Test for detection of phenolic compounds

Five milligram of the rhizome extract of *Kaempferia* was dissolved in 1.0 ml of water and 5.0 drops of 10 per cent ferric chloride was added to it. Development of dark blue colour indicated the presence of phenolic compounds.

Test for detection of tannins

Ferric chloride test

Two milligram of the rhizome extract of *Kaempferia* was mixed with three ml of 1.0 per cent ferric chloride solution. Development of a blue, green or brown colour indicated the presence of tannins.

Gelatin test

About 0.5 g of the rhizome extract of *Kaempferia* was mixed with a few drops of 1.0 per cent solution of gelatin containing 10 per cent sodium chloride. Development of a white precipitate indicated the presence of tannins.

Tests for detection of flavonoids

Ferric chloride test

To 2.0 ml of alcoholic solution of the rhizome extract of *Kaempferia* (0.5g extract in 10 ml methanol), a few drops of neutral ferric chloride solution was mixed. Development of green colour indicated the presence of flavonoids.

Lead acetate test

To 2.0 ml of alcoholic solution of the rhizome extract of *Kaempferia* (0.5 g extract in 10 ml methanol), a few drops of neutral ten per cent lead acetate was mixed. Development of yellow precipitate indicated the presence of flavonoids.

Tests for detection of glycosides

Sodium hydroxide test

A small amount of the rhizome extract of *Kaempferia* (about 5.0 mg) was mixed with 1.0 ml water and added five to six drops of sodium hydroxide solution (1 %). Development of yellow colour indicated the presence of glycosides.

Benedict's test

The rhizome extract of *Kaempferia* (0.5 g) was mixed with 1.0 ml of water and 5.0 ml of Benedict's reagent was added. The mixture was boiled for two minutes and cooled. Development of brown or red colour indicated the presence of glycosides.

Tests for detection of diterpenes

About 5.0 mg of the rhizome extract of *Kaempferia* was mixed with 3.0 ml of copper acetate solution (5 %). Development of green colour indicated the presence of diterpenes.

Tests for detection of triterpenes

Salkowski test

About 3.0 mg of the rhizome extract of *Kaempferia* was mixed with 3.0 ml of chloroform and then it was shaken with 3.0 ml concentrated sulphuric acid. Development of yellow colour in lower layer on standing indicated the presence of triterpenes.

Lieberman Burchardt test

About 3.0 mg of the rhizome extract of *Kaempferia* was mixed with 3.0 ml of chloroform in a test tube. Five drops of acetic acid and 1.0 ml of concentrated sulphuric acid were added to it through the sides of the test tube. Development of a deep red ring at the junction of two layers indicated the presence of triterpenes.

Tests for detection of saponins

Foam test

A small amount of the rhizome extract of *Kaempferia* (about five mg) was shaken with 5.0 ml of water. Development of the foam that persisted for ten minutes indicates the presence of saponins.

3.8 ANATOMY

3.8.1 Leaf epidermis

Epidermal peels of both adaxial (upper) and abaxial (lower) surfaces were made by placing the leaf on a clean glass slab with the surfaces to be studied facing downward. The specimens were sprinkled with water by holding it downwards from one end. The epidermis above the desired surfaces was scrapped off carefully with a sharp razor blade and loose cells were washed away from the epidermal

peel with the aid of soft camel hairbrush and water until the desired epidermis below was obtained. The epidermal peels were mounted in glass slide stained with aqueous solution of safranin for 4-8 minutes, then rinsed carefully in water to remove excess stain. Then it was examined using light power microscope at different magnification.

3.8.2 Leaf lamina, Petiole

Thin transverse sections of fresh leaf lamina and petiole was taken by hand section. The section was stained using safranin and it was examined using light power microscope.

3.8.3 Rhizome

Rhizomes were taken for transverse sections. Free hand sections were made in the apical and median regions. The sections were stained with Safranin. Then it was examined under microscope for vascular bundles, presence of fixed oil and starch.

To examine starch content, the section was stained with iodine. Starch grains turned blue on staining.

To examine fixed oil content, the sample was stained with sudan red. Presence of fixed oil was indicated by orange pink colour droplets.

3.8.4 Root tubers

Root tubers were also taken for transverse sections by free hand section method. Normal observation was made by staining with safranin and starch content was analysed by staining with sudan red.

3.9 PHARMACOLOGY

3.9.1 Plant Material

The rhizomes of *Kaempferia rotunda* and *Kaempferia parviflora* were collected from the field experiment. Two herbarium were prepared and voucher specimen with accession no. HERB/VPT/CVASMTY/3/2019 and HERB/VPT/CVASMTY/4/2019 were deposited at Department of Veterinary Pharmacology & Toxicology, College of Veterinary & Animal Sciences, Mannuthy.

3.9.2 Preparation of extracts

The rhizomes were shade dried followed by course pulverization. Plant extract was obtained using ethanol by soxhlet extraction. The extract was then dried using rotary evaporator.

3.9.3 *In vivo* studies

3.9.3.1 Acute toxicity study

Mice were administered with ethanolic extract of *K. rotunda* and *K. parviflora* in two groups of 5 animal each. They were monitored during the entire study period for the signs and symptoms of toxicity and/or mortality, behavioral alterations, food and water intake and changes in body weight. Relative change in body weight as observed on 7^{th} and 14^{th} day of the experiment. On day 14, the mice in each group were sacrificed and observed for lesions in all the internal organs.

3.9.3.2 Immunomodulatory studies

3.9.3.2.1 Experimental design

Experiment was conducted at Department of Veterinary Pharmacology & Toxicology, College of Veterinary & Animal Sciences, Mannuthy. The experiment protocol was approved by Institutional Animal Ethics Committee of College of Veterinary and Animal Sciences, Mannuthy (Order no. IAEC/CVASMTY 4/17-18). Swiss albino mice, procured from Small Animal Breeding Station, Mannuthy

were randomly divided in two sets of 72 each (Plate 2). First set of animals were used for delayed hypersensitivity with six groups of 12 animals each. Second set of animals were used for bone marrow cellularity with four group of 12 animals each. Sheep Red Blood Corpuscle (SRBC) antigen (1×10^8 cell /ml/100g BW) was injected i.p. to mice of all the groups on fifth day except A_I and B_I. The experiment protocol is illustrated below:

Table 2. Experiment protocol for *in vivo* immunomodulatory study

Group	**Treatments**
A_I & B_I	Control group receiving vehicle for 19 days
A_{II} & B_{II}	Mice administered with cyclophosphamide @ 30 mg/kg BW on 9^{th} , 10^{th} , 11^{th}, 16^{th}, 17^{th} and 18^{th} day
A_{III} & B_{III}	Mice administered with ethanolic extract of rhizome of *K. rotunda* @ 200mg/kg orally for 19 days
A_{IV} & B_{IV}	Mice administered with ethanolic extract of rhizome of *K. rotunda* @ 200mg/kg orally for 19 days and cyclophosphamide @ 30 mg/kg BW on 9^{th} , 10^{th} , 11^{th}, 16^{th}, 17^{th} and 18^{th} day
A_V & B_V	Mice administered with ethanolic extract of rhizome of *K. parviflora* @ 200mg/kg orally for 19 days
A_{VI} & B_{VI}	Mice administered with ethanolic extract of rhizome of *K. parviflora* @ 200mg/kg orally for 19 days and cyclophosphamide @ 30 mg/kg BW on 9^{th} , 10^{th} , 11^{th}, 16^{th}, 17^{th} and 18^{th} day

The first six animals in each group were sacrificed on 12^{th} day and the remaining six were sacrificed on 19^{th} day.

Plate 2: Swiss albino mice in laboratory condition

3.9.3.2.2 Immunization

Blood was collected from the sheep maintained at the University Sheep and Goat farm, Mannuthy, in equal volume of Alsever's solution following sterile procedure. This was used for antigen preparation and stored at 4°C until used.

3.9.3.2.3 Measurement of physiological parameters

The weight of individual mouse was recorded before, during (on 12th day) and at the end (19th day) of the experiment. The weight of the organs like spleen and liver were conjointly recorded at the time of sacrifice.

3.9.3.2.4 Measurement of haematological parameters

Blood samples were collected from submaxillary vein of all the mice from group A_I to A_{IV} on zero, 12th and 19th day of the experiment. Total and differential leucocyte count was taken as described by Schalm (1975).

3.9.3.2.5 Measurement of biochemical parameters

Blood collected from submaxillary vein of mice from group A_I to A_{IV} was taken test tube without anticoagulant, centrifuged at 2500 rpm for 10 minutes. Serum was separated for the estimation of protein, albumin and haemagglutination test (Plate. 3).

3.9.3.2.6 Measurement of immunological parameters

3.9.3.2.7 Haemagglutination test

Haemagglutination test was performed for the evaluation of cell mediated immune response. The blood was collected from mice groups A_I to A_{IV} on zero, 12th and 19th day of the experiment. Two fold dilutions of sera were prepared in 0.15 M PBS (PH 7.2) and 50 µl of each dilution were transferred into 96 well micro titre plates. 25 µl quantity of fresh 1per cent Sheep SRBC suspension in PBS was added into each well and mixed thoroughly. Thereafter they were incubated at 37°C for 1h. The reciprocal of highest dilution of the test serum giving 50 per cent agglutination had been expressed as HA titre (Ray *et al*., 1991).

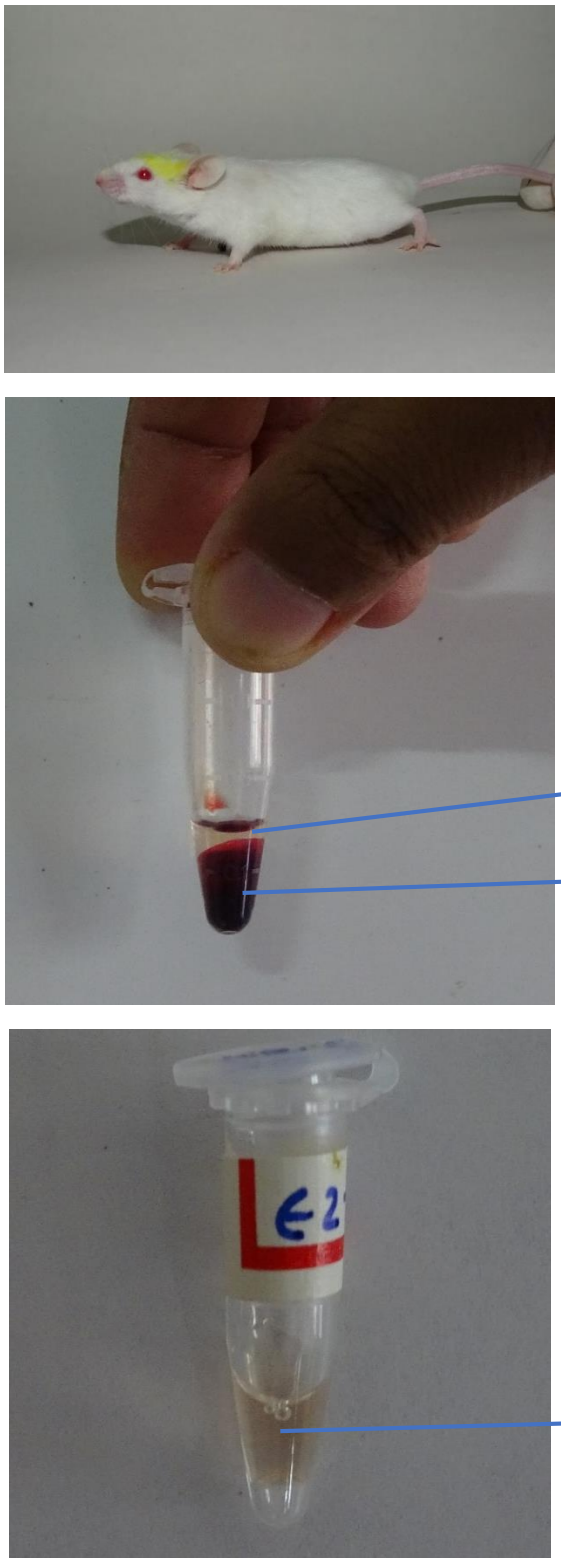


Plate 3: Steps for separation of serum from blood of Swiss albino mice

3.9.3.2.8 Bone marrow cellularity

Bone marrow cellularity was done for the evaluation of humoral immune response. Femurs of both the hind legs of the mice were dissected and the condyles of the femurs were removed using sharp scissors (Mehra and Vaidya, 1985). Then the bone marrow was flushed with five mL of 10 per cent foetal bovine serum. The number of cells was counted haemocytometrically on 12^{th} day and 19^{th} day after sacrificing the animals.

3.9.3.2.9 Histopathology of Spleen

On 12^{th} and 19^{th} days, the spleen of the animal was removed for histopathology. The tissue samples were fixed at 10 per cent formalin and embedded in paraffin and the sections were stained using haematoxylin and eosin (Bancroft and Stevens, 1990). It was observed for gross and histopathological lesions.

3.9.3.2.10 Delayed type Hypersensitivity (DTH)

Six mice from each groups of B_I, B_{II}, B_{III} and B_{IV} were primed with SRBC antigen i. p. on day 5 was then challenged on day 12 with SRBC antigen s. c. on right hind foot pad. The left hind foot pad received 0.025 mL of saline alone. The footpad swelling was measured at three different dimensions using vernier callipers after 24 h of challenge. The difference in footpad thickness was taken as a measure of DTH. The test was repeated on 19^{th} day in the remaining six mice of each group (Saraf *et al*., 1989).

3.10 *IN VITRO* STUDIES

3.10.1 Antioxidant activity

1, 1-Diphenyl-2- picryl- hydrazyl (DPPH) radical scavenging activity

Four millilitre of the reaction mixture containing 2.4 ml of DPPH (100µM in methanol) and1.6 ml of test solution at, 62.5, 125, 250 and 500 µg/ml concentrations of *K. rotunda* and *K. parviflora* were incubated at 37° C for 30 min (Cotelle *et al*., 1996). The absorbance of the resulting solution was measured at 517

nm using UV/VIS/NIR Spectrophotometer (Lambda 750, Perkin Elmer, Singapore). The reference standard used was ascorbic acid. The per cent inhibition of DPPH radical was calculated by the following equation:

Per cent inhibition = (1- AT/AC) x 100

AC= Absorbance of control, AT= Absorbance of extracts/ standard

3.10.2 *In vitro* anticancer studies

Cytotoxic evaluation of *K. rotunda* and *K. parviflora* were assessed using 3-(4,5- dimethyl thazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay in MCF-7 and MDA-MB-231 human breast carcinoma cell lines as per the method of Naidu *et al*. (2013). A pilot study was conducted to select the concentration at which cytotoxicity occurred. The concentrations used for pilot study were 2.5, 5, 10, 20, 40, 80, 160, 320 and 500 µg/ml. Based on the results of pilot study the main study was performed. The MCF-7 and MDA-MB- 231 breast cancer cell lines were seeded at a density of 5 x 10^3 cells per well, in 200 µl medium and were allowed to attach for overnight in a CO_2 incubator. Cells were treated with *K. rotunda* and *K. parviflora* extracts separately at concentrations of 10, 20, 40, 80, 160, 320 and 500 µg/ml for a period of 48 h. After the treatment, 20 µl of MTT (5 mg/ml) in 150 µL medium was added and incubated at 37°C for 4h after removing the medium with extracts of *K. rotunda* and *K. parviflora*. Then the media with MTT was removed and the formed purple formazan crystals were dissolved in 200 µl of DMSO and read at 570 nm in a ELISA plate reader (Varioskan flash, Thermo Fischer Scientific, Finland).

The IC_{50} values of extracts were calculated by plotting the concentration against per cent cell inhibition using the online software "GraphPad Prism version 8.0.0 for Windows."

3.10.3 Antimicrobial studies

Agar well diffusion method (Shah *et al*., 2011) was employed for the study. Solidified sterile Mueller Hinton agar petri dishes were incubated at 37°C for 24 h

for checking sterility. The broth suspension of the inoculum was taken using sterile cotton swab. Then it was streaked evenly on the agar surface repeated three times by turning the plate at 60^{o} angle. It was left for five minutes to dry. Equidistant wells of 8 mm were made on the medium using sterile borer and ethanolic extract of *K. rotunda* and *K. parviflora* at different concentrations (150, 200 and 250 mg/ml) were added to respective wells. The plates were incubated at 37^{o}C for 24 h. At the end of incubation, inhibition zones formed around the well were measured in millimetres. These procedures were performed in triplicates. The standard drug used for *Pseudomonas aeruginosa* and *Escheria coli* was Penicillin and for *Salmonella enterica* and *Staphylococcus aureus* was Azithromycin.

3.11 STATISTICAL ANALYSIS

Data of the field experiment was subjected to two factor analysis for *Kaempferia rotunda* and *Kaempferia parviflora* while T test was used for *Kaempferia galanga*. Analysis of Covariance (ANCOVA) was performed in Pharmacological studies. One way ANOVA was used for biochemical analysis and T-test was used for comparing vivipary and normal plants.

Results

4. RESULTS

The results of the study entitled "Performance analysis of medicinal *Kaempferia* species" carried out at the Department of Plantation crops and Spices, College of Horticulture, Kerala Agricultural University and the Department of Pharmacology and Toxicology, College of Veterinary and Animal Science, Kerala Veterinary and Animal Science University, Mannuthy are presented in this chapter.

4.1 MORPHOLOGICAL EVALUATION

A total of 18 genotypes belonging to three different species of *Kaempferia* as given below were evaluated in the study

1. Thirteen collections of *Kaempferia rotunda i.e*. seven from Manipur and six from Kerala
2. Three collections of *Kaempferia parviflora*, one each from Botanical Survey of India, Shillong, IISR, Kozhikode and Thailand.
3. Two collections of *Kaempferia galanga*, one each from Arunachal Pradesh and Kerala

Data on morphological parameters is presented species wise.

4.1.1 Morphological evaluation of *K. rotunda*

4.1.1.1 Qualitative parameters of K. rotunda

Qualitative parameters of *K. rotunda* are presented in table 3.

Table 3: Qualitative parameters of *K. rotunda*

Parameter	Observation
Rhizome colour: Scale Inner core	Brown (RHS 161B) Off-white (RHS NN155D)
Rhizome shape	Globose
Presence of root tubers	Present
Colour of root tubers	Creamy white (RHS NN155A)
Mature leaf shape	Erect/ lanceolate

Table 3 contd.

Parameter	Observation
Mature leaf colour	Green
Leaf tip shape	Cuspidate
Leaf margin	Entire
Growth habit	Sprawling

There was no variation in the qualitative parameters among the genotypes. The rhizome was globose shaped bearing numerous club shaped root tubers (Plate 11). The outer scale of the rhizome of *K. rotunda* was brown and the inner core was off white in colour. Leaves were erect and lanceolate in shape with cuspidate leaf tip and entire margin (Plate 9).

4.1.1.2 Number of days for sprouting in K. rotunda genotypes

The data on number of days required for sprouting in *K. rotunda* during the two years is presented in table 4.

Table 4: Number of days for sprouting in *K. rotunda* genotypes

Genotype	Number of days for sprouting		
	2017-18	2018-19	Pooled Mean
MCR-1	18.83	17.00	17.92
MCR-2	7.00	6.67	6.83
MCR-3	7.00	6.83	6.92
MCR-4	6.83	7.00	6.92
MCR-5	12.33	12.83	12.58
MCR-6	11.33	11.33	11.33
MCR-7	12.67	13.50	13.08
KCR-1	6.17	5.83	6.00
KCR-2	6.00	5.33	5.67
KCR-3	7.00	7.33	7.17
KCR-4	7.83	8.17	8.00
KCR-5	7.17	7.50	7.33
KCR-6	7.17	7.67	7.42
C.D (0.05)	0.71	1.10	1.31

Data given in table 4 showed that the rhizomes took 5.67 to 17.92 days for sprouting. There was significant difference in the number of days required for sprouting during the two years of observation. Lowest days for sprouting was recorded in KCR-2 (6.00) and highest days to sprout was recorded in the genotype MCR-1 (18.83) during 2017-18. During 2018-19, lowest days to sprout (5.33) was observed in KCR-2 and highest days to spout (17.00) in MCR-1. In the pooled mean of two years, the significantly lowest number of days (5.67) for sprouting was recorded by KCR-2 which was on par with KCR-1 and the highest days (17.92) was recorded by MCR-1.

4.1.1.3 Number of tillers per plant at 90 DAP in K. rotunda genotypes

The data on tillers produced per plant was recorded at monthly intervals and data recorded at 90 DAP are furnished in table 5.

Table 5: Number of tillers per plant of *K. rotunda* genotypes at 90 DAP

Genotype	Number of tillers/plant		
	2017-18	2018-19	Pooled Mean
MCR-1	0.33	0.17	0.25
MCR-2	0.00	1.67	0.83
MCR-3	0.00	0.00	0.00
MCR-4	0.33	1.17	0.75
MCR-5	0.33	0.17	0.25
MCR-6	0.00	1.33	0.67
MCR-7	0.67	3.00	1.83
KCR-1	1.83	2.17	2.00
KCR-2	1.17	1.83	1.50
KCR-3	1.00	0.33	0.67
KCR-4	0.50	1.00	0.75
KCR-5	0.67	1.33	1.00
KCR-6	0.00	1.33	0.67
C.D (0.05)	0.64	0.68	0.62

The tiller production was noticed only in nine genotypes at 90 DAP during 2017-18, whereas it was noticed at 90 DAP in 12 genotypes during 2018-19. There was no tiller production at all in genotype MCR-3 upto 90 DAP. There was significant difference in tiller production among the genotypes during the two years.

During 2017-18, highest number of tillers was produced by KCR-1and during 2018-19, MCR-7 recorded highest tiller production. In the pooled data, highest number of tillers (2.00) was recorded by genotype KCR-1.

4.1.1.4 Number of tillers per plant at 120 DAP in K. rotunda genotypes

The data on tillers produced per plant recorded at 120 DAP are presented in table 6.

Table 6: Number of tillers per plant in *K. rotunda* at 120 DAP

Genotype	Number of tillers per plant		
	2017-18	**2018-19**	**Pooled Mean**
MCR-1	1.17	1.17	1.17
MCR-2	1.17	1.83	1.50
MCR-3	0.67	1.50	1.08
MCR-4	0.50	1.17	0.83
MCR-5	0.50	1.67	1.08
MCR-6	0.33	2.33	1.33
MCR-7	0.83	2.33	1.58
KCR-1	1.67	2.00	1.83
KCR-2	1.00	1.83	1.42
KCR-3	1.33	1.33	1.33
KCR-4	0.50	2.00	1.25
KCR-5	1.00	2.33	1.67
KCR-6	1.33	1.50	1.42
C.D (0.05)	0.63	0.72	NS

All the genotypes produced tillers by 120 DAP. There was significant difference in tiller production among the genotypes during 2017-18 as well as 2018-19. The highest number of tillers (1.67) was recorded in KCR-1 and it was on par with KCR-3 and KCR-6 during 2017-18. The highest number of tillers during 2018-19 was recorded in the genotypes MCR-6, MCR-7 and KCR-5 (2.33). No significant difference was observed between the genotypes in the pooled mean.

4.1.1.5 Number of tillers per plant at 150 DAP in K. rotunda genotypes

The data on tiller production by *K. rotunda* genotypes at 150 DAP are given in table 7.

Table 7: Number of tillers per plant in *K. rotunda* at 150 DAP

Genotype	Number of tillers/plant		
	2017-18	2018-19	Pooled Mean
MCR-1	2.33	1.83	2.08
MCR-2	1.50	1.83	1.67
MCR-3	1.83	1.50	1.67
MCR-4	1.50	1.67	1.58
MCR-5	1.67	2.00	1.83
MCR-6	1.83	2.50	2.17
MCR-7	2.50	2.67	2.58
KCR-1	2.83	3.50	3.17
KCR-2	3.50	3.50	3.50
KCR-3	3.83	3.17	3.50
KCR-4	1.50	2.67	2.08
KCR-5	3.67	4.17	3.92
KCR-6	2.50	3.17	2.83
C.D (0.05)	0.71	0.91	0.59

The tiller production per plant ranged from 1.50 to 3.83 at 150 DAP. Genotype KCR-3 produced the highest number of tillers (3.83) during 2017-18, while during 2018-19, highest number of tillers was produced by the genotype KCR-5 (4.17). In the pooled mean data, significantly highest number of tillers was recorded in KCR-5 (3.92) and the lowest was recorded in MCR-4 (1.58).

4.1.1.6 Number of tillers per plant in K. rotunda genotypes at 180 DAP

The data on tiller production by *K. rotunda* genotypes at 180 DAP are given in table 8.

Table 8: Number of tillers per plant in *K. rotunda* at 180 DAP

Genotype	Number of tillers/plant		
	2017-18	2018-19	Pooled Mean
MCR-1	2.50	2.50	2.50
MCR-2	1.33	2.00	1.67
MCR-3	1.33	2.17	1.75
MCR-4	1.33	1.50	1.42
MCR-5	1.83	2.17	2.00

Table 8 contd.

Genotype	2017-18	2018-19	Pooled Mean
MCR-6	1.67	2.00	1.83
MCR-7	1.83	2.50	2.17
KCR-1	2.67	3.00	2.83
KCR-2	3.33	3.83	3.58
KCR-3	3.50	4.17	3.83
KCR-4	1.33	1.67	1.50
KCR-5	2.00	2.67	2.33
KCR-6	1.00	1.50	1.25
C.D (0.05)	0.70	1.05	1.33

The data revealed that the tiller production was highest at 180 DAP. During both the years significantly highest tiller production was recorded by KCR-3 (3.50 and 4.17). In the pooled mean of two years also KCR-3 produced significantly highest number of tillers (3.83) which was on par with KCR-2.

4.1.1.7 Number of tillers per plant in K. rotunda genotypes at 210 DAP

The data on tiller production by *K. rotunda* genotypes at 210 DAP are given in Table 9.

Table 9: Number of tillers per plant in *K. rotunda* at 210 DAP

Genotype	Number of tillers/plant		
	2017-18	2018-19	Pooled Mean
MCR-1	1.83	1.83	1.83
MCR-2	2.00	2.17	2.08
MCR-3	1.67	1.50	1.58
MCR-4	1.33	1.33	1.33
MCR-5	0.83	1.17	1.00
MCR-6	1.67	1.50	1.58
MCR-7	1.67	2.00	1.83
KCR-1	0.67	1.33	1.00
KCR-2	2.17	2.33	2.25
KCR-3	1.67	1.67	1.67
KCR-4	1.00	1.00	1.00
KCR-5	0.83	1.17	1.00
KCR-6	1.83	1.67	1.75
C.D (0.05)	0.76	0.80	0.53

During both the years, significantly highest number of tillers was recorded in the genotype KCR-2 (2.17 and 2.33, respectively). In the pooled mean data also KCR-2 recorded significantly highest number of tillers, it was on par with MCR-2.

4.1.1.8 Number of leaves per plant in K. rotunda genotypes at 30 DAP

The data on number of leaves per plant at 30 DAP during two years are presented in table 10.

The pooled data given in the table revealed that number of leaves per plant at 30 DAP ranged from 1.33 to 3.42. The leaf production was significantly different among the genotypes in both the years. The highest number of leaves was seen on the genotype KCR-1 (3.83) during 2017-18. In 2018-19, leaf production was significantly high in KCR-3 and KCR-4 (3.33). In the pooled mean of two years, significantly highest number of leaves was seen in KCR-3 and KCR-4 (3.42) closely followed by KCR-1 (3.08).

Table 10: Number of leaves per plant in *K. rotunda* genotypes at 30 DAP

Genotype	Number of leaves/plant		
	2017-18	2018-19	Pooled Mean
MCR-1	1.17	1.50	1.33
MCR-2	3.00	2.83	2.92
MCR-3	2.67	2.50	2.58
MCR-4	2.67	2.50	2.58
MCR-5	1.67	2.00	1.83
MCR-6	2.00	1.83	1.92
MCR-7	1.83	1.67	1.75
KCR-1	3.83	2.33	3.08
KCR-2	2.33	2.33	2.33
KCR-3	3.50	3.33	3.42
KCR-4	3.50	3.33	3.42
KCR-5	1.67	2.17	1.92
KCR-6	1.67	1.50	1.58
C.D (0.05)	0.60	0.77	0.50

4.1.1.9 Number of leaves per plant at 60 DAP

The data on number of leaves per plant at 60 DAP during both the years are depicted in table 11.

Table 11: Number of leaves per plant in *K. rotunda* genotypes at 60 DAP

Genotype	Number of leaves/plant		
	2017-18	2018-19	Pooled Mean
MCR-1	3.83	3.50	3.67
MCR-2	4.50	4.83	4.67
MCR-3	4.83	4.67	4.75
MCR-4	4.83	4.67	4.75
MCR-5	3.67	3.50	3.58
MCR-6	3.50	4.00	3.75
MCR-7	4.00	3.67	3.83
KCR-1	5.50	5.50	5.50
KCR-2	4.83	4.83	4.83
KCR-3	5.67	5.67	5.67
KCR-4	4.33	4.50	4.42
KCR-5	3.50	3.33	3.42
KCR-6	2.50	2.33	2.42
C.D (0.05)	0.91	0.90	0.61

The leaf production increased in all the genotypes and at 60 DAP the leaf production showed significant variation during both the years as well as in the pooled mean. During 2017-18 and in the pooled mean, highest (5.67) leaf production was observed in KCR-3 and it was on par with KCR-1. In 2018-19 also highest leaf production was noticed in genotype KCR-3 (5.67).

4.1.1.10 Number of leaves per plant in K. rotunda genotypes at 90 DAP

The data on number of leaves per plant at 90 DAP during both the years are depicted in table 12.

Table 12: Number of leaves per plant in *K. rotunda* genotypes at 90 DAP

Genotype	Number of leaves/plant		
	2017-18	2018-19	Pooled Mean
MCR-1	7.00	7.67	7.33
MCR-2	6.83	9.50	8.17
MCR-3	6.17	8.17	7.17
MCR-4	7.83	14.33	11.08
MCR-5	6.00	7.33	6.67
MCR-6	6.33	11.67	9.00
MCR-7	6.67	14.00	10.33
KCR-1	9.17	12.83	11.00
KCR-2	8.50	10.33	9.42
KCR-3	11.17	6.33	8.75
KCR-4	7.83	8.67	8.25
KCR-5	9.83	11.17	10.50
KCR-6	4.67	7.00	5.83
C.D (0.05)	1.12	1.55	1.82

The data reveal that the number of leaves at 90 DAP ranged from 5.83 to 11.08. During 2017-18, significantly highest number of leaves (11.17) was produced by the genotype KCR-3 and lowest (4.67) by the genotype KCR-6 while during 2018-19, the highest (14.33) leaves was produced by genotype MCR-4 which was on par with MCR-7 (14.00) and least (6.33) was observed in genotype KCR-3. In the pooled mean, MCR-4 (11.08) recorded the significantly highest leaf production and it was on par with MCR-7, KCR-1 and KCR-5.

4.1.1.11 Number of leaves per plant in K. rotunda genotypes at 120 DAP

The data on number of leaves per plant of different genotypes recorded at 120 DAP during both the years are depicted in table 13.

Table 13: Number of leaves per plant in *K. rotunda* genotypes at 120 DAP

Genotype	2017-18	2018-19	Pooled Mean
MCR-1	8.00	7.00	7.50
MCR-2	9.17	10.83	10.00
MCR-3	9.00	10.00	9.50
MCR-4	9.17	9.33	9.25
MCR-5	8.67	10.17	9.42
MCR-6	8.50	12.00	10.25
MCR-7	8.67	10.83	9.75
KCR-1	9.67	11.50	10.58
KCR-2	11.00	12.83	11.92
KCR-3	10.00	9.17	9.58
KCR-4	10.17	12.50	11.33
KCR-5	11.50	15.33	13.42
KCR-6	5.17	9.67	7.42
C.D (0.05)	1.67	2.35	1.67

The number of leaves produced increased at 120 DAP, during 2017-18 it was significantly highest in KCR-5 (11.50) which was on par with KCR-2, KCR-3, KCR-4 and lowest in genotype KCR-6 (5.17). During 2018-19, highest (15.33) number of leaves was produced by the genotype KCR-5 and lowest (7.00) was recorded by the genotype MCR-1. Pooled mean data also showed significant difference in leaf production which ranged from 7.42 to 13.42.

4.1.1.12 Number of leaves per plant in K. rotunda genotypes at 150 DAP

The data on the number of leaves per plant at 150 DAP during both the years are furnished in table 14.

Table 14: Number of leaves per plant in *K. rotunda* genotypes at 150 DAP

Genotype	Number of leaves/plant		
	2017-18	2018-19	Pooled Mean
MCR-1	13.17	13.17	13.17
MCR-2	10.33	12.00	11.17
MCR-3	10.00	11.50	10.75
MCR-4	9.67	10.17	9.92
MCR-5	9.67	11.83	10.75
MCR-6	9.50	11.17	10.33
MCR-7	11.00	11.50	11.25
KCR-1	12.67	12.67	12.67
KCR-2	13.83	13.17	13.50
KCR-3	13.00	13.17	13.08
KCR-4	12.17	13.17	12.67
KCR-5	13.67	12.33	13.00
KCR-6	6.50	8.83	7.67
C.D (0.05)	2.00	2.19	1.49

The number of leaves per plant ranged from 7.67 to 13.50 at 150 DAP as shown by the pooled mean. The leaf production was significantly different among the genotypes in both the years and in the pooled mean. During 2017-18, highest leaf production at 150 DAP was recorded in the genotype KCR-2 (13.83) which was on par with MCR-1 and KCR-5 while during 2018-19 highest leaf production was recorded in the genotypes MCR-1, KCR-2, KCR-3 and KCR-4 (13.17). The pooled mean of genotypes revealed that the leaf production was significantly highest (13.50) in the genotype KCR-2 and it was on par with MCR-1, KCR-3 and KCR-5.

4.1.1.13 Number of leaves per plant in K. rotunda genotypes at 180 DAP

The data on the number of leaves per plant at 180 DAP during both the years are given in table 15.

Table 15: Number of leaves per plant in *K. rotunda* genotypes at 180 DAP

Genotype	Number of leaves per plant		
	2017-18	2018-19	Pooled Mean
MCR-1	16.83	17.33	17.08
MCR-2	13.17	13.50	13.33
MCR-3	13.33	13.50	13.42
MCR-4	12.00	12.50	12.25
MCR-5	13.50	13.33	13.42
MCR-6	14.83	16.50	15.67
MCR-7	14.83	15.50	15.17
KCR-1	16.83	17.50	17.17
KCR-2	17.67	16.67	17.17
KCR-3	16.67	11.00	13.83
KCR-4	15.00	13.33	14.17
KCR-5	15.50	15.67	15.58
KCR-6	10.00	10.33	10.17
C.D (0.05)	3.47	2.89	2.24

The leaf production increased and reached maximum at 180 DAP. There was significant variation in leaf production among the genotypes during both the years. In 2017-18, highest leaf production was observed in genotype KCR-2 (17.67) which was on par with MCR-1, MCR-6, MCR-7, KCR-1, KCR-3, KCR-4 and KCR-5 while during 2018-19, highest number of leaves was recorded in genotype KCR-1 and it was on par with MCR-1, MCR-6 and KCR-2. The significantly highest value (17.17) was recorded by the genotypes KCR-1 and KCR-2 and it was on par with MCR-1, KCR-5, MCR-6 and MCR-7 in the pooled mean.

4.1.1.14 Number of leaves per plant in K. rotunda genotypes at 210 DAP

The data on the number of leaves per plant at 120 DAP during both the years are given in table 16.

Table 16: Number of leaves per plant in *K. rotunda* genotypes at 210 DAP

Genotype	Number of leaves per plant		
	2017-18	2018-19	Pooled Mean
MCR-1	7.83	7.67	7.75
MCR-2	9.67	9.83	9.75
MCR-3	13.00	12.33	12.67
MCR-4	9.83	10.67	10.25
MCR-5	11.00	10.67	10.83
MCR-6	9.67	11.17	10.42
MCR-7	10.83	11.17	11.00
KCR-1	8.83	10.50	9.67
KCR-2	8.67	8.67	8.67
KCR-3	9.00	9.33	9.17
KCR-4	7.00	6.33	6.67
KCR-5	6.50	6.83	6.67
KCR-6	12.67	12.67	12.67
C.D (0.05)	1.75	1.67	1.17

The number of leaves per plant declined after 180 DAP in all the genotypes. Significant difference was observed among the genotypes during both the years as well as in the pooled mean. During 2017-18 and in pooled mean value, significantly highest number of leaves was recorded in the genotype MCR-3 (13.00 and 12.67, respectively) which was on par with KCR-6 whereas during 2018-19, highest number of leaves was observed in genotype KCR-6 which was on par with MCR-3. The data on pooled mean of genotypes showed that number of leaves ranged from 6.67 to 12.67.

4.1.1.15 Leaf length and breadth in K. rotunda genotypes at 60 DAP

The data on leaf length and breadth recorded at 60 DAP are furnished in table 17.

Table 17: Leaf length and breadth in *K. rotunda* genotypes at 60 DAP

Genotype	Leaf length (cm)			Leaf breadth (cm)		
	2017-18	2018-19	Pooled Mean	2017-18	2018-19	Pooled Mean
MCR-1	16.93	17.42	17.18	4.38	4.28	4.33
MCR-2	27.68	25.72	26.70	5.97	5.73	5.85
MCR-3	31.85	29.58	30.72	6.13	6.45	6.29
MCR-4	25.22	24.18	24.70	5.20	5.18	5.19
MCR-5	35.32	33.52	34.42	6.53	6.38	6.46
MCR-6	32.53	34.17	33.35	6.98	6.45	6.72
MCR-7	26.77	24.20	25.48	4.13	4.37	4.25
KCR-1	22.27	22.53	22.40	8.43	5.85	7.14
KCR-2	29.33	27.33	28.33	3.45	3.58	3.52
KCR-3	21.03	22.82	21.93	2.70	2.85	2.78
KCR-4	29.52	28.82	29.17	5.12	4.70	4.91
KCR-5	21.83	22.97	22.40	3.88	3.67	3.78
KCR-6	17.70	19.38	18.54	3.72	3.47	3.59
C.D (0.05)	2.41	3.15	1.98	0.72	0.62	0.55

At 60 DAP, leaf length increased steadily and significant difference was observed among the genotypes during both the years and the pooled mean. During 2017-18, significantly highest leaf length was noticed in genotype MCR-5. In 2018-19 significantly highest leaf length was observed in genotype MCR-6 (34.17) and it was on par with MCR-5. In the pooled mean significantly highest value was recorded in genotype MCR-5 (34.42) and it was on par with MCR-6.

The observation on leaf breadth at 60 DAP ranged from 2.78 to 7.14. During 2017-18, significantly highest (8.43 cm) leaf breadth was observed in the genotype KCR-1, whereas during 2018-19 significantly highest (6.45 cm) leaf breadth was noticed in MCR-3, MCR-6 and it was on par with MCR-5. In the pooled mean, significantly highest value (7.14 cm) was observed KCR-1 and it was on par with MCR-6.

4.1.1.16 Leaf length and breadth in K. rotunda genotypes at 90 DAP

The data on leaf length and breadth recorded at 90 DAP are presented in table 18.

Table 18: Leaf length and breadth in *K. rotunda* genotypes at 90 DAP

Genotype	Leaf length (cm)			Leaf breadth (cm)		
	2017-18	2018-19	Pooled Mean	2017-18	2018-19	Pooled Mean
MCR-1	27.47	27.55	27.51	5.70	5.48	5.59
MCR-2	41.23	40.78	41.01	7.25	7.20	7.23
MCR-3	37.87	40.00	38.93	7.32	8.22	7.77
MCR-4	34.13	34.83	34.48	9.60	7.07	8.33
MCR-5	40.95	45.00	42.98	7.07	7.08	7.08
MCR-6	44.37	43.00	43.68	7.60	6.27	6.93
MCR-7	33.77	34.87	34.32	5.45	5.38	5.42
KCR-1	29.13	29.00	29.07	5.53	6.97	6.25
KCR-2	41.67	41.33	41.50	5.37	5.17	5.27
KCR-3	32.88	31.83	32.36	4.50	4.37	4.43
KCR-4	32.90	31.17	32.03	5.45	5.28	5.37
KCR-5	30.10	29.58	29.84	4.30	4.05	4.18
KCR-6	20.77	23.92	22.34	4.30	4.15	4.23
C.D (0.05)	2.80	3.75	2.30	0.72	0.52	0.57

There was increase in leaf length at 90 DAP and the increase was significant among the genotypes. The leaf length ranged from 22.34 cm to 43.68 cm. During 2017-18, significantly highest leaf length (44.37 cm) was observed in genotype MCR-6 while during 2018-19, MCR-5 (45.00 cm) recorded the highest length and it was on par with MCR-6. In the pooled mean, significantly highest value (43.68 cm) was observed in the genotype MCR-6 and it was on par with MCR-5

Leaf breadth increased steadily after 60 DAP. During 2017-18, leaf breadth ranged from 4.30 to 9.60 cm while during 2018-19, leaf brea dth ranged from 4.05 cm to 8.22 cm. In case of pooled mean data, MCR-4 recorded significantly highest value (8.33 cm) and lowest (4.18 cm) was observed in KCR-5.

4.1.1.17 Leaf length and breadth in K. rotunda genotypes at 120 DAP

The data on leaf length and breadth recorded at 120 DAP is presented in table 19.

Table 19: Leaf length and breadth in *K. rotunda* genotypes at 120 DAP

Genotype	Leaf length (cm)			Leaf breadth (cm)		
	2017-18	2018-19	Pooled Mean	2017-18	2018-19	Pooled Mean
MCR-1	43.10	38.18	40.64	7.35	7.22	7.28
MCR-2	41.90	42.35	42.13	9.23	8.50	8.87
MCR-3	47.37	45.95	46.66	8.93	8.42	8.68
MCR-4	47.30	44.00	45.65	8.08	9.20	8.64
MCR-5	50.73	52.03	51.38	6.97	6.92	6.94
MCR-6	44.20	45.00	44.60	6.82	6.45	6.63
MCR-7	43.60	43.10	43.35	6.03	6.08	6.06
KCR-1	37.30	36.50	36.90	7.20	6.95	7.08
KCR-2	43.67	44.17	43.92	7.22	7.18	7.20
KCR-3	38.05	34.67	36.36	6.02	5.58	5.80
KCR-4	37.90	37.33	37.62	5.52	5.62	5.57
KCR-5	34.10	34.00	34.05	6.40	6.07	6.23
KCR-6	27.08	28.30	27.69	5.17	4.95	5.06
C.D (0.05)	2.87	4.02	2.46	0.76	0.70	0.52

The leaf length at 120 DAP ranged from 27.69 cm to 51.38 cm. There was significant difference among the genotypes during both the years as well as pooled mean. In both the years and the pooled mean, the significantly highest leaf length was recorded in the genotype MCR-5 (50.73 cm, 52.03 cm, and 51.38 cm, respectively) and lowest in the genotype KCR-6.

The leaf breadth kept increasing at 120 DAP. There was significant difference among the genotypes and the pooled mean. During 2017-18, highest leaf breadth was noticed in genotype MCR-2 (9.23 cm) and it was on par with MCR-3 while during 2018-19, MCR-4 (9.23 cm) recorded significantly highest leaf breadth. In the pooled mean, genotype MCR-2 showed significantly highest (8.87) leaf breadth which was on par with MCR-3 and MCR-4, the lowest (5.06) was recorded by the genotype KCR-6.

4.1.1.18 Leaf length and breadth in K. rotunda genotype at 150 DAP

The data on leaf length and breadth at 150 DAP are given in table 20.

Table 20: Leaf length and breadth in *K. rotunda* genotype at 150 DAP

Genotype	Leaf length (cm)			Leaf breadth (cm)		
	2017-18	2018-19	Pooled Mean	2017-18	2018-19	Pooled Mean
MCR-1	46.33	44.32	45.33	7.63	8.17	7.90
MCR-2	46.02	49.83	47.93	9.38	8.17	8.78
MCR-3	52.87	51.38	52.13	8.98	9.92	9.45
MCR-4	59.63	56.10	57.87	8.25	8.23	8.24
MCR-5	54.78	53.18	53.98	7.63	7.20	7.42
MCR-6	59.87	55.22	57.54	7.48	7.48	7.48
MCR-7	50.10	46.97	48.53	6.78	6.25	6.52
KCR-1	48.97	48.17	48.57	7.28	6.98	7.13
KCR-2	49.17	48.00	48.58	7.38	7.17	7.28
KCR-3	44.72	44.17	44.44	6.78	6.38	6.58
KCR-4	42.57	42.17	42.37	6.07	5.98	6.03
KCR-5	44.27	43.87	44.07	6.58	6.17	6.38
KCR-6	28.75	29.67	29.21	5.48	5.27	5.38
C.D (0.05)	2.59	3.67	2.32	0.66	0.59	0.47

There was significant difference observed among the genotypes in leaf length at 150 DAP. In both the years and the pooled mean, genotype MCR-4 recorded the highest (59.63 cm, 56.10 cm and 57.87 cm, respectively) leaf length and it was on par with MCR-4, the lowest was recorded in genotype KCR-6.

Leaf breadth at 150 DAP also showed the increasing trend. At 2017-18, leaf breadth ranged from 5.48 cm to 9.38 cm, while at 2018-19, it ranged from 5.27 cm to 9.92 cm. Pooled mean data showed that MCR-3 recorded the significantly highest (9.45 cm) leaf breadth and lowest (5.38 cm) was recorded by genotype KCR-6.

4.1.1.19 Leaf length and breadth in K. rotunda genotypes at 180 DAP

The data on leaf length and breadth recorded at 180 DAP are given in table 21.

Table 21: Leaf length and breadth in *K. rotunda* genotypes at 180 DAP

Genotype	Leaf length (cm)			Leaf breadth (cm)		
	2017-18	2018-19	Pooled Mean	2017-18	2018-19	Pooled Mean
MCR-1	49.17	50.83	50.00	8.13	8.38	8.26
MCR-2	51.02	51.00	51.01	8.27	8.63	8.45
MCR-3	52.80	53.28	53.04	7.60	8.47	8.03
MCR-4	58.85	59.23	59.04	7.90	8.28	8.09
MCR-5	53.15	52.78	52.97	7.70	8.55	8.13
MCR-6	60.47	60.07	60.27	8.07	8.28	8.18
MCR-7	49.60	50.73	50.17	7.12	6.77	6.94
KCR-1	49.05	50.15	49.60	7.82	7.93	7.88
KCR-2	47.07	48.97	48.02	6.70	7.03	6.87
KCR-3	46.32	48.77	47.54	8.10	7.88	7.99
KCR-4	41.07	42.83	41.95	7.28	7.08	7.18
KCR-5	44.10	46.65	45.38	8.18	8.00	8.09
KCR-6	29.50	32.47	30.98	6.47	6.27	6.37
C.D (0.05)	2.29	3.39	2.03	0.64	0.67	0.47

Leaf length increased after 150 DAP and it ranged from 30.98 cm to 60.27 cm at 180 DAP. Significant difference was observed among the genotypes during both the years and the pooled mean. In both the years and pooled mean, significantly highest leaf length was recorded in the genotype MCR-6 (60.47 cm, 60.07 cm and 60.27 cm, respectively) which was on par with MCR-4 and the least was noticed in the genotype KCR-6.

There was significant difference among the genotypes in both the years and pooled mean. During 2017-18, leaf breadth ranged from 6.47 cm to 8.27 cm while during 2018-19, the range was 6.27 cm to 8.63 cm. In the pooled mean, leaf breadth was observed in the range of 6.37 cm to 8.45 cm at 180 DAP.

4.1.1.20 Leaf area in K. rotunda genotypes at 60 DAP

The data on leaf area of different genotypes recorded at 60 DAP are given in table 22.

Table 22: Leaf area in *K. rotunda* genotypes at 60 DAP

Genotype	Leaf area (cm^2)		
	2017-18	2018-19	Pooled Mean
MCR-1	143.42	144.80	144.11
MCR-2	122.41	119.83	121.12
MCR-3	133.60	134.20	133.90
MCR-4	76.06	71.24	73.65
MCR-5	216.55	215.27	215.91
MCR-6	176.51	175.34	175.93
MCR-7	155.28	154.64	154.96
KCR-1	193.45	195.58	194.51
KCR-2	182.91	178.96	180.93
KCR-3	163.47	160.48	161.97
KCR-4	178.36	179.35	178.86
KCR-5	182.46	183.51	182.98
KCR-6	133.62	133.18	133.40
C.D (0.05)	8.20	7.35	5.24

There was significant difference among the genotypes during both the years as well as the pooled mean for leaf area at 60 DAP. In both the years and pooled mean, MCR-5 showed significantly highest (216.55, 215.27, 215.91 cm^2, respectively) leaf area.

4.1.1.21 Leaf area in K. rotunda genotypes at 90 DAP

The data on leaf area of different genotypes recorded at 90 DAP are presented in table 23.

Table 23: Leaf area in *K. rotunda* genotypes at 90 DAP

Genotype	Leaf area (cm^2)		
	2017-18	2018-19	Pooled Mean
MCR-1	189.29	189.06	189.18
MCR-2	181.81	181.05	181.43
MCR-3	150.92	153.41	152.16
MCR-4	192.46	193.64	193.05
MCR-5	218.22	216.80	217.51
MCR-6	231.70	229.13	230.42

Table 23 contd.

Genotype	Leaf area (cm^2)		
	2017-18	2018-19	Pooled Mean
KCR-1	207.26	211.40	209.33
KCR-2	231.37	214.86	223.11
KCR-3	215.23	216.41	215.82
KCR-4	195.48	196.72	196.10
KCR-5	224.59	226.46	225.53
KCR-6	209.45	206.64	208.04
C.D (0.05)	12.43	11.74	8.33

At 90 DAP, leaf area showed increasing trend. There was significant difference among the genotypes during both the years and the pooled mean. During 2017-18, significantly highest leaf area was recorded in genotype MCR-6 (231.70 cm^2) which was on par with KCR-2 and KCR-5. In 2018-19 also, MCR-6 (229.13 cm^2) showed significantly highest leaf area which was on par with KCR-5. In the pooled mean of genotypes, significantly highest (230.42 cm^2) leaf area was noticed in the genotype MCR-6 (230.42 cm^2) which was on par with KCR-5 and KCR-2.

4.1.1.22 Leaf area in K. rotunda genotypes at 120 DAP

The leaf area of different genotypes recorded at 120 DAP are presented in table 24.

Table 24: Leaf area in *K. rotunda* genotypes at 120 DAP

Genotype	Leaf area (cm^2)		
	2017-18	2018-19	Pooled Mean
MCR-1	245.84	246.16	246.00
MCR-2	238.88	237.78	238.33
MCR-3	274.91	273.76	274.33
MCR-4	223.97	225.25	224.61
MCR-5	226.19	224.44	225.31
MCR-6	263.85	268.63	266.24
MCR-7	296.07	294.37	295.22
KCR-1	263.49	260.09	261.79
KCR-2	240.50	237.81	239.15
KCR-3	255.18	254.58	254.88

Table 24 contd.

Genotype	Leaf area (cm^2)		
	2017-18	2018-19	Pooled Mean
KCR-4	230.95	238.32	234.64
KCR-5	292.59	292.74	292.66
KCR-6	235.34	237.56	236.45
C.D (0.05)	10.08	10.45	6.90

The leaf area at 120 DAP also showed the increasing trend. The range of leaf area was 224.61 cm^2 to 295.22 cm^2. There was significant difference among the genotypes. The genotype MCR-7 registered the significantly highest value (296.07, 294.37, 295.22 cm^2, respectively) during both the years and pooled mean and it was on par with KCR-5.

4.1.1.23 Leaf area in K. rotunda genotypes at 150 DAP

The data on leaf area of different genotypes recorded at 150 DAP are depicted in table 25.

Table 25: Leaf area in *K. rotunda* genotypes at 150 DAP

Genotype	Leaf area (cm^2)		
	2017-18	2018-19	Pooled Mean
MCR-1	245.95	239.06	242.51
MCR-2	273.62	274.16	273.89
MCR-3	316.39	314.71	315.55
MCR-4	299.17	300.09	299.63
MCR-5	280.29	267.22	273.75
MCR-6	366.07	363.20	364.63
MCR-7	323.33	316.10	319.71
KCR-1	270.58	266.39	268.48
KCR-2	263.60	240.59	252.10
KCR-3	277.81	273.85	275.83
KCR-4	245.50	246.61	246.05
KCR-5	320.92	282.56	301.74
KCR-6	265.44	261.66	263.55
C.D (0.05)	12.86	15.97	11.11

At 150 DAP, the leaf area ranged from 242.51 cm^2 to 364.633 cm^2. The genotype MCR-6 recorded significantly highest leaf area during both the years and pooled mean while KCR-4 recorded the lowest leaf area during 2017-18, while MCR-1 recorded lowest leaf area during 2018-19.

4.1.1.24 Leaf area in K. rotunda genotypes at 180 DAP

The data on leaf area of different genotypes recorded at 180 DAP are depicted in table 26.

Table 26: Leaf area in *K. rotunda* genotypes at 180 DAP

Genotype	Leaf area (cm^2)		
	2017-18	**2018-19**	**Pooled Mean**
MCR-1	248.09	248.16	248.12
MCR-2	276.58	276.05	276.32
MCR-3	318.64	319.46	319.05
MCR-4	299.79	300.32	300.06
MCR-5	286.71	276.80	281.75
MCR-6	373.62	372.28	372.95
MCR-7	330.90	326.85	328.87
KCR-1	277.26	273.23	275.24
KCR-2	278.38	270.13	274.25
KCR-3	280.75	276.41	278.58
KCR-4	255.48	256.72	256.10
KCR-5	324.48	294.92	309.70
KCR-6	269.45	266.64	268.04
C.D (0.05)	14.27	11.87	9.50

After 150 DAP, there was only very little increase in leaf area. Significant difference was observed among the genotypes during both the years and the pooled mean. MCR-6 recorded significantly highest (372.95 cm^2) leaf area and the lowest (248.12 cm^2) was recorded in MCR-1 in the pooled mean.

4.1.1.25 Plant spread of K. rotunda genotypes

The data regarding the plant spread in the North-South as well as East West direction are presented in the table 27.

Table 27: Plant spread of *K. rotunda* genotypes at 180 DAP

Genotype	North-South (N-S) (cm)			East-West (E-W) (cm)		
	2017-18	2018-19	Pooled Mean	2017-18	2018-19	Pooled Mean
MCR-1	21.50	25.00	23.25	48.00	45.67	46.83
MCR-2	65.50	57.67	61.58	61.33	60.00	60.67
MCR-3	71.33	71.33	71.33	55.50	53.33	54.42
MCR-4	67.83	69.50	68.67	49.83	49.17	49.50
MCR-5	61.67	60.17	60.92	55.33	53.50	54.42
MCR-6	67.17	71.17	69.17	45.33	48.83	47.08
MCR-7	55.50	56.17	55.83	46.33	45.67	46.00
KCR-1	61.33	57.50	59.42	67.67	65.17	66.42
KCR-2	50.00	50.67	50.33	61.67	61.00	61.33
KCR-3	57.50	55.67	56.58	61.83	59.67	60.75
KCR-4	54.67	47.33	51.00	57.33	56.50	56.92
KCR-5	41.50	54.33	47.92	56.00	33.83	44.92
KCR-6	60.17	50.33	55.25	30.17	42.50	36.33
C.D (0.05)	3.59	6.35	0.49	3.95	5.69	4.44

The plant spread in the North-South (N-S) direction ranged from 23.25cm to 71.33 cm while in the East-West direction it ranged from 36.33 cm to 66.42 cm in the pooled mean. The N-S plant spread was significantly highest (71.33) in the genotype MCR-3 and lowest (21.50) in MCR-1 during 2017-18. In 2018-19 and the pooled mean also MCR-3 recorded the highest N-S spread and it was on par MCR-4 and MCR-6. In the E-W spread, KCR-1 recorded significantly highest (67.67, 65.17, 66.42) spread in both the years and the pooled mean.

4.1.1.26 Plant height in K. rotunda genotypes

The data on plant height recorded at 180 DAP are presented in the table 28.

Table 28: Plant height in *K. rotunda* genotypes

Genotype	Plant height (cm)		
	2017-18	2018-19	Pooled Mean
MCR-1	100.17	83.85	92.01
MCR-2	94.67	85.45	90.06
MCR-3	98.67	101.08	99.88
MCR-4	105.00	101.33	103.17
MCR-5	93.33	95.67	94.50
MCR-6	118.17	114.33	116.25
MCR-7	85.00	103.00	94.00
KCR-1	108.67	113.50	111.08
KCR-2	106.83	100.83	103.83
KCR-3	106.83	103.33	105.08
KCR-4	117.33	92.33	104.83
KCR-5	103.17	97.33	100.25
KCR-6	68.17	64.50	66.33
C.D (0.05)	6.11	7.38	6.30

The plant height at 180 DAP ranged from 68.17 to 118.17 cm during 2017-18. Significantly highest value (118.17 cm) was observed in the genotype MCR-6 and it was on par with genotype KCR-4 during 2017-18. During 2018-19 also, significantly highest plant height was noticed in the genotype MCR-6 and it was on par with genotype KCR-1. In both the years, plants of KCR-6 were the shortest (68.17, 64.50 cm respectively). In the pooled mean of genotypes too significantly highest plant height was recorded in the genotype MCR-6 and it was on par with KCR-1.

4.1.1.27 Fresh and dry weight of leaves per plant in K. rotunda genotypes

The data on fresh weight and dry weight of leaves per plant recorded at 180 DAP in all the genotypes are given in table 29.

Table 29: Fresh weight and dry weight of leaves per plant in *K. rotunda* genotypes

Genotype	Fresh weight (g)			Dry weight (g)		
	2017-18	2018-19	Pooled Mean	2017-18	2018-19	Pooled Mean
MCR-1	159.00	162.00	160.50	13.23	11.76	12.49
MCR-2	153.00	151.67	152.33	14.50	13.51	14.01
MCR-3	138.00	137.50	137.75	15.13	12.99	14.06
MCR-4	187.40	188.50	187.95	20.00	20.38	20.19
MCR-5	153.40	151.67	152.53	15.10	16.02	15.56
MCR-6	183.70	182.58	183.14	36.58	36.56	36.57
MCR-7	83.80	89.00	86.40	12.20	18.07	15.14
KCR-1	121.20	119.00	120.10	11.12	11.04	11.08
KCR-2	90.60	105.00	97.80	10.70	11.07	10.88
KCR-3	130.17	125.67	127.92	14.45	13.96	14.21
KCR-4	113.67	113.50	113.58	12.34	11.39	11.87
KCR-5	119.17	125.50	122.33	11.30	20.32	15.81
KCR-6	56.60	53.67	55.13	5.26	8.26	6.76
C.D (0.05)	14.72	9.88	8.55	2.73	5.11	3.03

The data presented in table 29 showed that the fresh weight of leaves ranged from 55.13 g to 187.95 g in the pooled mean. Significant difference was observed among the genotypes during both the years and in the pooled mean. The significantly highest (187.40, 188.50, 187.95 g, respectively) fresh weight of leaves was recorded in the genotype MCR-4 which was on par with MCR-6 during both the years and in the pooled mean. Dry weight of leaves per plant was found to be significantly highest in the genotype MCR-6 in both the years and the pooled mean. The mean data ranged from 6.76 g to 36.57 g.

4.1.1.28 Length and girth of rhizomes in K. rotunda genotypes

The data on length and girth of rhizomes recorded in the genotypes at harvest are presented in table 30.

Table 30: Length and girth of rhizomes in *K. rotunda* genotypes

Genotypes	Length of rhizome (cm)			Girth of rhizome (cm)		
	2017-18	2018-19	Pooled Mean	2017-18	2018-19	Pooled Mean
MCR-1	5.98	5.17	5.57	1.53	1.97	1.75
MCR-2	5.88	5.02	5.45	1.83	2.02	1.93
MCR-3	6.04	3.95	5.00	2.02	1.82	1.92
MCR-4	8.14	5.17	6.65	2.22	1.87	2.04
MCR-5	6.82	5.92	6.37	2.07	2.03	2.05
MCR-6	7.90	5.40	6.65	2.10	2.55	2.33
MCR-7	5.08	4.17	4.62	2.03	1.62	1.83
KCR-1	11.40	5.27	8.33	2.78	2.33	2.56
KCR-2	5.46	4.82	5.14	1.62	2.78	2.20
KCR-3	7.56	4.28	5.92	2.57	2.32	2.44
KCR-4	7.64	3.63	5.64	2.05	2.02	2.03
KCR-5	10.58	4.80	7.69	2.53	1.82	2.18
KCR-6	5.70	5.35	5.53	1.98	1.77	1.88
C.D (0.05)	0.65	1.27	1.44	0.15	0.38	0.28

During 2017-18, rhizome length was observed to be significantly highest (11.40 cm) in the genotype KCR-1 and the least (5.08 cm) was observed in the genotype MCR-7. During 2018-19, MCR-5 recorded the highest value (5.92 cm). The pooled mean showed that KCR-1 recorded significantly highest (8.33 cm) rhizome length.

The girth of rhizome showed significant variation among the genotypes during both the years and in the pooled mean. The highest (2.78) girth was registered in the genotype KCR-1 and least (1.53) in the genotype MCR-1 during 2017-18. In 2018-19, significantly highest (2.78) rhizome girth was recorded by the genotype KCR-2. In case of pooled mean, KCR-1 recorded significantly highest value (2.56).

4.1.1.29 Fresh and dry yield of rhizomes in K. rotunda genotypes

The data on fresh and dry yield of rhizomes of *K. rotunda* genotypes are presented in the table 31.

Table 31: Fresh and dry yield of rhizome in *K. rotunda* genotypes

Genotype	Fresh yield of rhizome (g/plant)			Dry yield of rhizome (g/plant)		
	2017-18	2018-19	Pooled Mean	2017-18	2018-19	Pooled Mean
MCR-1	17.50	16.94	17.22	11.18	11.75	11.47
MCR-2	14.92	15.49	15.21	4.68	5.31	5.00
MCR-3	17.60	15.03	16.32	11.10	10.46	10.78
MCR-4	25.74	17.13	21.43	11.16	10.15	10.65
MCR-5	20.24	17.36	18.80	8.23	8.21	8.22
MCR-6	46.95	57.94	52.44	17.58	17.87	17.73
MCR-7	17.34	19.16	18.25	11.24	11.85	11.54
KCR-1	27.75	19.67	23.71	13.78	13.68	13.73
KCR-2	14.44	21.32	17.88	11.86	14.18	13.02
KCR-3	28.22	28.72	28.47	13.72	14.23	13.97
KCR-4	34.00	29.65	31.83	14.72	14.38	14.55
KCR-5	38.56	29.59	34.07	9.62	11.20	10.41
KCR-6	16.76	16.58	16.67	10.83	11.55	11.19
C.D (0.05)	3.17	4.32	3.47	2.02	2.23	1.46

Table 31 shows the fresh and dry rhizome yield of *K. rotunda* genotypes during two years. The fresh rhizome yield ranged from 15.21 g/plant to 52.44 g/plant in the pooled data. During 2017-18, 2018-19 and in the pooled mean, genotype MCR-6 recorded the significantly highest (46.95 g, 57.94 g, 52.44 g, respectively) fresh yield of rhizome.

In the pooled data, the dry yield of rhizome ranged from 5.00 to 17.73 g/plant. There was significant difference in dry yield of rhizome among the genotypes during both the years and in the pooled mean. During both the years and the pooled mean, genotype MCR-6 gave the highest (17.58, 17.87, 17.73 g/plant, respectively) dry yield and lowest (4.68, 5.31, 5.00 g/plant, respectively) yield was recorded by the genotype MCR-2.

4.1.1.30 Driage of rhizome in K. rotunda genotypes

The data on the driage of rhizome in *K. rotunda* genotypes are furnished in the table 32.

Table 32: Driage in *K. rotunda* genotypes

Genotype	Driage (%)		
	2017-18	2018-19	Pooled Mean
MCR-1	64.78	63.18	63.98
MCR-2	60.63	51.16	55.89
MCR-3	63.22	58.86	61.04
MCR-4	64.92	48.99	56.95
MCR-5	52.74	53.93	53.34
MCR-6	58.39	55.51	56.95
MCR-7	61.81	51.32	56.57
KCR-1	56.50	56.65	56.58
KCR-2	65.35	59.21	62.28
KCR-3	50.32	54.49	52.41
KCR-4	61.41	54.42	57.91
KCR-5	54.95	53.81	54.38
KCR-6	61.45	59.06	60.25
C.D (0.05)	8.12	8.96	6.72

The driage ranged from 52.41 to 63.98 and varied significantly among the genotypes. During 2017-18, KCR-2 recorded significantly highest (65.35) drying per cent which was on par with MCR-1, MCR-2, MCR-3, MCR-4, MCR-6, MCR-7, KCR-2, KCR-4 and KCR-6. In 2018-19, significantly highest (63.18) drying per cent was observed in the genotype MCR-1 which was on par with MCR-3, KCR-1, KCR-2 and KCR-6. In case of pooled mean, MCR-1 registered the significantly highest (63.98) value and it was on par with MCR-2, KCR-2 and KCR-6.

4.1.1.31 Number of root tubers in K. rotunda genotypes

Root tubers arising from rhizome were observed and the data is presented in table 33.

Table 33: Number of root tubers in *K. rotunda* genotypes

Genotype	Number of root tubers		
	2017-18	2018-19	Pooled Mean
MCR-1	46.17	33.83	40.00
MCR-2	44.00	33.50	38.75
MCR-3	43.00	21.17	32.08
MCR-4	63.33	45.33	54.33
MCR-5	49.67	32.50	41.08
MCR-6	57.83	53.67	55.75
MCR-7	44.67	37.33	41.00
KCR-1	118.00	35.83	76.92
KCR-2	42.17	38.33	40.25
KCR-3	61.17	45.17	53.17
KCR-4	114.17	33.00	73.58
KCR-5	52.33	53.17	52.75
KCR-6	23.83	32.00	27.92
C.D (0.05)	3.08	2.69	14.37

In 2017-18, root tuber production was significantly higher (118.00) in the genotype KCR-1. During 2018-19, genotype MCR-6 produced significantly highest number of root tubers (53.17). In the pooled mean, significantly highest number of root tubers was recorded in genotype KCR-1 (76.92).

4.1.1.32 Length and girth of tubers in K. rotunda genotypes

The data on dimensions of root tubers recorded in the genotypes at harvest are presented in table 34.

Table 34: Length and girth of tubers in *K. rotunda* genotypes

Genotype	Length of root tubers (cm)			Girth of root tubers (cm)		
	2017-18	2018-19	Pooled Mean	2017-18	2018-19	Pooled Mean
MCR-1	7.62	9.38	8.50	1.33	1.52	1.42
MCR-2	6.83	6.77	6.80	1.39	1.60	1.49
MCR-3	6.50	4.35	5.42	1.41	1.63	1.52
MCR-4	9.48	11.66	10.57	1.53	1.55	1.54
MCR-5	7.00	8.73	7.87	1.50	1.70	1.60
MCR-6	6.52	7.42	6.97	1.50	1.33	1.42

Table 34 contd.

Genotype	Length of root tubers (cm)			Girth of root tubers (cm)		
	2017-18	2018-19	Pooled Mean	2017-18	2018-19	Pooled Mean
MCR-7	4.77	6.00	5.38	0.98	1.13	1.05
KCR-1	5.02	6.48	5.75	1.74	1.59	1.67
KCR-2	4.20	6.72	5.46	1.24	1.41	1.33
KCR-3	4.68	6.00	5.34	1.49	1.49	1.49
KCR-4	4.75	5.02	4.88	1.14	1.34	1.24
KCR-5	5.24	5.77	5.50	1.35	1.48	1.42
KCR-6	5.95	8.63	5.97	1.03	1.29	1.16
C.D (0.05)	0.98	1.52	1.25	0.13	0.17	0.12

The length and girth of tubers varied significantly among the genotypes during both the years and also in the pooled mean. During 2017-18, highest (9.48 cm) tuber length was observed in the genotype MCR-4 and lowest (4.20 cm) in the genotype KCR-2. In 2018-19, significantly highest (11.66 cm) tuber length was noticed in the genotype MCR-4 but the lowest (5.02 cm) was recorded in the genotype KCR-4. The pooled mean of genotypes also depicted significant difference and the tuber length ranged from 4.88 cm to 10.57 cm.

In the case of tuber girth, genotype KCR-1 showed the highest (1.74 cm) girth and lowest (0.98 cm) was recorded in the genotype MCR-7 during 2017-18. During 2018-19, MCR-5 recorded the highest tuber girth (1.70 cm) and lowest tuber girth (1.13 cm) was recorded in the genotype MCR-7. In the pooled mean, genotype KCR-1 recorded the highest (1.67 cm) tuber girth.

4.1.1.33 Fresh and dry weight of root tubers in K. rotunda genotypes

The data on fresh and dry weight of root tubers is presented in table 35.

Table 35: Fresh and dry weight of root tubers in *K. rotunda* genotypes

Genotype	Fresh weight of root tubers (g/plant)			Dry weight of root tubers (g/plant)		
	2017-18	2018-19	Pooled Mean	2017-18	2018-19	Pooled Mean
MCR-1	141.72	102.88	122.30	35.42	26.07	30.74
MCR-2	162.97	124.22	143.59	37.57	28.60	33.08
MCR-3	145.87	69.97	107.92	22.63	11.15	16.89
MCR-4	262.10	186.70	224.40	45.83	32.85	39.34
MCR-5	194.38	124.42	159.40	50.50	33.05	41.78
MCR-6	300.08	276.52	288.30	69.82	64.93	67.38
MCR-7	81.88	68.53	75.21	24.10	20.15	22.13
KCR-1	255.22	77.28	166.25	69.72	21.20	45.46
KCR-2	101.42	92.58	97.00	30.08	27.47	28.78
KCR-3	165.33	121.02	143.18	44.68	33.30	38.99
KCR-4	130.00	37.55	83.78	37.27	10.75	24.01
KCR-5	166.92	169.75	168.33	41.28	42.03	41.66
KCR-6	70.83	94.58	82.71	21.67	29.22	25.44
C.D (0.05)	7.32	12.80	29.88	2.04	3.62	7.64

Fresh weight of root tubers per plant was significantly highest in the genotype MCR-6 during both the years and in the pooled mean (300.08 g, 276.52 g, and 288.30 g, respectively).

Dry weight of root tubers also showed significant difference among the genotypes during both the years and in the pooled mean. During both years and pooled mean, genotype MCR-6 recorded significantly higher dry weight of root tubers (69.82 g, 64.93 g and 67.38 g, respectively).

4.1.1.34 Biological yield in K. rotunda genotypes

The biological yield in *K. rotunda* genotypes are furnished in the table 36.

Table 36: Biological yield in *K. rotunda* genotypes

Genotype	Biological yield (g/plant)		
	2017-18	2018-19	Pooled Mean
MCR-1	59.92	51.30	55.61
MCR-2	56.93	46.84	51.89
MCR-3	49.13	37.04	43.09
MCR-4	77.58	63.50	70.54
MCR-5	74.02	56.49	65.25
MCR-6	124.03	120.29	122.16
MCR-7	47.61	46.19	46.90
KCR-1	94.65	44.52	69.59
KCR-2	52.68	52.85	52.76
KCR-3	72.94	62.57	67.75
KCR-4	64.57	36.07	50.32
KCR-5	62.23	74.74	68.48
KCR-6	37.86	47.69	42.78
C.D (0.05)	5.66	7.81	11.18

The data showed that during both the years and pooled data (124.03, 120.29, 122.16 respectively) genotype MCR-6 registered significantly higher biological yield.

4.1.1.35 Harvest index in K. rotunda genotypes

The harvest index in *K. rotunda* genotypes is presented in table 37.

Table 37: Harvest index in *K. rotunda* genotypes

Genotype	Harvest index (%)		
	2017-18	2018-19	Pooled Mean
MCR-1	34.99	36.73	35.86
MCR-2	16.17	21.05	18.61
MCR-3	32.67	36.52	34.60
MCR-4	25.68	26.05	25.86
MCR-5	24.33	25.84	25.08

Table 37. Contd.

Genotypes	Harvest index (%)		
	2017-18	2018-19	Pooled Mean
MCR-7	37.99	38.01	38.00
KCR-1	32.47	47.87	40.17
KCR-2	39.27	44.04	41.66
KCR-3	34.81	37.73	36.27
KCR-4	40.16	51.44	45.80
KCR-5	30.67	25.82	28.24
KCR-6	50.02	44.84	47.43
C.D (0.05)	3.62	4.55	3.45

Harvest index was significantly highest in the genotype KCR-6 (50.02 %) during 2017-18 and the lowest was noticed in the genotype MCR-2 (16.71 %). During 2018-19, genotype KCR-4 showed significantly highest harvest index (51.44 %). In the pooled mean, KCR-6 showed significantly highest index of 47.43 per cent which was on par with KCR-4 (45.80 %).

4.1.2 Morphological evaluation of *K. parviflora*

There were three genotypes, one each from Thailand, IISR, Kozhikode and BSI, Shillong. The genotypes did not differ in their qualitative features.

4.1.2.1. Qualitative parameters in K. parviflora

Qualitative parameters in *K. parviflora* are given in table 38.

Table 38: Qualitative parameters of *K. parviflora*

Parameter	Details
Rhizome colour: scale inner core	Brown (RHS 164B) Purple (RHS N187A)
Rhizome shape	Irregular shape, branch or palmate
Presence of root tubers	Present
Colour of root tubers	Off white (RHB 155D)
Mature leaf shape	Semi-erect/ lanceolate
Mature leaf colour	Light green

Table 38 contd.

Parameter	Details
Leaf tip shape	Cuspidate
Leaf margin	Undulated
Growth habit	Sprawling

The rhizome of *K. parviflora* is brown coloured in the outer scale and purple coloured in the inner core. Rhizomes are irregular in shape with branch or palmate. Few root tubers arise from rhizome which have white long stem, enlarged at the top with pointed beaks (Plate 11). Leaves are light green and lanceolate shaped. Leaf tips are cuspidate and the margin of leaves are undulated (Plate 9).

4.1.2.2 Number of days for sprouting in K. parviflora genotypes

The data on number of days required for sprouting in *K. parviflora* during both the seasons are presented in table 39.

Table 39: Number of days for sprouting in *K. parviflora* genotypes

Genotype	Number of days to sprout		
	2017-18	2018-19	Pooled mean
KCP-1	19.33	17.17	18.25
KCP-2	15.17	15.67	15.42
BSI-1	16.67	17.67	17.17
C.D (0.05)	1.08	1.40	1.01

The data presented in table 39 showed that there was significant difference in the days to sprout among the genotypes. The genotype KCP-2 took lowest (15.17) days to sprout and KCP-1 took the highest (19.33) days during 2017-18. During 2018-19, genotype KCP-2 took the lowest (15.67) days and it was on par with KCP-1. In case of pooled mean, genotype KCP-2 took lowest (15.42) days to sprout and KCP-1 took highest (18.25) days which was on par with BSI-1.

4.1.2.3 Number of tillers per plant at 60 DAP in K. parviflora genotypes

The tillers produced per plant was recorded at monthly intervals and data recorded at 60 DAP are furnished in table 40.

Table 40: Number of tillers per plant in *K. parviflora* genotypes at 60 DAP

Genotype	Number of tillers/plant		
	2017-18	2018-19	Pooled mean
KCP-1	1.33	1.33	1.33
KCP-2	0.50	0.67	0.58
BSI-1	1.50	1.50	1.50
C.D (0.05)	0.80	0.65	0.47

The tillers per plant at 60 DAP ranged from 0.58 to 1.50 in the pooled mean. There was significant difference among the genotypes during both the years as well as in the pooled mean. In both the years and pooled mean, significantly highest (1.50) tiller production was observed in the genotype BSI-1 and it was on par with KCP-1.

4.1.2.4 Number of tillers per plant at 90 DAP in K. parviflora genotypes

The tillers produced per plant at 90 DAP are given in table 41.

Table 41: Number of tillers per plant in *K. parviflora* genotypes at 90 DAP

Genotype	Number of tillers/plant		
	2017-18	2018-19	Pooled mean
KCP-1	2.17	4.83	3.50
KCP-2	3.00	2.50	2.75
BSI-1	2.83	2.83	2.83
C.D (0.05)	NS	1.06	NS

Tiller production increased at 90 DAP in all the genotypes. No significant difference in tiller production was noticed during 2017-18 and in the pooled mean. During 2018-19, KCP-1 produced significantly highest (4.83) number of tillers.

4.1.2.5 Number of tillers per plant K. parviflora genotypes at 120 DAP

The tillers produced per plant recorded at 120 DAP is presented in table 42.

Table 42: Number of tillers per plant in *K. parviflora* genotypes at 120 DAP

Genotype	Number of tillers/plant		
	2017-18	2018-19	Pooled mean
KCP-1	4.33	6.17	5.25
KCP-2	3.50	4.50	4.00
BSI-1	4.50	7.00	5.75
C.D (0.05)	NS	0.92	1.01

The tiller production ranged from 4.00 to 5.75 at 120 DAP in pooled mean. There was no significant variation among the genotypes during 2017-18. During 2018-19 and the pooled mean, significantly highest tiller production was recorded in the genotype BSI-1 (7.00 and 5.75 respectively) and it was on par with KCP-1.

4.1.2.6 Number of tillers per plant at 150 DAP in K. parviflora genotypes

The tillers produced per plant at 150 DAP are presented in table 43.

Table 43: Number of tillers per plant in *K. parviflora* genotypes at 150 DAP

Genotype	Number of tillers/plant		
	2017-18	2018-19	Pooled mean
KCP-1	4.67	4.33	4.50
KCP-2	5.00	4.67	4.83
BSI-1	5.50	6.33	5.92
C.D (0.05)	NS	1.10	0.73

The data revealed that there was no significant difference among the genotypes during 2017-18 in the number of tillers produced at 150 DAP. In 2018-19 and the pooled mean, BSI-1 recorded significantly highest (6.33, 5.92) tiller production and KCP-1 recorded the least (4.33, 4.50).

4.1.2.7 Number of tillers per plant in K. parviflora genotypes at 180 DAP

The tillers produced per plant at 180 DAP are presented in table 44.

Table 44: Number of tillers per plant in *K. parviflora* genotypes at 180 DAP

Genotype	Number of tillers/plant		
	2017-18	2018-19	Pooled mean
KCP-1	4.67	5.17	4.92
KCP-2	4.67	5.33	5.00
BSI-1	6.33	7.00	6.67
C.D (0.05)	0.78	0.79	0.57

The tiller production was maximum at 180 DAP. There was significant difference in tiller production among the genotypes during both the years and the pooled mean. During both the years and the pooled mean, BSI-1 produced the significantly highest (6.33, 7.00, 6.67) number of tillers and lowest number of tillers was produced by the genotype KCP-1.

4.1.2.8 Number of tillers per plant at 210 DAP in K. parviflora genotypes

The tillers produced per plant at 210 DAP are given in table 45.

Table 45: Number of tillers per plant in *K. parviflora* genotypes at 210 DAP

Genotype	Number of tillers/plant		
	2017-18	2018-19	Pooled mean
KCP-1	3.33	3.17	3.25
KCP-2	3.67	3.67	3.67
BSI-1	4.83	5.67	5.25
C.D (0.05)	0.59	1.08	0.60

After 180 days, the tiller production declined in all the genotypes. Significant difference was observed among the genotypes during two years and in the pooled mean at 210 DAP. In both the years and the pooled mean, BSI-1 recorded the significantly highest (4.83, 5.67, 5.25) tiller production and lowest value was recorded in the genotype KCP-1 (3.33, 3.17, 3.25).

4.1.2.9 Plant spread in K. parviflora genotypes

The data regarding the plant spread are presented in the table 46.

Table 46: Plant spread in *K. parviflora* genotypes

Genotype	North-South spread (cm)			East-West Spread (cm)		
	2017-18	2018-19	Pooled mean	2017-18	2018-19	Pooled mean
KCP-1	51.33	58.33	54.83	48.50	55.00	51.75
KCP-2	60.33	50.50	55.42	54.67	44.83	49.75
BSI-1	55.00	55.50	55.25	53.33	51.33	52.33
C.D (0.05)	6.21	NS	NS	4.84	7.00	NS

During 2017-18, N-S spread was highest (60.33 cm) in the genotype KCP-2 and lowest (51.33 cm) plant spread was recorded by the genotype KCP-1. The year 2018-19 and the pooled mean showed no significant difference among the genotypes. In case of E-W spread during 2017-18, highest (54.67 cm) plant spread was observed in the genotype KCP-2 and it was on par with BSI-1. During 2018-19, E-W plant spread was highest (55.00 cm) in the genotype KCP-1 and it was on par with BSI-1. Pooled mean of both N-S and E-W spread showed no significant difference among the genotypes.

4.1.2.10 Number of leaves per plant in K. parviflora genotypes at 30 DAP

The data on number of leaves per plant at 30 DAP during both the years are given in table 47.

Table 47: Number of leaves per plant in *K. parviflora* genotypes at 30 DAP

Genotype	Number of leaves per plant		
	2017-18	2018-19	Pooled mean
KCP-1	1.00	1.00	1.00
KCP-2	1.00	1.00	1.00
BSI-1	1.00	1.00	1.00
C.D (0.05)	NS	NS	NS

It is seen from the data that all the genotypes had only single leaf at 30 DAP.

4.1.2.11 Number of leaves per plant in K. parviflora genotypes at 60 DAP

The number of leaves per plant at 60 DAP in the genotypes during both the years and the pooled mean are furnished in table 48.

Table 48: Number of leaves per plant in *K. parviflora* genotypes at 60 DAP

Genotype	Number of leaves/plant		
	2017-18	2018-19	Pooled mean
KCP-1	1.00	1.33	1.17
KCP-2	2.33	2.17	2.25
BSI-1	1.67	1.50	1.58
C.D (0.05)	0.69	NS	0.48

The number of leaves increased at 60 DAP. There was significant variation among the genotypes during 2017-18 and the pooled mean. Genotype KCP-2 showed significantly highest (2.33, 2.25) number of leaves during 2017-18 and in the pooled mean.

4.1.2.12 Number of leaves per plant in K. parviflora genotypes at 90 DAP

The number of leaves per plant at 90 DAP in all the genotypes during two years and the pooled mean are presented in table 49.

Table 49: Number of leaves per plant in *K. parviflora* genotypes at 90 DAP

Genotype	Number of leaves/plant		
	2017-18	2018-19	Pooled mean
KCP-1	5.17	6.83	6.00
KCP-2	5.33	5.33	5.33
BSI-1	5.83	5.67	5.75
C.D (0.05)	NS	1.17	NS

The leaf production showed an increasing trend at 90 DAP. The number of leaves ranged from 5.33 to 6.00 in pooled mean. No significant difference was noticed among the genotypes during 2017-18 and in the pooled mean. During 2018-19, genotype KCP-1 produced significantly highest number of leaves (6.83).

4.1.2.13 Number of leaves per plant in K. parviflora genotypes at 120 DAP

The table 50 denotes the number of leaves per plant at 120 DAP in the genotypes during both years.

Table 50: Number of leaves per plant in *K. parviflora* genotypes at 120 DAP

Genotype	Number of leaves/plant		
	2017-18	2018-19	Pooled mean
KCP-1	11.17	13.33	12.25
KCP-2	8.00	10.50	9.25
BSI-1	8.00	8.00	8.00
C.D (0.05)	NS	2.03	1.83

The data showed that there was an increase in leaf production at 120 DAP. There was no significant difference in leaf production among the genotypes during 2017-18. The genotype KCP-1 produced the highest (13.33, 12.25) number of leaves during 2018-19 and in the pooled mean.

4.1.2.14 Number of leaves per plant in K. parviflora genotypes at 150 DAP

The table 51 presents the number of leaves per plant at 150 DAP during both the years under observation.

Table 51: Number of leaves per plant in *K. parviflora* genotypes at 150 DAP

Genotype	Number of leaves/plant		
	2017-18	2018-19	Pooled mean
KCP-1	13.00	15.67	14.33
KCP-2	9.00	12.67	10.83
BSI-1	10.33	12.83	11.58
C.D (0.05)	1.07	2.45	1.78

At 150 DAP, number of leaves produced increased in all the genotypes. In both the years and the pooled mean, significantly highest (13.00, 15.67, 14.33 respectively) leaf production was recorded by the genotype KCP-1.

4.1.2.15 Number of leaves per plant in K. parviflora genotypes at 180 DAP

The data on the number of leaves per plant at 180 DAP in both the years are presented in table 52.

Table 52: Number of leaves per plant in *K. parviflora* genotypes at 180 DAP

Genotype	Number of leaves/plant		
	2017-18	2018-19	Pooled mean
KCP-1	15.67	17.17	16.42
KCP-2	16.00	17.00	16.50
BSI-1	17.67	18.17	17.92
C.D (0.05)	NS	NS	NS

The highest leaf production was recorded in all the genotypes at 180 DAP and it ranged from 16.42 to 17.92. However, there was no significant difference among the genotypes during both the years and also in the pooled mean.

4.1.2.16 Number of leaves per plant in K. parviflora genotypes at 210 DAP

The data on the number of leaves per plant at 210 DAP during both the years is given in table 53.

Table 53: Number of leaves per plant in *K. parviflora* genotypes at 210 DAP

Genotype	Number of leaves/plant		
	2017-18	2018-19	Pooled mean
KCP-1	13.17	13.17	13.17
KCP-2	13.33	12.83	13.08
BSI-1	16.00	14.17	15.08
C.D (0.05)	1.80	NS	1.65

The data indicated that there was decline in leaf production at 210 DAP. Significantly highest (16.00, 15.08) leaf production was observed in the genotype BSI-1 during 2017-18 and in the pooled mean. No significant difference was observed among the genotypes during 2018-19.

4.1.2.17 Leaf length and leaf breadth in K. parviflora genotypes at 60 DAP

The data on leaf length and breadth at 60 DAP are presented in table 54.

Table 54: Leaf length and leaf breadth in *K. parviflora* genotypes at 60 DAP

Genotype	Leaf length (cm)			Leaf breadth (cm)		
	2017-18	2018-19	Pooled mean	2017-18	2018-19	Pooled mean
KCP-1	17.40	17.45	17.43	9.90	10.10	10.00
KCP-2	20.32	20.90	20.61	10.72	10.93	10.83
BSI-1	18.93	19.60	19.27	9.87	9.90	9.88
C.D (0.05)	0.69	1.22	0.68	NS	NS	0.45

There was an increase in both the leaf length and breadth at 60 DAP as evident from the data presented in table 54. In both the years and the pooled mean, genotype KCP-2 had the highest (20.32, 20.90, and 20.61) leaf length while, the lowest (17.40, 17.45, 17.43) was observed in genotype KCP-1. In case of leaf breadth, no significant difference was observed among the genotypes during both the years. However, pooled mean showed significantly highest (10.83) leaf breadth in the genotype KCP-2 and it was on par with KCP-2.

4.1.2.18 Leaf length and leaf breadth in K. parviflora genotypes at 90 DAP

The data on leaf length and breadth at 90 DAP are presented in table 55.

Table 55: Leaf length and leaf breadth in *K. parviflora* genotypes at 90 DAP

Genotype	Leaf length (cm)			Leaf breadth (cm)		
	2017-18	2018-19	Pooled mean	2017-18	2018-19	Pooled mean
KCP-1	20.92	21.50	21.21	10.87	10.83	10.85
KCP-2	23.42	23.72	23.57	13.03	13.47	13.25
BSI-1	23.02	23.58	23.30	12.57	12.75	12.66
C.D (0.05)	0.72	1.54	0.80	0.51	0.74	0.43

The data presented in table 55 showed that there was an increase in leaf length and breadth at 90 DAP. Significant difference was observed in both the leaf length and breadth among the genotypes. In both the years and the pooled mean of leaf length, genotype KCP-2 showed the highest value (23.42, 23.72, 23.57) which was on par with BSI-1. In case of leaf breadth also, genotype KCP-2 recorded highest values (13.03, 13.47, 13.25) during both the years and in the pooled mean.

4.1.2.19 Leaf length and leaf breadth in K. parviflora genotypes at 120 DAP

The data on leaf length and breadth at 120 DAP are presented in table 56.

Table 56: Leaf length and leaf breadth in *K. parviflora* genotypes at 120 DAP

Genotype	Leaf length (cm)			Leaf breadth (cm)		
	2017-18	2018-19	Pooled mean	2017-18	2018-19	Pooled mean
KCP-1	23.32	24.38	23.85	13.07	13.42	13.24
KCP-2	25.95	26.18	26.07	14.40	14.65	14.52
BSI-1	26.80	27.02	26.91	13.83	13.98	13.91
C.D (0.05)	1.52	NS	1.23	0.57	0.57	0.39

Increasing trend of both the leaf length and leaf breadth was also observed at 120 DAP. Significant difference was observed among the genotypes except 2018-19. In 2017-18 and in the pooled mean of leaf length, genotype BSI-1 recorded significantly highest value (26.80, 26.91) which was on par with KCP-2. In case of leaf breadth during both the years and in the pooled mean, genotype KCP-2 showed the highest value (14.40, 14.65, 14.52, respectively).

4.1.2.20 Leaf length and leaf breadth in K. parviflora genotypes at 150 DAP

The data on leaf length and breadth at 150 DAP are presented in table 57.

Table 57: Leaf length and leaf breadth in *K. parviflora* genotypes at 150 DAP

Genotype	Leaf length (cm)			Leaf breadth (cm)		
	2017-18	2018-19	Pooled mean	2017-18	2018-19	Pooled mean
KCP-1	25.38	25.87	25.63	13.33	13.68	13.51
KCP-2	26.28	26.52	26.40	14.80	14.98	14.89
BSI-1	26.68	27.27	26.98	14.20	14.12	14.16
C.D (0.05)	NS	NS	NS	0.77	0.54	0.44

There was no significant difference in leaf length among the genotypes during both the years and in the pooled mean. Genotype KCP-2 recorded significantly highest (14.80, 14.98, 14.89) leaf breadth in both the years and pooled mean.

4.1.2.21 Leaf length and leaf breadth in K. parviflora genotypes at 180 DAP

The data on leaf length and breadth at 180 DAP are presented in table 58.

Table 58: Leaf length and leaf breadth in *K. parviflora* genotypes at 180 DAP

Genotype	Leaf length (cm)			Leaf breadth (cm)		
	2017-18	2018-19	Pooled mean	2017-18	2018-19	Pooled mean
KCP-1	26.65	27.12	26.88	14.32	13.88	14.10
KCP-2	26.88	27.60	27.24	14.78	14.95	14.87
BSI-1	26.72	27.18	26.95	13.73	14.20	13.97
C.D (0.05)	NS	NS	NS	NS	NS	0.66

The leaf length and leaf breadth reached their peak at 180 DAP. However, no significant difference was observed in both the parameters during both the years and in the pooled mean. But, the pooled mean of leaf breadth showed significant difference which ranged from 13.97 to 14.87 cm.

4.1.2.22 Leaf area in K. parviflora genotypes at 60 DAP

The data on leaf area recorded in different genotypes of *K. parviflora* at 60 DAP are tabulated in table 59.

Table 59: Leaf area in *K. parviflora* genotypes at 60 DAP

Genotype	Leaf area (cm^2)		
	2017-18	2018-19	Pooled mean
KCP-1	77.51	78.22	77.86
KCP-2	124.19	127.04	125.62
BSI-1	130.26	130.41	130.34
C.D (0.05)	5.22	5.88	3.65

Leaf area increased at 60 DAP in all the genotypes. Significantly highest leaf area in both the years and the pooled mean was recorded in the genotype BSI-1 (130.26, 130.41 and 130.34 respectively) and lowest (77.51, 78.22 and 77.86, respectively) by the genotype KCP-1.

4.1.2.23 Leaf area in K. parviflora genotypes at 90 DAP

The data on leaf area recorded in different genotypes of *K. parviflora* at 90 DAP are given in table 60.

Table 60: Leaf area in *K. parviflora* genotypes at 90 DAP

Genotype	Leaf area (cm^2)		
	2017-18	**2018-19**	**Mean**
KCP-1	160.02	176.54	168.28
KCP-2	188.04	184.73	186.38
BSI-1	172.76	171.77	172.27
C.D (0.05)	14.90	NS	10.61

The data shown in table 60 indicated that there was increase in leaf area at 90 DAP. During 2017-18, significantly highest (188.04 cm^2) leaf area was observed in the genotype KCP-2 and lowest value (160.02 cm^2) was recorded in the genotype KCP-1. During 2018-19, no significant difference was observed. The pooled mean showed that leaf area ranged from 169.28 cm^2 to 186.38 cm^2.

4.1.2.24 Leaf area in K. parviflora genotypes at 120 DAP

The data on leaf area recorded in different genotypes of *K. parviflora* at 120 DAP are given in table 61.

Table 61: Leaf area in *K. parviflora* genotypes at 120 DAP

Genotype	Leaf area (cm^2)		
	2017-18	**2018-19**	**Mean**
KCP-1	206.45	203.43	204.94
KCP-2	247.34	246.36	246.85
BSI-1	236.17	234.77	235.47
C.D (0.05)	13.57	13.84	8.86

The data in table 61 showed an increasing trend of leaf area at 120 DAP. There was a significant variation among the genotypes during two years and the pooled mean. Highest leaf area was recorded in the genotype KCP-2 (247.34, 246.36 and 246.85) in both the years and pooled mean.

4.1.2.25 Leaf area in K. parviflora genotypes at 150 DAP

The data on leaf area recorded in different genotypes of *K. parviflora* at 150 DAP are given in table 62.

Table 62: Leaf area in *K. parviflora* genotypes at 150 DAP

Genotype	Leaf area (cm^2)		
	2017-18	**2018-19**	**Mean**
KCP-1	207.12	204.73	205.92
KCP-2	248.01	238.44	243.23
BSI-1	227.74	221.54	224.64
C.D (0.05)	13.66	13.66	9.27

The leaf area at 150 DAP ranged from 205.92 cm^2 to 243.23 cm^2 in the pooled mean. A significant difference was observed among the genotypes. In both the years and pooled mean, significantly highest (248.01, 238.44, 243.23 cm^2 respectively) leaf area was recorded in the genotype KCP-2 and least was recorded in the genotype KCP-1.

4.1.2.26 Leaf area in K. parviflora genotypes at 180 DAP

The data on leaf area recorded in different genotypes of *K. parviflora* at 180 DAP are given in table 63.

Table 63: Leaf area in *K. parviflora* genotypes at 180 DAP

Genotype	Leaf area (cm^2)		
	2017-18	**2018-19**	**Mean**
KCP-1	206.66	203.77	205.21
KCP-2	252.49	246.36	249.42
BSI-1	231.46	234.77	233.11
C.D (0.05)	12.54	13.77	8.69

At 180 DAP, leaf area ranged from 205.21 cm^2 in KCP-2 to 249.42 cm^2 in BSI-1 in the pooled mean. A significant difference was observed among the genotypes.

4.1.2.27 Fresh weight and dry weight of leaves per plant in K. parviflora genotypes

The data on fresh as well as dry weight of leaves per plant recorded at 180 DAP in the genotypes are given in table 64.

Table 64: Fresh weight and dry weight of leaves per plant in *K. parviflora* genotypes

Genotype	Fresh weight of leaves (g/plant)			Dry weight of leaves (g/plant)		
	2017-18	2018-19	Pooled mean	2017-18	2018-19	Pooled mean
KCP-1	121.17	118.67	119.92	9.30	8.96	9.13
KCP-2	122.33	122.83	122.58	9.07	13.40	11.23
BSI-1	104.33	104.33	104.33	6.99	8.16	7.58
C.D (0.05)	12.42	11.65	7.78	0.86	2.26	1.57

There was a significant difference among the genotypes during both the years and in the pooled mean. The genotype KCP-2 showed significantly highest (122.58 g) fresh weight of leaves which was on par with KCP-1 and lowest value was recorded in the genotype BSI-1 during both the years and in the pooled mean. A significant variation was noticed among the genotypes in pooled mean in case of dry weight of leaves per plant also. Significantly highest value was recorded in the genotype KCP-2 during both the years and in the pooled mean. The dry weight of leaves ranged from 7.58 g to 11.23 g.

4.1.2.28 Plant height in K. parviflora genotypes

The data on plant height in *K. parviflora* genotypes recorded at 180 DAP are presented in table 65.

Table 65: Plant height in *K. parviflora* genotypes at 180 DAP

Genotype	Plant height (cm)		
	2017-18	2018-19	Pooled mean
KCP-1	48.00	52.90	50.45
KCP-2	54.67	53.50	54.08
BSI-1	49.00	53.50	51.25
C.D (0.05)	5.10	NS	NS

The genotype KCP-2 showed significantly higher plant height during 2017-18. No significant difference was noticed in plant height among the genotypes during 2018-19 and the pooled mean.

4.1.2.29 Length and girth of rhizomes in K. parviflora genotypes

The data on length and girth of rhizomes recorded in the genotypes at harvest are presented in the table 66.

Table 66: Length and girth of rhizomes in *K. parviflora* genotypes

Genotype	Length of rhizome (cm)			Girth of rhizome (cm)		
	2017-18	2018-19	Pooled mean	2017-18	2018-19	Pooled mean
KCP-1	10.90	9.32	10.11	2.32	2.14	2.23
KCP-2	10.42	10.92	10.67	2.57	2.27	2.42
BSI-1	10.75	9.15	9.95	2.37	2.15	2.26
C.D (0.05)	NS	1.46	NS	NS	NS	NS

The data showed that there was no significant difference in the length as well as in the girth of rhizomes among the genotypes and the pooled mean, except 2018-19 where the genotype KCP-2 recorded the significantly highest length of rhizome.

4.1.2.30 Fresh and dry yield of rhizomes in K. parviflora genotypes

The data on fresh yield of rhizomes of *K. parviflora* genotypes are depicted in table 67.

Table 67: Fresh and dry yield of rhizome in *K. parviflora* genotypes

Genotype	Fresh yield (g/plant)			Dry yield (g/plant)		
	2017-18	2018-19	Pooled mean	2017-18	2018-19	Pooled mean
KCP-1	109.92	119.29	114.60	36.14	35.66	35.90
KCP-2	62.15	80.57	71.36	27.02	33.35	30.18
BSI-1	82.23	98.62	90.43	34.05	36.21	35.13
C.D (0.05)	7.57	11.58	9.12	5.83	NS	4.63

A significant variation was observed among the genotypes during both the years and in the pooled mean in both fresh and dry weight of rhizome except 2018-19 in dry yield. Significantly highest (109.92 g, 119.29 g, 114.60 g) fresh weight in both the years and the pooled mean was recorded by the genotype KCP-1. The dry yield of rhizome showed no significant variation among the genotypes during 2018-19. A significant variation was observed among the genotypes during 2017-18 and in the pooled mean, highest value (36.14 g, 35.90 g) was recorded by genotype KCP-1 which was on par with BSI-1.

4.1.2.31 Driage of rhizomes in K. parviflora genotypes

The data on the driage of rhizomes of *K. parviflora* genotypes are given in table 68.

Table 68: Driage in *K. parviflora* genotypes

Genotype	Driage (%)		
	2017-18	2018-19	Pooled mean
KCP-1	32.87	30.15	31.51
KCP-2	43.32	40.98	42.15
BSI-1	41.64	37.00	39.32
C.D (0.05)	7.13	7.20	4.84

The driage ranged from 31.51 to 42.15 and it was significant among the genotypes in both the years and pooled mean. During both the years and pooled mean, significantly highest (43.32 %, 40.98 %, 42.15 %) drying per cent was noticed in the genotype KCP-1 and it was on par with BSI-1.

4.1.2.32 Length and girth of root tubers in K. parviflora genotypes

The data on the length and girth of root tubers recorded in the genotypes at harvest is presented in the table 69.

Table 69: Length and girth of root tubers in *K. parviflora* genotypes

Genotype	Length of root tubers (cm)			Girth of root tubers (cm)		
	2017-18	2018-19	Pooled mean	2017-18	2018-19	Pooled mean
KCP-1	15.87	14.63	15.25	0.76	0.76	0.76
KCP-2	15.92	13.72	14.82	0.95	0.76	0.86
BSI-1	13.72	14.30	14.01	0.84	0.67	0.76
C.D (0.05)	NS	NS	NS	0.12	NS	NS

There was no significant variation among the genotypes during both the years and in the pooled mean, for both the length and girth of root tubers except during 2017-18, where genotype KCP-2 recorded significantly highest root tuber girth which was on par with BSI-1.

4.1.2.33 Number of root tubers in K. parviflora genotypes

The data on number of root tubers arising from rhizome are presented in table 70.

Table 70: Number of root tubers in *K. parviflora* genotypes

Genotype	Number of root tubers		
	2017-18	2018-19	Mean
KCP-1	17.67	9.67	**13.67**
KCP-2	16.17	6.00	11.08
BSI-1	13.50	3.67	8.58
C.D (0.05)	3.23	1.44	NS

There was significant difference among genotypes during both the years. In both the years KCP-1 showed significantly highest number (17.67, 9.67) of root tubers.

4.1.2.34 Fresh and dry weight of root tubers in K. parviflora genotypes

The data on fresh and dry weight of root tubers are presented in table 71.

Table 71: Fresh and dry weight of root tubers in *K. parviflora* genotypes

Genotype	Fresh yield (g/plant)			Dry yield (g/plant)		
	2017-18	2018-19	Pooled mean	2017-18	2018-19	Pooled mean
KCP-1	33.13	13.33	23.23	6.35	2.59	4.47
KCP-2	26.17	8.13	17.15	3.97	1.26	2.61
BSI-1	20.38	5.04	12.71	3.12	0.77	1.94
C.D (0.05)	3.97	0.93	7.95	0.46	0.35	1.33

The data on fresh weight and dry weight of root tubers showed significant difference during both the years and in the pooled mean. Genotype KCP-1 showed significantly highest (33.13 g, 13.33 g, 23.23 g) fresh weight of root tubers.

In case of dry weight of root tubers also, during both the years and pooled mean, KCP-1 showed significantly highest (6.35 g, 2.59 g and 4.47 g, respectively) dry weight of root tubers.

4.1.2.35 Biological yield in K. parviflora genotypes

The data on biological yield of the genotypes are given in table 72.

Table 72: Biological yield in *K. parviflora* genotypes

Genotype	Biological yield (g/plant)		
	2017-18	2018-19	Mean
KCP-1	49.72	47.64	48.68
KCP-2	39.57	49.32	44.44
BSI-1	44.25	46.78	45.51
C.D (0.05)	7.98	NS	NS

The data on biological yield indicated that there was significant difference among the genotypes during 2017-18 where KCP-1 showed significantly highest (49.72 g) biological yield which was on par with BSI-1. In both 2018-19 and pooled mean, no significant difference was noticed in biological yield.

4.1.2.36 Harvest index in K. parviflora genotypes

The data on harvest index calculated in *K. parviflora* genotypes are depicted in table 73.

Table 73: Harvest index in *K. parviflora* genotypes

Genotype	Harvest index (%)		
	2017-18	2018-19	Mean
KCP-1	72.63	74.82	**73.73**
KCP-2	67.85	67.06	67.45
BSI-1	76.58	76.96	**76.77**
C.D (0.05)	4.41	6.65	3.68

There was a significant difference among the genotypes during both the years and the pooled mean. During both the years and in the pooled mean, genotype BSI-1 showed significantly highest (76.58, 76.96, 76.77) harvest index which was on par with KCP-1.

4.1.3 Vivipary in *K. parviflora*

The phenomenon of vivipary was noticed in *K. parviflora* in all the accessions. The observations were recorded on various aspects of vivipary. The viviparous seedlings were observed for growth and yield parameters for two years. As a confirmatory test, recalcitrance of the seed was also examined.

4.1.3.1 Recalcitrance of seeds

Table 74: Test for recalcitrance in *K. parviflora* seeds

Parameter	Dried seeds	Fresh seeds
Number of seeds tested	20	20
No. of germinated seeds	0	11
% germination	0	55

Germination was noticed only in fresh seeds from 4th day onwards. Fifty five percent germination was noticed in freshly harvested seeds, whereas there was no germination at all in the dried seeds of *K. parviflora* (Table 74 and plate 5). Development of viviparous seedling from the inflorescence is depicted in plate 6.

4.1.3.2 Characters of viviparous seedlings

The data on days to seed set, number of seeds per inflorescence and viviparous seedlings produced per plant are given in the table 75.

Table 75: Characters of viviparous seedlings

Parameter	Mean value
No. of seeds per inflorescence	3
Days to seed set	22.5
No. of viviparous seedlings/plants	2-3

The data in table 75 showed that average number of seeds per inflorescence was three. On an average a plant produced 2-3 viviparous seedlings.

4.1.3.3 Growth characters of viviparous seedlings

The viviparous seedlings were transplanted to grow bags and growth parameters of transplanted viviparous seedlings were observed and recorded which are presented in the table 76.

Table 76: Growth characters of viviparous seedlings of *K. parviflora*

Parameter	30DAT	60 DAT	90 DAT	120 DAT	150 DAT
No. of leaves	3	4.7	7.3	9.8	9.1
No. of tillers	0	0	1.9	2.7	2.1
Leaf length (cm)	2.6	7.4	13.5	15.3	14.2
Leaf breadth (cm)	1.4	3.0	5.1	6.2	5.7
Leaf area (cm^2)	3.09	14.93	24.66	37.39	35.51

DAT-Days after transplanting

Data given in the table 76 showed that leaf production increased from 30 DAT (3) to 120 DAT (9.8) after which it declined.

There was no tiller production upto 60 DAT. Number of tillers was highest (2.7) at 120 DAT.

Leaf length, leaf breadth and leaf area increased upto 120 DAT after which all the parameters recorded declining values. Highest leaf length (15.3 cm), leaf breadth (6.2 cm) and leaf area (37.39 cm^2) was observed at 120 DAT.

Plate 4: Vivipary in *K. parviflora*

Plate 5: Recalcitrance test in *K. parviflora* seeds

Plate 6: Development of Viviparous plant

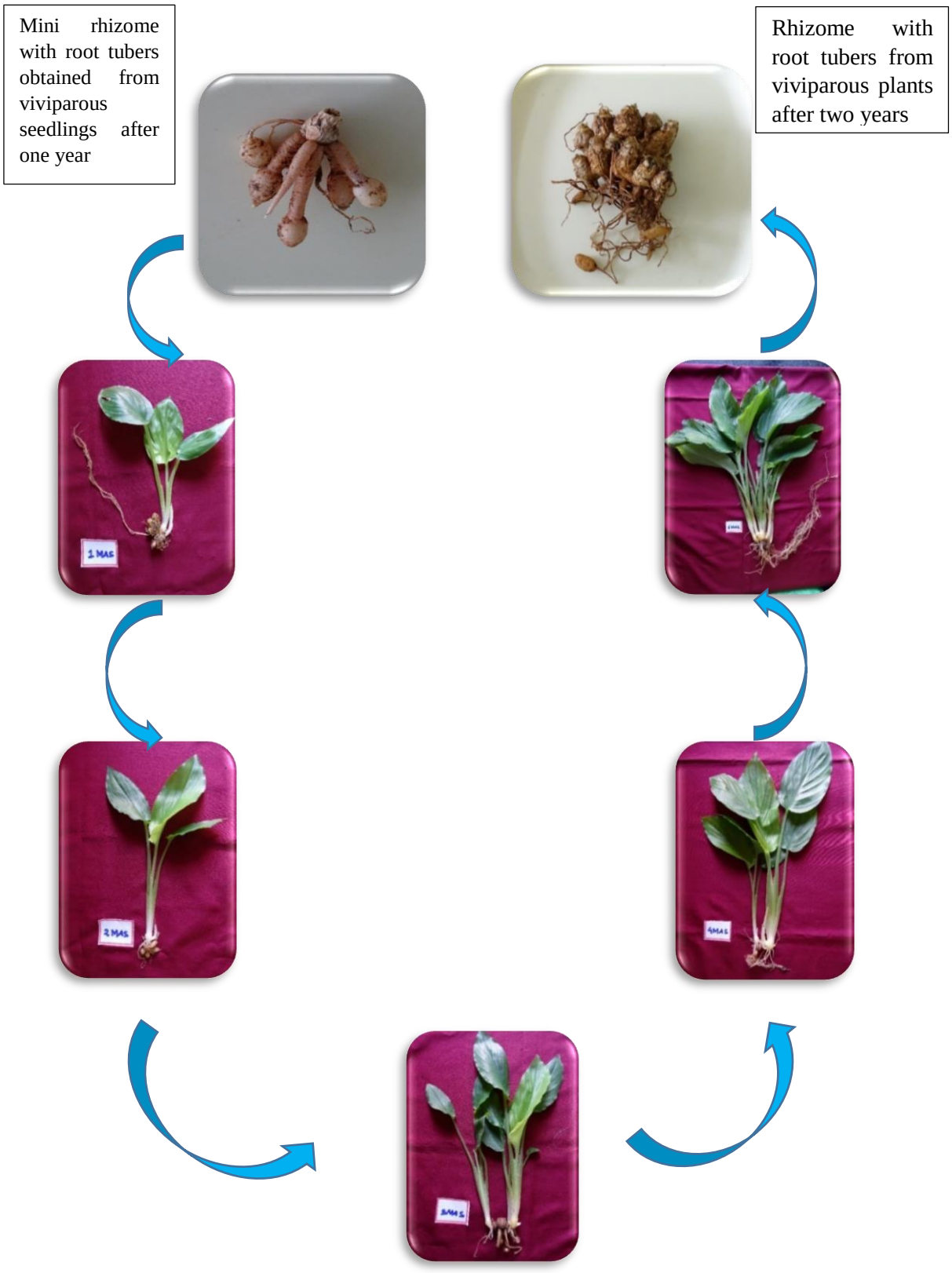


Plate 7: Growth and development of viviparous plants

Plate 8 (a): Comparison of viviparous and rhizome borne plants

Plate 8 (b): Comparison of the rhizome of viviparous and rhizome borne

4.1.3.4 Rhizome characters of viviparous seedlings

The average values of rhizome characters of viviparous seedlings are given in table 77.

Table 77: Rhizome characters of viviparous seedlings of *K. parviflora*

Parameter	Mean value
No. of root tubers	10.4
Length of tuber (mm)	4.21
Girth of tuber (mm)	4.36
Fresh weight of tuber (g)	1.86
Length of rhizome (mm)	20.18
Girth of rhizome (mm)	15.80
Fresh weight of rhizome (g)	1.41

The data showed that the average number of root tubers produced by viviparous seedling was 10.4 (Table 77). The root tuber dimensions were: length 4.21 mm, girth 4.36mm and fresh weight 1.86 g. At the end of the season, the rhizome was too small with a length of 20.18 mm, girth of 15.80 mm and fresh weight of 1.41 g.

4.1.3.5 Comparison of viviparous and normal plants of K. parviflora

The rhizome harvested from the viviparous seedling was planted in next season along with the normal rhizome as check and comparison was made between the viviparous plants and normal rhizome borne plants (Table 78-79).

Days to sprout varied significantly between the two types. Normal plants sprouted early (16.7) compared to the viviparous plants (28.5).

Number of leaves was significantly higher in the viviparous plants upto 150 DAP, it was on par at 180 DAP, while at 210 DAP, normal plants showed significantly higher leaf production. Leaf production at 30 DAP for viviparous plant was 3.3, while normal plants produced only single leaf. At 210 DAP, viviparous plants had 9.4 leaves, whereas the normal plants had 13.1 leaves.

Table 78: Comparison of viviparous and normal plants of *K. parviflora*

Parameter	Type	30 DAP	60 DAP	90 DAP	120 DAP	150 DAP	180 DAP	210 DAP
Days to sprout	Viviparous	28.5[a]						
	Normal (Check)	16.7						
No. of leaves	Viviparous	3.3[a]	5.7[a]	8.1[a]	12.9[a]	13.8[a]	16.5	9.4
	Normal	1.0	1.9	5.2	10	9.9	15.5	13.1[a]
No. of tillers	Viviparous	0	1.6	3.9[a]	3.6	5.0[a]	6.4[a]	4.3[a]
	Normal	0	1.2	2.7	3.1	3.6	4.7	3.3
Leaf length (cm)	Viviparous	7.75	9.96	17.53	19.72	20.93	22.13	20.91
	Normal	15.61[a]	19.22[a]	22.49[a]	25.02[a]	26.06[a]	26.73[a]	24.78[a]
Leaf breadth (cm)	Viviparous	3.61	4.58	10.63	13.29	13.64	15.38[a]	15.22
	Normal	9.23[a]	10.23[a]	12.40[a]	14.00	14.21	14.40	16.19[a]
Leaf area (cm^2)	Viviparous	21.13	36.76	48.57	76.32	87.80	112.91	109.97
	Normal	52.51[a]	118.06[a]	124.93[a]	220.77[a]	220.42[a]	224.62[a]	221.64[a]
Height of plant	Viviparous	-	-	-	-	-	44.4	-
	Normal	-	-	-	-	-	50.34[a]	-
Fresh weight of leaves (g)	Viviparous	-	-	-	-	-	294.39[a]	-
	Normal	-	-	-	-	-	121.6	-
Dry weight of leaves (g)	Viviparous	-	-	-	-	-	10.92	-
	Normal	-	-	-	-	-	10.26	-

[a] indicates significant difference between viviparous and normal plants for the given parameter. (t test, p=0.05, n=10)

With respect to tiller production, at 60 DAP and 120 DAP, number of tillers showed no significant difference between the two types whereas it was significantly higher in viviparous plants at 90, 150, 180 and 210 DAP. Highest tiller production was observed at 180 DAP in both the types with values 6.4 and 4.7, respectively and there after it declined.

Leaf length and leaf area were significantly lower in viviparous plants when compared with normal plants at all the growth periods. The peak was observed at 180 DAP with values of 22.13 cm, 26.73 cm for leaf length and 112.91 cm^2, 224.62 cm^2 for leaf area, respectively. The plant height was significantly higher in normal plants.

In case of leaf breadth, normal plant recorded significantly higher values at 30 DAP (9.23cm), 60 DAP (10.23 cm), 90 DAP (12.40 cm) and 210 DAP (16.19 cm). There was no significant difference between the two types at 120 DAP and 150 DAP. At 180 DAP, viviparous plants showed significantly higher (15.22 cm) leaf breadth.

Fresh weight of leaves was significantly higher (294.39 g) in viviparous plants, while there was no significant difference in dry weight of leaves.

4.1.3.6 Yield parameters of viviparous and normal plants of K. parviflora

Table 79: Yield parameters of viviparous and normal plants of *K. parviflora*

Yield parameter	Viviparous plants	Normal plants
Length of rhizome (cm)	7.68	10.64[a]
Girth of rhizome (mm)	19.70	22.46[a]
Number of tubers	5.4	8.1[a]
Length of tuber (cm)	11.44	13.44
Girth of tuber (mm)	8.00	7.74
Fresh weight of rhizome (g)	30.05	102.06[a]
Dry weight of rhizome (g)	8.20	34.26[a]

[a] indicates significant difference between viviparous plants and normal plants in the given parameters. (t test, p=0.05, n=10)

The data on yield parameters of viviparous and normal plants of *Kaempferia parviflora* are furnished in table 79.

All the rhizome characters recorded significantly higher values in the normal plants. They recorded a rhizome length of 10.64 cm, girth of 22.46 mm, fresh weight of 102.06 g and dry weight of 34.26 g.

With respect to tuber characters, there was not much difference noticed between the two types of plants. However, tuber number was higher in normal plants.

4.1.4 Morphological observations of *K. galanga*

Kaempferia galanga was used as a reference species in the study. There were two genotypes, one each from Arunachal Pradesh and Kerala

4.1.4.1 Qualitative parameters

Qualitative parameters of *K. galanga* is given in table 80.

Rhizome of *K. galanga* are dark reddish brown in colour with pearl white inner core. Rhizome bears tuberous roots (Plate 11). Dark green leaves are round ovate with acute tip and entire margin (Plate 9).

Table 80: Qualitative parameters in *K. galanga*

Parameter	Observation
Rhizome colour :Scale Inner core	Dark reddish brown (RHS N199C) Pearl white
Rhizome shape	Globus
Presence of root tubers	Present
Colour of root tubers	Creamy white (RHS)
Mature leaf shape	Round ovate
Mature leaf colour	Dark green
Leaf tip shape	Acute
Leaf margin	Entire
Growth habit	Sprawling

4.1.4.2 Growth parameters in K. galanga genotypes

Growth parameters of *K. galanga* are given in the table 81.

Table 81: Growth parameters in *K. galanga* genotypes

Parameter	Year	Genotypes	Mean
Days to sprout	2016-17	KCG-1	2.5
		ArPCG-1	2.8
	2018-19	KCG-1	2.67
		ArPCG-1	2.67
Plant spread (NS) (cm)	2016-17	KCG-1	28.50
		ArPCG-1	30.83
	2018-19	KCG-1	30.67
		ArPCG-1	32.17
Plant spread (EW) (cm)	2016-17	KCG-1	23.67
		ArPCG-1	29.16[a]
	2018-19	KCG-1	23.83
		ArPCG-1	28.50[a]
Fresh weight of leaves (g/plant)	2016-17	KCG-1	96.17[a]
		ArPCG-1	60.00
	2018-19	KCG-1	91.0[a]
		ArPCG-1	62.33
Dry weight of leaves (g/plant)	2016-17	KCG-1	7.33[a]
		ArPCG-1	4.06
	2018-19	KCG-1	8.71[a]
		ArPCG-1	5.14

[a] indicates significant difference between genotypes in the given parameters. (t test, p=0.05, n=10)

The days to sprout and plant spread (NS) did not show any significant difference during both the years and between genotypes. Whereas, during both years, ArPCG-1 showed significantly higher (29.16, 28.50) E-W spread.

The fresh and dry weight of leaves were significantly higher in the genotype KCG when compared with ArPCG-1 in both the years. Fresh weight of leaves for KCG was 96.17g and 91.0 g, respectively during 2017-18 and 2018-19. Dry weight of leaves in KCG-1 was 7.33g and 8.71g, respectively during 2017-18 and 2018-19.

4.1.4.3 Growth parameters of K. galanga genotypes at monthly intervals

The data on various growth parameters of *K. galanga* measured at monthly intervals is presented in the table 82.

The tiller production increased upto 180 DAP and thereafter it declined in both the genotypes during two years. No significant difference was observed between genotypes upto 60 DAP. Genotype ArPCG-1 showed significantly higher tiller production at 90 DAP (4.00, 4.16), 120 DAP (3.33, 4.50), 150 DAP (4.83, 5.83), 180 DAP (6.17, 6.67), 201 DAP (5.33, 5.57) respectively during two years.

The leaf production reached peak at 180 DAP in both the genotypes and two years. During 2017-18, there was no significant difference between the genotypes upto 60 DAP, while from 90 DAP onwards ArPCG-1 produced significantly higher number of leaves with values at 90 DAP (6.50), 120 DAP (11.0), 150 DAP (13.67), 180 DAP (16.17), and at 210 DAP (13.5). During 2018-19, ArPCG-1 produced significantly higher number of leaves when compared with KCG-1 in all the growth periods except 60 DAP which showed no significant difference.

In case of leaf length, genotype KCG-1 showed significantly higher leaf length at 60 DAP (16.55 cm), 180 DAP (23.28 cm) and 210 DAP (24.18 cm) during 2017-18. During 2018-19, ArPCG-1 showed significantly higher (13.63 cm) leaf length at 30 DAP, while KCG showed significantly higher value at 60 DAP (16.25 cm), 150 DAP (22.43 cm), 180 DAP (24.47 cm) and 210 DAP (23.73 cm).

With respect to leaf breadth, no significant difference was observed at 30 DAP between the genotypes and two years. Genotype KCG-1 showed significantly higher leaf breadth from 60 DAP to 210 DAP during 2017-18. During 2018-19, no significance difference was observed at 150 DAP, while KCG showed significantly

higher value at 60 DAP (8.22 cm), 90 DAP (9.58 cm), 120 DAP (12.47), 180 DAP (13.48 cm) and 210 DAP (13.60 cm).

Leaf area reached peak at 210 DAP. During 2017-18, no significant difference was observed upto 60 DAP, thereafter genotype KCG-1 showed significantly higher leaf area. Genotype KCG-1 showed significantly higher leaf area in all the growing periods during 2018-19.

4.1.4.4 Rhizome characters in K. galanga genotypes

The data in table 83 indicate the rhizome characters of two *K. galanga* genotypes.

Length of rhizome was slightly higher in the genotype KCG-1 even though no significant difference was observed between the genotypes. In case of girth of rhizome, genotype KCG-1 recorded significantly higher values (2.13 cm, 2.07 cm) during both the years.

Number of tubers was significantly higher in the genotype KCG-1 when compared with ArPCG-1 during both the years with mean value of 28.67 and 23.50 respectively. Length of tubers showed no significant difference between the genotypes during 2017-18, whereas during 2018-19 genotype KCG-1 had significantly higher (12.86 cm) tuber length. In case of girth of root tuber, genotype KCG-1 recorded significantly higher value (0.91 cm, 0.85 cm) when compared with ArPCG-1 during both the years.

Fresh and dry yield of rhizome showed no significant difference between the genotypes in both the years. The highest fresh yield was 46.84 g during 2017-18 and 55.23 g during 2018-19. The same trend was observed in dry yield of rhizome also. Driage of KCG-1 was 45.55 and 41.12 per cent, while that of ArPCG-1 was 38.66 and 38.30 per cent, respectively during 2017-18 and 2018-19.

Table 82: Growth parameters of *K. galanga* genotypes

Parameter	Year	Genotype	30 DAP	60 DAP	90 DAP	120 DAP	150 DAP	180 DAP	210 DAP
No. of tillers/plant	2016-17	KCG-1	0.67	0.50	1.50	1.83	3.00	4.17	3.50
		ArPCG-1	0.83	0.67	4.00[a]	3.33[a]	4.83[a]	6.17[a]	5.33[a]
	2018-19	KCG-1	0.50	0.50	1.67	2.67	4.00	5.00	4.17
		ArPCG-1	0.67	0.67	4.16[a]	4.50[a]	5.83[a]	6.67[a]	5.57[a]
No. of leaves	2016-17	KCG-1	2.00	2.50	5.00	6.33	7.17	6.83	4.83
		ArPCG-1	3.00	2.83	6.50[a]	11.0[a]	13.67[a]	16.17[a]	13.5[a]
	2018-19	KCG-1	1.83	2.67	5.50	6.83	7.00	8.17	5.50
		ArPCG-1	2.66[a]	2.67	6.50[a]	11.3[a]	13.67[a]	16.00[a]	13.0[a]
Leaf length (cm)	2016-17	KCG-1	11.85	16.55[a]	19.78	21.38	22.40	23.28[a]	24.18[a]
		ArPCG-1	13.25	14.58	19.87	20.70	22.00	20.17	21.20
	2018-19	KCG-1	11.55	16.25[a]	20.12	21.68	22.43[a]	24.47[a]	23.73[a]
		ArPCG-1	13.63[a]	14.98	20.25	20.97	20.25	20.53	20.02
Leaf breadth (cm)	2016-17	KCG-1	5.26	7.77[a]	8.78[a]	12.98[a]	13.28[a]	13.35[a]	12.97[a]
		ArPCG-1	5.12	5.75	6.02	9.68	9.95	9.45	10.03
	2018-19	KCG-1	5.27	8.22[a]	9.58[a]	12.47[a]	12.22	13.48[a]	13.60[a]
		KCG-1	5.12	6.42	6.07	9.32	9.55	9.20	10.15
Leaf area (cm^2)	2016-17	ArPCG-1	39.12	79.47	131.7[a]	141.05[a]	144.27[a]	144.03[a]	147.27[a]
		KCG-1	32.0	76.86	105.78	130.22	127.33	131.76	132.71
	2018-19	ArPCG-1	37.27[a]	81.80[a]	136.76[a]	147.27[a]	144.26[a]	147.27[a]	145.28[a]
		KCG-1	30.36	75.19	105.29	130.89	126.97	131.89	131.28

[a] indicates significant difference between genotypes in the given parameters. (t test, p=0.05, n=10)

Table 83: Rhizome characters in *K. galanga* genotypes

Parameter	Year	Genotype	Mean
Length of rhizome (cm)	2016-17	KCG-1	8.87
		ArPCG-1	7.88
	2018-19	KCG-1	8.33
		ArPCG-1	7.90
Girth of rhizome (cm)	2016-17	KCG-1	2.13[a]
		ArPCG-1	1.49
	2018-19	KCG-1	2.07[a]
		ArPCG-1	1.62
No. of root tuber	2016-17	KCG-1	28.67[a]
		ArPCG-1	14.40
	2018-19	KCG-1	23.50[a]
		ArPCG-1	11.67
Length of root tuber (cm)	2016-17	KCG-1	13.78
		ArPCG-1	15.08
	2018-19	KCG-1	12.86[a]
		ArPCG-1	7.73
Girth of root tuber (cm)	2016-17	KCG-1	0.91[a]
		ArPCG-1	0.74
	2018-19	KCG-1	0.85[a]
		ArPCG-1	0.72
Fresh yield of rhizome (g/plant)	2016-17	KCG-1	46.12
		ArPCG-1	46.84
	2018-19	KCG-1	53.70
		ArPCG-1	55.23
Dry yield of rhizome (g/plant)	2016-17	KCG-1	21.45
		ArPCG-1	22.27
	2018-19	KCG-1	20.83
		ArPCG-1	25.33
Driage (%)	2016-17	KCG-1	45.55
		ArPCG-1	38.66
	2018-19	KCG-1	41.12
		ArPCG-1	38.30

[a] indicates significant difference between genotypes in the given parameters. (t test, p=0.05, n=10)

Plate 9: Full grown plants of (A) *K. rotunda*, (B) *K. parviflora* and (C) *K. galanga*

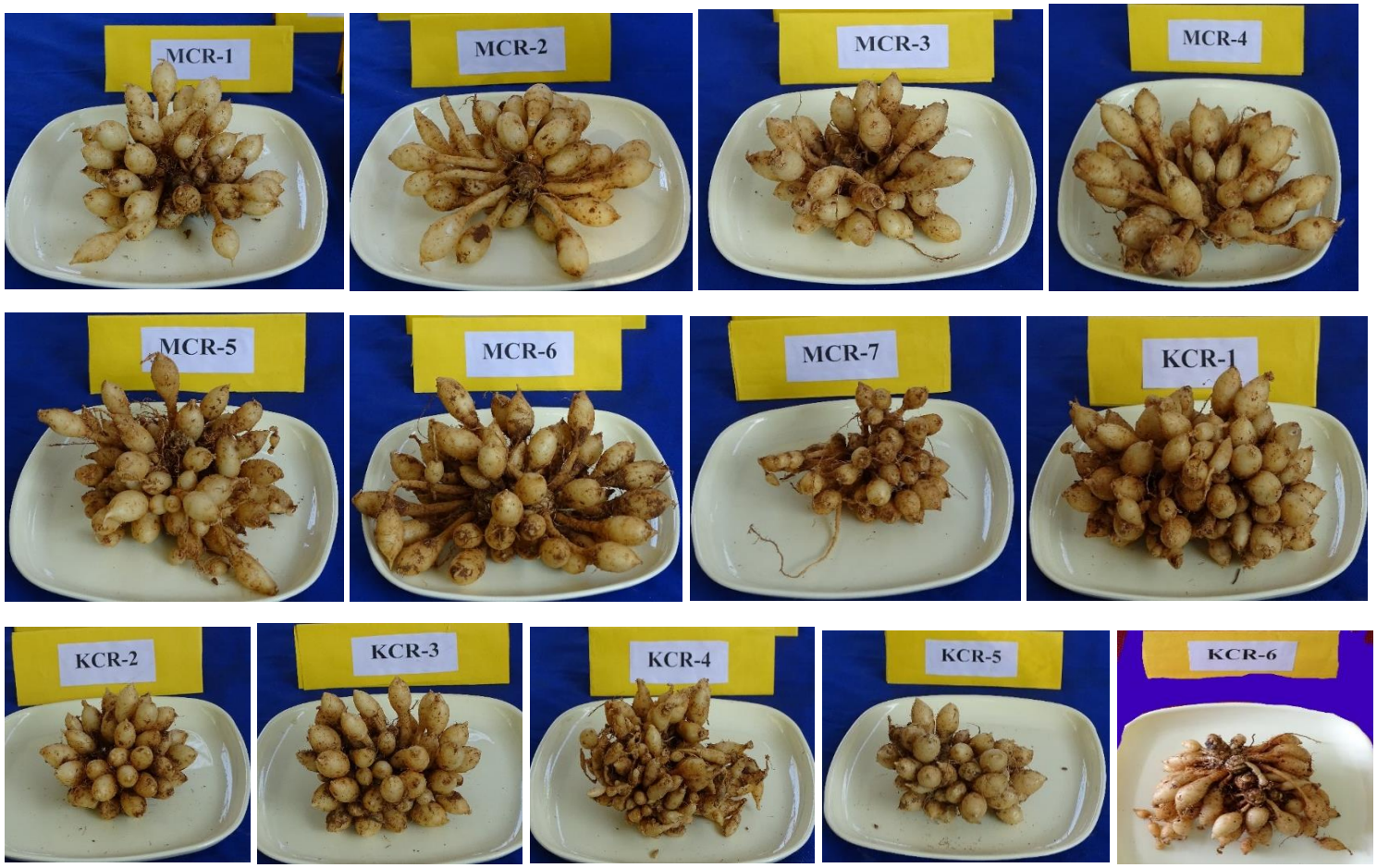


Plate 10 : *Kaempferia rotunda* rhizome with root tubers

Plate 11: Rhizome with root tubers of *Kaempferia* species

4.2 FLORAL BIOLOGY OF *Kaempferia* SPECIES

4.2.1 Flowering and floral parameters of *Kaempferia* species

The season of flowering, type of inflorescence and flower colour of the three Kaempferia species is depicted in table 84.

Table 84: Flowering and floral parameters of *Kaempferia* species

Parameter	*K. parviflora*	*K. rotunda*	*K. galanga*
Season of flowering	Last week of May to second week of November	First week of March to last week of April	First week of June to first week of July
Type of inflorescence	Scape	Scape	Scape
Flower size	Small	Large	Medium
Colour of sepal	Greenish white	White (RHSCF1A)	White (RHSCF1A)
Colour of petal	Purple blue with white margin labellum (RHSCF N81A)	Purple blue with white margin labellum (RHSCF N80A)	White with Purple blue in the middle (RHSCF N74A)
Colour of stamen	Off-white	Light yellow	Off-white
Colour of anther	Off-white	Light yellow	Off-white

*RHS-Royal Horticulture Society Colour Chart

The flowering was observed from last week of May to second week of November in *K. parviflora*. *K. rotunda* started flowering by first week of March which continued upto last week of April. *K. galanga* flowered for one month only *i.e.* from first week of June to first week of July.

All the three *Kaempferia* species possessed scape type of inflorescence. Colour of sepal was greenish white in *K. parviflora*, while it was white for *K. rotunda* and *K. galanga*.

Two out of three petals were white colour in all the three species. Labellum, the conspicuous part had different shades of purple blue colour in the three species.

K. parviflora had purple blue colour (RHSCF N81A) labellum with white margins, while *K. rotunda* had purple blue colour (RHSCF N80A) labellum with white margins. In case of *K. galanga*, labellum was white with Purple blue (RHSCF N74A) in the middle. Colour of stamen and anther was off-white for *K. parviflora* and *K. galanga* and light yellow for *K. rotunda*.

4.2.2 Floral morphology

Comprehensive floral morphology of *K. parviflora*, *K. rotunda* and *K. galanga* is given in Figure 30-32. Floral morphology of the three species did not vary much. The inflorescence of *Kaempferia* species is a scape directly arising from the rhizome. Floral parts are large in *K. rotunda*, medium in *K. galanga* and small in *K. parviflora*. Flowers are bisexual, complete, trimerous and zygomorphic in all the species. Perianth is connate at the base, forming a long tube and free at the apex. The floral parts are arranged on the trimerous ground plan of the monocotyledons. The five whorls of flora parts alternate with one another in *K. parviflora* and *K. galanga*, while two petals overlapped in *K. rotunda*. The stamen are arranged in two whorls. The outer whorl of stamens is represented by two staminoides in *K. parviflora* and *K. galanga* and two to four in *K. rotunda*, which are situated at the base of tubular perianth. The posterior stamen of the inner whorl is the only fertile one in all the species. The other two stamens are united and form a large bilobed showy labellum, which is the most conspicuous part of the flower. The fertile stamen is bilobed and the connective tissue forms a hood above the stamen. The anther lobe forms a groove through which style passes so that the stigma comes very close to the anther. Gynoecium consists of tricarpellary syncarpous inferior ovary with ovules arranged in axile placentation. Style is long and ends in a papillae stigma. Papillae of *K. parviflora* was 0.07 mm and for that of *K. rotunda* was 0.13 mm and *K. galanga* was 0.06 mm.

4.2.3 Floral biology and pollen morphology of *Kaempferia* species

Floral biology of three species of *Kaempferia* is presented in table 85.

Table 85: Floral biology and pollen morphology of three *Kaempferia* species

Parameter	*K. parviflora*	*K. rotunda*	*K. galanga*
No. of flowers/plant	14.2	8.9	5.6
Anthesis (am)	5.00-7.15	3.00-5.00	4.00-5.00
Anther dehiscence (am)	5:15-6:30	4:30-5.30	4.30-5.00
Stamen length (mm)	1.84	8.28	2.41
Length of pollen (µm)	176.25	149.94	116.38
Diameter of pollen (µm)	146.83	140.67	107.66
Pollen fertility (%)	85.18	94.24	90.71
Stigma receptivity (hrs.)	8	24	9
Style length (cm)	6.19	7.72	4.6
No. of ovules	12.4	15	8
Length of ovule (mm)	0.40	4.15	0.56
Diameter of ovule (mm)	0.28	2.94	0.44
Days to seed set	22.5	-	-

The mean number of flowers per plant was 14.2 in *K. parviflora*, 8.9 in *K. rotunda* and 5.6 in *K. galanga*.

The time of anthesis in *K. parviflora* was 5.00 am to 7.15 am. *K. rotunda* flowers started splitting at 3 am, while peak anthesis was at 4.00 to 5.00 am. In case of *K. galanga*, peak anthesis time was 4.00 to 5.00 am.

The pollen grain of *K. parviflora* was spherical to spherical elongated shape, slightly heterogeneous, off white in colour with average size of 176.25^{x}146.83 µm. In case of *K. rotunda*, pollen grains were spherical, homogenous and yellowish in colour with average size of 149.94^{x}140.67 µm. Pollen grain of *K. galanga* were spherical, homogenous size and white in colour with size of 116.38^{x}107.66 µm.

Pollen fertility assessed using acetocarmine stain showed that it was 85.18 per cent for *K. parviflora*, 94.24 per cent for *K. rotunda* and 90.71 per cent for *K. galanga*.

Stigma was receptive upto 8 hours after the anthesis in *K. parviflora* whereas *K. rotunda* and *K. galanga* remained receptive for 24 hours and 9 hours, respectively.

The style was long with 6.19 cm in *K. parviflora*, 7.72 cm in *K. rotunda* and 4.6 cm in *K. galanga*.

The average number of ovules recorded per ovary was highest for *K. rotunda* with value 15 while it was 12.4 for *K. parviflora* and 8 for *K galanga*.

Microscopic measurement of ovule showed an average length of 0.40 mm, 4.15 mm and 0.56 mm respectively in *K. parviflora*, *K. rotunda* and *K. galanga*, while average diameter of ovule was 0.28 mm, 2.94 mm and 0.44 mm, respectively for the three species.

Kaempferia parviflora produced seeds 22.5 days after flowering, while no seed set was noticed in both *K. galanga* and *K. rotunda*.

4.2.4 *In vivo* stigmatic pollination

Gynoecium after *in vivo* stigmatic pollination was observed for pollen germination and pollen tube growth under fluorescence microscope and images are presented in Plate 28. In *K. parviflora*, germinated pollen tube reached ovule 12 hours after pollination (Plate 39 CV). Pollen germination occurred on the spiny stigma/papillae of *K. galanga* and growth was observed upto $3/4^{th}$ of the style length. In case of *K. rotunda*, pollen germination did not occur at all.

4.3 BIOCHEMICAL PARAMETERS

Rhizomes of all the three *Kaempferia* species were subjected to biochemical analysis for estimating the contents of volatile oil, oleoresin, starch, sugars, amino acids and flavonoids. GCMS analysis of the volatile oil was also carried out in the three species. Ethanol extract of rhizome of *K. parviflora* and *K. rotunda* was also

subjected to GCMS analysis. Phytochemical screening for various secondary metabolites was done in *K. rotunda* and *K. parviflora*.

4.3.1 Volatile oil and oleoresin content in *Kaempferia* species

The data on volatile oil and oleoresin content in three *Kaempferia* species are given in table 86.

Table 86: Volatile oil and oleoresin content in *Kaempferia* genotypes

Species/genotype	Volatile oil (%)	Oleoresin (%)
K. parviflora		
KCP-1	0.20	4.17
KCP-2	0.21	2.03
BSI-1	0.21	2.27
CD (5%)	**NS**	**0.87**
K. rotunda		
MCR-1	0.15	3.11
MCR-2	0.06	3.76
MCR-3	0.23	0.60
MCR-4	0.16	2.67
MCR-5	0.31	0.83
MCR-6	0.31	2.16
MCR-7	0.21	3.77
KCR-1	0.21	1.14
KCR-2	0.21	2.40
KCR-3	0.317	4.95
KCR-4	0.12	2.05
KCR-5	0.12	0.82
KCR-6	0.16	1.34
C.D (5%)	0.04	0.43
***K. galanga* (t test-5%)**		
KCG	0.71	2.67[a]
ARPCG	0.64	2.12

Volatile oil content of *K. parviflora* genotypes ranged from 0.20 to 0.21 per cent (table 86). No significant difference was observed among the *K. parviflora* genotypes. Oleoresin content ranged from 2.03 to 4.17 per cent, KCP-1 recorded significantly highest (4.17 %) value and KCP-2 recorded the lowest (2.03 %) content.

Kaempferia rotunda genotypes exhibited variable oil content. KCR-3 recorded significantly highest (0.32 %) oil content which was on par with the genotypes MCR-6 and MCR-5. Significantly lowest value was observed in the genotype MCR-2 (0.06 %).

Oleoresin content of *K. rotunda* genotypes ranged from 0.82 to 4.95 per cent. Significantly highest (4.95 %) value was recorded in the genotype KCR-3 and lowest (0.82%) was recorded in the genotype KCR-5.

No significant difference in oil content was observed between the two genotypes of *K. galanga*, whereas oleoresin content was significantly higher in the genotype KCG (2.67 %)**.**

4.3.2 Biochemical parameters of *Kaempferia* species

The data on starch, total sugar, flavonoids and total free amino acids content in the rhizomes of Kaempferia species are presented in table 87.

Table 87: Biochemical parameters of *Kaempferia* species

Species	Starch (mg/100mg)	Total sugar (mg/100mg)	Total free amino acids (mg/100mg)	Flavonoids (mg eq. of quercetin/100mg)
K. parviflora	13.2	9 .00	0.49	26.1
K. rotunda	3.8	1.74	1.15	4.9
K. galanga	4.4	1.23	0.76	5.81

The starch content of *K. parviflora* was 13.2 mg, while that of *K. rotunda* was 3.8 mg and *K. galanga* was 4.4 mg. Total sugar content was 9.00, 1.74 and 1.23 mg, respectively for *K. parviflora*, *K. rotunda* and *K. galanga*. With respect to

total free amino acid content, it was 0.49 mg for *K. parviflora*, 1.15 mg for *K. rotunda* and 0.76 mg for *K. galanga*. Flavonoid content of *K. parviflora* was 26.07 mg, while that of *K. rotunda* and *K. galanga* were 4.9 and 5.81 mg, respectively.

4.3.3 GCMSMS profile for volatile oil of *K. parviflora*

GCMSMS profile with respect to major components of *K. parviflora* volatile oil is presented in table 88.

Table 88: GCMSMS profile of *K. parviflora* volatile oil

Sl. No.	Compound	RT	Mol. Wt.	Area
1.	Ethanol, 2-(trimethylsilyl)-	2.08	118	89.6
2.	à-D-Glucopyranoside, methyl 2-(acetylamino)-2-deoxy-3-O-(trimethylsilyl)-, cyclic methylboronate	2.67	331	0.92
3.	1H-2,8a-Methanocyclopenta[a]cyclopropa[e]cyclodecen-11-one, 1a,2,5,5a,6,9,10,10a-octahydro-5,5a,6-trihydroxy-1,4-bis(hydroxymethyl)-1,7,9-tr imethyl-, [1S-(1à,1aà,2à,5á,5aá,6á,8aà,9à,10aà)]	2.87	364	0.42
4.	Butanoic acid, 4-chloro-, 1,1a,1b,4,4a,5,7a,7b,8,9-decahydro-4a,7b-dihydroxy-3-(hydroxymethyl)-1,1,6,8-t etramethyl-5-oxo-9aH-cyclopropa[3,4]benz[1,2-e]azulene-9,9a-diyl ester, [1ar-(1aà,1bá,4aá,7aà,7bà,8à,9á,9aà)]	2.98	572	0.11
5.	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis-	3.05	326	0.45
6.	Silanediol, dimethyl-	3.60	92	1.26
7.	3-Benzoylmethyl-3-hydroxy-5-nitro-2-indolinone	3.71	326	0.84
8.	3-tert-Butyl-5-chloro-2-hydroxybenzophenone	3.80	288	0.40
9.	3-(3-Carboxy-4-hydroxyphenyl)-D-alanine	3.86	225	0.03
10.	.psi.,.psi.-Carotene, 3,4-didehydro-1,1',2,2'-tetrahydro-1'-hydroxy-1-methoxy-	4.00	584	0.08
11.	Phenylpropylamine, N-acetyl-3,4,5-trimethoxy-	4.21	267	0.01
12.	Bicyclo[2.2.0]hex-2-ene-1-carboxylic acid, 5,5,6,6-tetracyano-2,3,4-tri(1,1-dimethylethyl)-, 1,1-dimethylethyl ester	4.28	448	0.02

Table 88. Contd.

Sl. No.	Compound	RT	Mol. Wt.	Area
13.	1-Dimethylisopropylsilyloxynonane	4.38	244	0.04
14.	Quinoline, 8-bromo-	4.46	207	
15.	.psi.,.psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy-	6.05	600	0.09
16.	o-Ethyl o-4-methylcyclohexyl methylphosphonate	8.99	220	0.01
17.	Haloxazolam	9.03	376	0.02
19.	Rhodoviolascin	9.79	596	0.01
19.	3-Fluoro-3-(2-oxo-2-phenyl-ethylsulfanyl)-2-trifluoromethyl-acrylic acid methyl ester	11.69	322	0.04
20.	Milbemycin b, 13-chloro-5-demethoxy-28-deoxy-6,28-epoxy-5-(hydroxyimino)-25-(1-methylethy l)-, (6R,13R,25R)	13.71	603	0.04
21.	7-methoxy-4'-hydroxyisoflavone	15.35	268	0.01
22.	6-Methylquinolinic acid diamide	16.25	179	0.02
23.	3'H-Cycloprop(1,2)cholesta-1,4,6-trien-3-one,	16.48 (steroid comp.)	491	0.15
24.	1,2,4-Triazol-3-amine, 5-(1,3,5-trimethyl-4-pyrazolyl)amino-	16.65	207	0.02
25.	Benzene, [3-(2-cyclohexylethyl)-6-cyclopentylhexyl]-	17.56	340	0.16
26.	Pyridine, 2-amino-5-iodo-	21.56	220	0.02
27.	1H,3H-Pyrano[3,4-c]pyran-5-carboxaldehyde, 4,4a,5,6-tetrahydro-6-methyl-1-oxo-, [4as-(4aà,5à,6á)]	26.71	196	0.01
28.	3,5-Dihydroxy-4',7-dimethoxyflavone	30.31	314	0.01
29.	3-Methoxy-3-methylbutanol	30.55	118	0.02
30.	3',4',5,7-Tetramethoxyflavone	30.63	342	0.01
31.	7aH-Cyclopenta[a]cyclopropa[f]cycloundecene-2,4,7,7a,10,11-hexol, 1,1a,2,3,4,4a,5,6,7,10,11,11a-dodecahydro-1,1,3,6,9-pentamethyl-, 2,4,7,10,11-pentaacetate	37.85	511	0.01
32.	2á,4a-Epoxymethylphenanthrene-7-methanol, 1,1-dimethyl-2-methoxy-8-(1,3-dithiin-2-ylidene)methyl-1,2,3,4,4a,4b,5,6,7,8,8a, 9-dodecahydro-, acetate	44.83	490	0.05
33.	Acacetin	54.20	284	0.01
34.	Hematoporphyrin	57.96	598	0.17

There were 34 chemical compounds. Ethanol, 2-(trimethylsilyl) was present in high quantity (89.6 %). Compounds belonging to flavone groups present in the volatile oil are 3,5-Dihydroxy-4',7-dimethoxyflavone; 3',4',5,7-Tetramethoxyflavone; Flavanone, 3,5,7-trihydroxy-4'-methoxy; 4',5,7-Trihydroxyflavanone, tris(trimethylsilyl) ether; Flavanone, 3-hydroxy-2',4',5,7-tetramethoxy-, acetate, trans; 7-methoxy-4'-hydroxyisoflavone and Acacetin.

4.3.4 GCMSMS profile of *K. rotunda* volatile oil

GCMSMS profile with respect to major components of *K. rotunda* volatile oil is given in table 89.

Table 89: GCMSMS profile of *K. rotunda* volatile oil

Sl. No.	Compound	RT	Molecular weight	Area
1	Ethanol, 2-(trimethylsilyl)-	2.07	118	0.84
2	(1,2,2-trimethyl-3-cyclopenten-1-yl)acetaldehyde	4.77	152	0.00
3	Camphene	7.69	136	32.88
4	Eucalyptol	10.60	154	0.77
5	1,6-Octadien-3-ol, 3,7-dimethyl-	13.38	154	0.19
6	Camphor	15.58	152	11.01
7	Endo-Borneol	16.50	154	2.03
8	Bornyl acetate	21.85	196	18.52
9	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1à,2á,4á)]-	25.69	204	0.04
10	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-	26.87	204	0.04
11	à-ylangene	28.44	204	1.05
12	1S,2S,5R-1,4,4-Trimethyltricyclo[6.3.1.0(2,5)]dodec-8(9)-ene	28.88	0.19	0.19
13	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1S-(1à,7à,8aà)]	29.05	204	0.41
14	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	29.65	202	0.54

Table 89. Contd.

Sl. No.	Compound	RT	Molecular weight	Area
15	Pentadecane	30.80	212	6.73
16	Di-epi-à-cedrene-(I)	31.06	204	0.22
17	Ar-tumerone	36.55	216	0.06
18	Benzyl Benzoate	41.16	212	15.39
19	Benzoic acid, 2-hydroxy-, phenylmethyl ester	44.31	228	0.19
20	1H-Naphtho[2,1-b]pyran, 3-ethenyldodecahydro-3,4a,7,7,10a-pentamethyl-, [3R-(3à,4aá,6aà,10aá,10bà)]	48.56	290	0.05
21	Podocarp-7-en-3á-ol, 13á-methyl-13-vinyl-	52.51	288	6.67
22	1-Carbomethoxy-1,2,5,5-tetramethyl-cis-decalin(1R,2S,4as,8as)	54.94	252	0.08

Twenty two compounds were identified from volatile oil of *K. rotunda*. Major compounds were Camphene (32.88 %), Bornyl acetate (18.52 %), Benzyl Benzoate (15.39 %), Camphor (11.01 %), Pentadecane (6.73 %), Podocarp-7-en-3á-ol, 13á-methyl-13-vinyl- (6.67 %), endo-Borneol (2.03 %) and à-ylangene (1.05 %).

4.3.5 GCMSMS profile of *K. galanga* volatile oil

GCMSMS profile with respect to major components of *K. galanga* volatile oil is given in table 90.

Table 90: GCMSMS profile of *K. galanga* volatile oil

Sl. No.	Compound	RT	Molecular weight	Area
1.	Ethanol, 2-(trimethylsilyl)-	2.09	118	0.86
2.	Camphene	7.62	136	10.82
3.	à-Pinene	10.20	136	12.76
4.	2,6-Dimethyl-1,3,5,7-octatetraene, E,E-	10.46	134	4.75
5.	Eucalyptol	10.97	154	20.94
6.	ç-Terpinene	11.65	136	0.01
7.	Farnesene epoxide, E-	13.53	152	0.07

Table 90 contd.

Sl. No.	Compound	RT	Mol. Wt.	Area
8.	(+)-2-Bornanone	15.21	152	0.04
9.	endo-Borneol	16.85	154	5.54
10.	trans-2-Caren-4-ol	17.03	152	0.13
11.	Thymol	17.34	150	0.05
12.	L-à-Terpineol	17.63	154	0.07
13.	2,4-Cycloheptadien-1-one, 2,6,6-trimethyl-	18.34	150	0.16
14.	Linalyl acetate	18.41	196	0.03
15.	1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1aà,4à,4aá,7bà)]	26.34	204	0.77
16.	Epizonarene	26.89	204	0.06
17.	2-Propenoic acid, 3-phenyl-, ethyl ester	28.89	176	8.15
18.	Pentadecane	31.37	212	15.63
19.	ç-Muurolene	31.82	204	1.24
20.	1-Carbethoxy-3-[à-hydroxy-á-N-phenylpiperazinoethyl]-4-[h]-quinolizine-4-one	31.95	421	0.10
21.	Cubenol	35.14	222	0.06
22.	Pyridine, 4-(4-methyl-5-cis-phenyl-1,3-oxazolidin-2-yl)-	41.23	240	0.11
23.	Ethyl 5-(5-methyl-2-furyl)-2,4-pentadienoate	41.79	206	0.15
24.	Ethyl p-methoxycinnamate	43.47	206	16.44
25.	Kaur-16-ene, (8á,13á)-	47.35	272	0.05
26.	Aristolene epoxide	51.61	220	0.03
27.	Cyclopenta[a,d]cycloocten-5-one, 1,2,3,3a,4,5,6,8,9,9a,10,10a-dodecahydro-7-(1-methylethyl)-1,9a-dimethyl-4-met hylene	58.08	286	0.03

A total of 27 compounds were identified from volatile oil of *K. galanga*. Major compounds identified were Eucalyptol (20.94 %), Ethyl p-methoxycinnamate (16.44 %), Pentadecane (15.63 %), à-Pinene (12.76 %), Camphene (10.82 %), 2-Propenoic acid, 3-phenyl-, ethyl ester (8.15 %), endo-

Borneol (5.54 %), 2,6-Dimethyl-1,3,5,7-octatetraene, E,E- (4.75 %) and ç-Muurolene (1.24 %). Other compounds were present in traces.

4.3.6 GCMS profile of ethanolic rhizome extract of K. parviflora

GCMS profile with respect to major components of ethanolic extract of *K. parviflora* is given in table 91.

Table 91: GCMS profile of ethanolic rhizome extract of *K. parviflora*

Sl. No.	Name of compound	Area (%)	R.T. (min)
1.	Hexadecanoic acid, ethyl ester	0.72	19.5
2.	Ethyl linolate	0.90	23.4
3.	Techtochrysin	8.73	32.0
4.	Benzaldehyde,(diphenylmethylidene)hydrazone	7.15	32.5
5.	Coumaran-7-ol-3-one, 2-[4-methoxybenzylidene]-6-methoxy-	2.94	32.5
6.	Dimethylchrysin	74.43	35.7
7.	2-(4-Hydroxy-3-methoxyphenyl)-3,7-dimethoxy-4H-chromen-4-one	0.79	37.6
8.	Tri-o-methylapigenin	4.32	40.8

A total of eight compounds were identified from ethanolic extract of *K. parviflora*. Major compounds were Dimethylchrysin (74.43 %), Techtochrysin (8.73 %), Benzaldehyde,(diphenylmethylidene)hydrazine (7.15 %), Tri-o-methylapigenin (4.32 %), Coumaran-7-ol-3-one, 2-[4-methoxybenzylidene]-6-methoxy- (2.94 %).

4.3.7 GCMS profile of ethanolic rhizome extract of K. rotunda

GCMS profile with respect to major components of ethanolic extract of *K. rotunda* is given in table 92.

Table 92: GCMS profile of ethanolic rhizome extract of *K. rotunda*

Sl. No.	Compound	Area (%)	R.T (min)
1.	L-.Alpha.-Bornyl Acetate	7.90	9.691
2.	Alpha-Ionol	0.99	12.139
3.	Aromatic Curcumene	1.46	12.335
4.	Pentadecane	16.35	12.554
5.	.beta.-curcumene	0.69	12.710
6.	Germacrene-D	0.84	12.833
7.	1-Chlorooctadecane	0.52	13.724
8.	Heptadecane	4.32	14.901
9.	Benzoic Acid, Phenylmethyl Ester	34.49	16.020
10.	Benzyl salicylate	1.97	17.473
11.	Sandaracopimar-15-en-8.beta.-yl acetate	3.24	19.365
12.	Andrographolide	8.09	23.148
13.	trans-Z-.alpha.-Bisabolene epoxide	3.31	23.439
14.	Tricyclo[20.8.0.0e7,16]Triacontan, 1(22),7(16)-Diepoxy-	1.42	28.501
15.	Glycerol 2-Hexadecanoate	1.77	30.635
16.	4,8,13-Cyclotetradecatriene-1,3-Diol, 1,5,9-Trimethyl-12-(1-Methylethyl)-	7.27	31.011
17.	9-Octadecenoic Acid, (2-Phenyl-1,3-Dioxolan-4-Yl)Methyl Ester, Cis-	1.04	32.127
18.	2,5-Furandione, 3-(Dodecenyl)Dihydro-	4.34	32.473

Eighteen compounds were identified from ethanolic extract of *K. rotunda*. Major compounds identified were Benzoic Acid, Phenylmethyl Ester (34.49 %), Pentadecane (16.35 %), Andrographolide (8.09 %), L-.Alpha.-Bornyl Acetate (7.90 %), 4,8,13-Cyclotetradecatriene-1,3-Diol, 1,5,9-Trimethyl-12-(1-Methylethyl)- (7.27 %), Heptadecane (4.32 %), 2,5-Furandione, 3-(Dodecenyl)Dihydro- (4.34 %), trans-Z-.alpha.-Bisabolene epoxide (3.31 %), Sandaracopimar-15-en-8.beta.-yl acetate (3.24 %), Glycerol 2-Hexadecanoate (1.77 %).

4.3.8. Phytochemical screening of ethanolic rhizome extract of *Kaempferia* species

Results of the qualitative tests conducted for the presence of various phytochemical compounds in the ethanolic extract of two *Kaempferia* species are presented in table 93.

Table 93: Phytochemical screening of ethanolic rhizome extract of *K. parviflora* and *K. rotunda*

Sl. No.	Tests	*K. parviflora*	K. *rotunda*
1.	**Steroids**		
a.	Salkowski test	-	+
b.	Lieberman Burchardt test	-	+
2.	**Alkaloids**		
a.	Mayer's test	-	-
b.	Wagner's test	-	-
c.	Hager's test	-	-
d.	Dragendroff's Test	-	-
3.	**Phenolic compounds**	-	-
4.	**Tannins**		
a.	Ferric chloride test	-	-
b.	Gelatin test	-	-
5.	**Flavonoids**		
a.	Ferric chloride test	+	+
b.	Lead acetate test	+	+
6.	**Glycosides**		
a.	Sodium hydroxide test	+	+
b.	Benedict's test	+	+
7.	**Diterpenes**		

Table 93 contd.

Sl. No.	Tests	*K. parviflora*	K. *rotunda*
a.	Diterpenes test	-	-
8.	**Triterpenes**		
a.	Salkowski test	-	-
b.	Lieberman Burchardt test		-
9.	**Saponins**		
a.	Foam test	-	+

Results of phytochemical screening of ethanolic extract of *K. parviflora* indicated the presence of flavonoids and glycosides and that for *K. rotunda* showed the presence of steroids, flavonoids, glycosides and saponins.

4.4 ANATOMICAL STUDIES

4.4.1 Anatomy of *K. galanga*

Anatomy of leaf, stem, rhizome and tubers is presented in Plates 1 to 5 and table 97.

4.4.1.1 Epidermal characters of K. galanga

Microscopic observations of both the adaxial and abaxial surface of leaves of *K. galanga* are presented in plate 12 and table 94.

Table 94: Stomatal characters of *K. galanga*

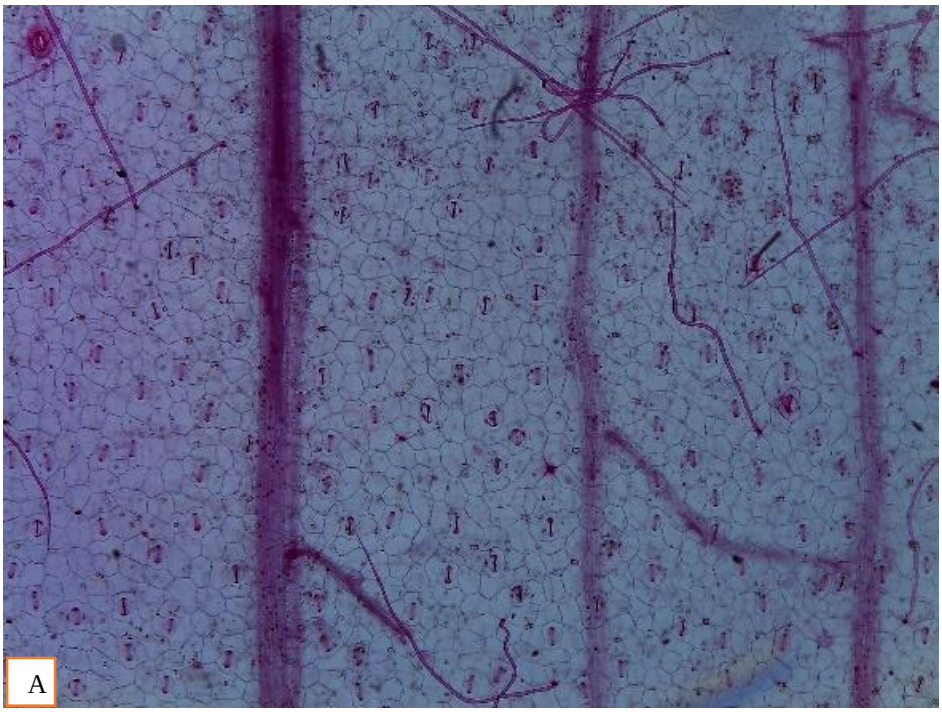
Type of stomata	Length of stomata (µm)	Breadth of stomata (µm)	Stomatal index (%)
Hexacytic	(U) 50.4	39.9	10
	(L) 70.4	28.9	24.26

Epidermal cells are irregular or polygonal and usually run perpendicular to veins. Stomata are hexacytic and more in the lower surface (abaxial) than the upper surface (adaxial). Stomatal length for upper surface was 50.4 µm, while that of lower surface was 70.4 µm. In case of breadth of stomata, it was 39.9 µm for upper surface and 28.9 µm for lower surface. Stomatal index was 24.26 for lower surface and 10 for upper surface. Unicellular trichomes were present in the epidermal cells of abaxial side (Plate 12).

4.4.1.2 TS of leaf lamina of K. galanga

TS of leaf lamina of *K. galanga* described in plate 13.

Transverse section of leaf of *K. galanga* consisted of upper and lower epidermis (Plate 13). The lower and upper epidermal layers are uniserriate and cells are thin walled. The continuity of cells are interrupted by stomata. The outline of the mesophyll cells are irregular. It had collateral and closed type of vascular bundle. Presence of oil globule was also noticed in the TS of leaf.



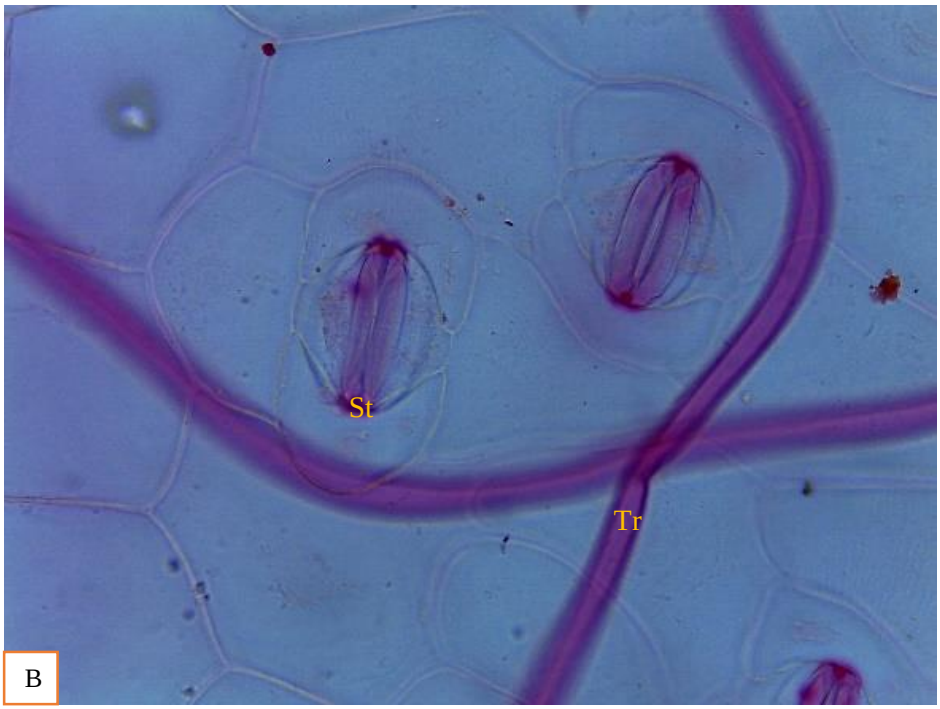


Plate12: Epidermal characters of *K. galanga.* (A)- Leaf epidermis of *K. galanga* (10X). (B)- Leaf epidermis of *K. galanga* (40X). (St-Stomata, Tr-Trichome)

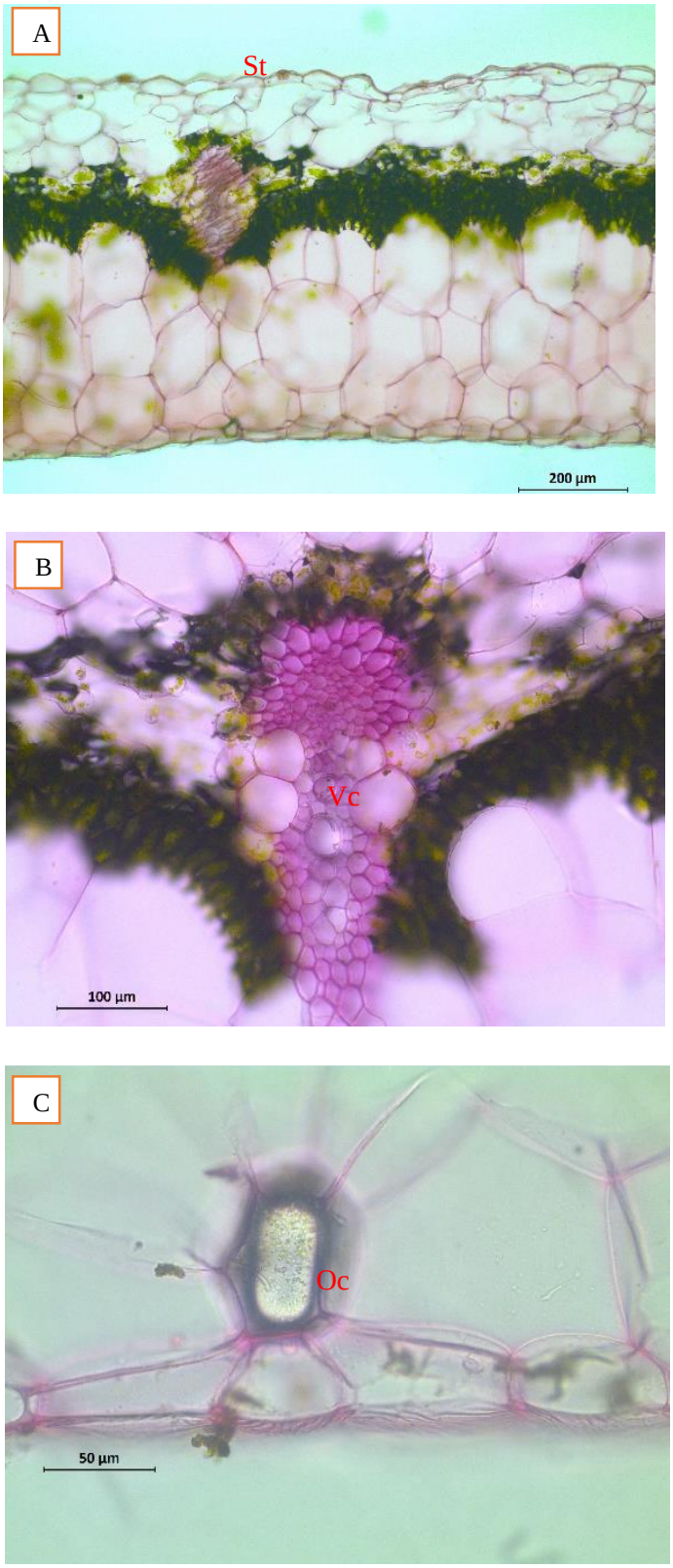


Plate 13: TS of leaf lamina of *K. galanga*. (A)-TS of leaf lamina 10X. (B)-TS of leaf lamina showing vascular bundles. (C)- TS of leaf lamina showing oil globules (St-Stomata, Vc-Vascular bundles, Oc-Oil globules)

Plates 14: TS of stem of *K. galanga*. (A)- TS of stem (10X), (B)-Oil globule (40X), (C)- Vascular bundle with epidermis (10X). (Vc-Vascular bundles, Oc-Oil globules)

4.4.1.3 TS of stem of K. galanga

TS of stem of *K. galanga* is described in plate 14.

Epidermis was single layered with small cells (Plate 14). Mesophyll cells were irregular in outline. A line of small vascular bundles were visible just beneath the epidermal layer. Next layer of vascular bundles were bigger in size situated near to vacuoles. Below this layer again smaller bundles were visible. Presence of oil globules were also noticed in the TS of stem.

4.4.1.4 TS of rhizome in K. galanga

TS of rhizome in *K. galanga* is given in Plates 15.

The TS of rhizome showed outer brown coloured cork cells in 6-7 layers followed by parenchymatous tissue. It had collateral and closed vascular bundles which were scattered in both cortical and ground tissues as well as embedded in the endodermal layer (Plate 15). Starch granules were more abundant near to endodermis and are oval to spherical in shape. Orange coloured oil globules were present and oleoresin like mass were also detected with light orange colour.

4.4.1.5 TS of root tuber of K. galanga

TS of root tuber of *K. galanga* is given in plate 16.

The transverse section of root tuber was circular in outline. Exodermis was 2-3 layered and had thin walls (Plate 5). Inner cortex was marked by one layer of endodermis. Vascular tissues were polyarch in arrangement. Starch granules were oval shaped and were present abundantly near the endodermal layer and in the pith.

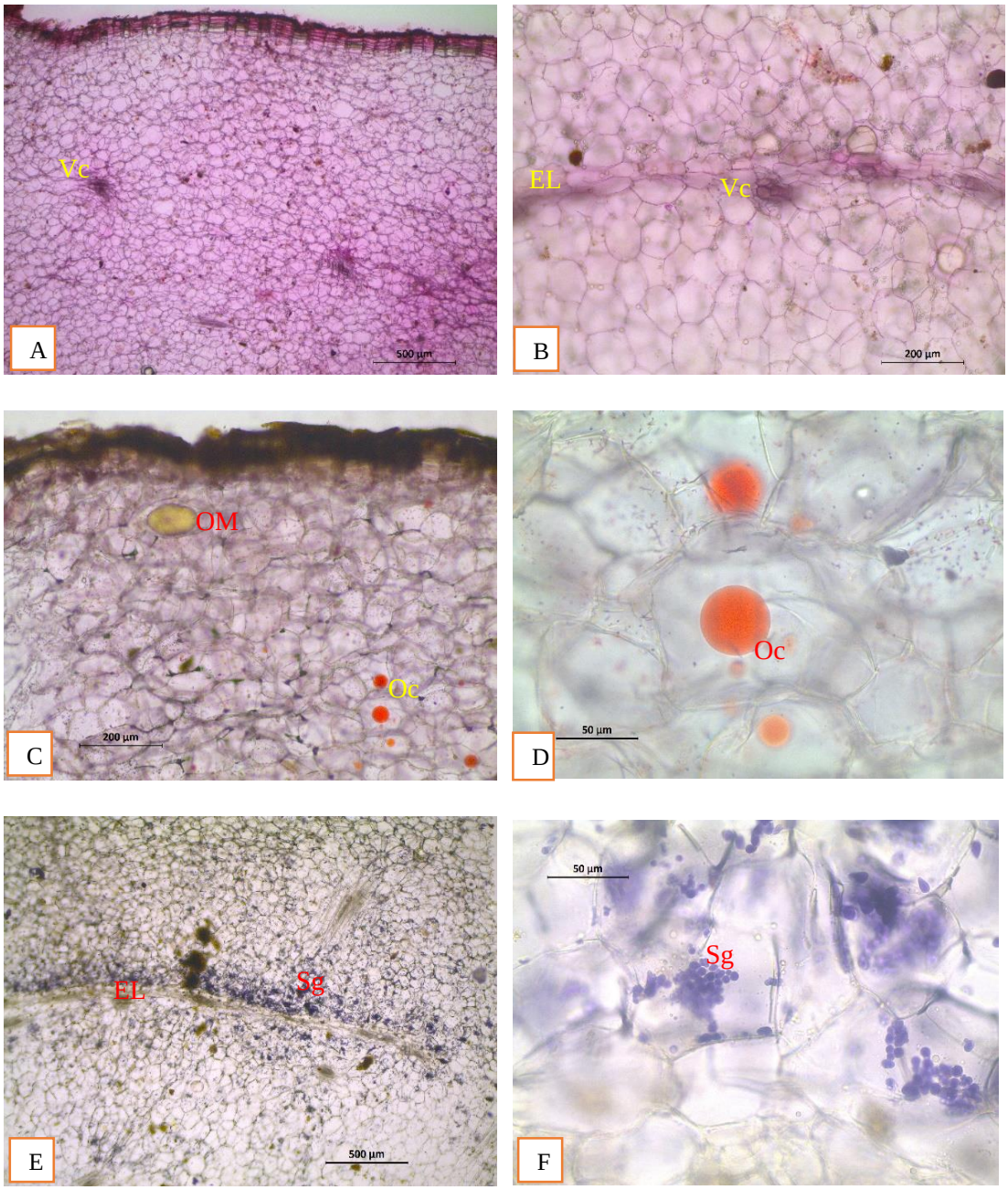
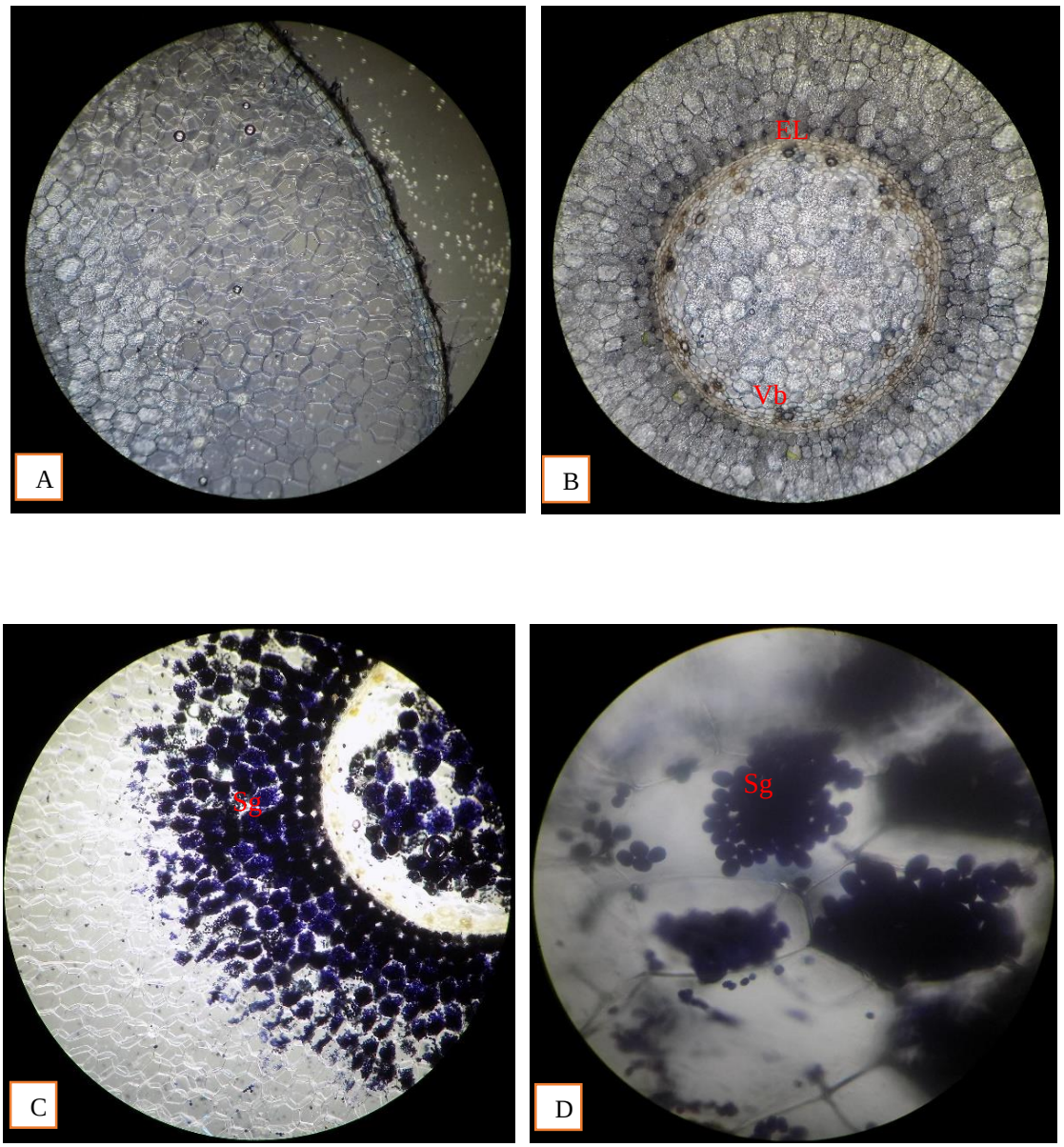


Plate 15: TS of rhizome in *K. galanga*. (A)-TS of rhizome (4X), (B)-TS of rhizome with endodermal layer, (C)-TS of rhizome with dark orange colour oil globules and light orange colour oleoresin mass (10X), (D)- Oil globules (40X), (E): Starch granules distribution near endodermal layer (4X), (F)- Starch granules (40X). (VC-Vascular bundles, EL- Endodermal layer, OM-Oleoresin mass, Oc-Oil globules, Sg-Starch granules)



Plates 16: TS of root tuber of *K. galanga*. (A)-Epidermal layer (10X), (B)-Endodermal layer with polyarch arrangement of vascular tissues (10X), (C)- Starch distribution near endodermal layer and pith (4X), (D)- Starch granules (40X). (EL-Endodermal layer, VC- Vascular bundles, Sg-Starch granules)

4.4.2 Anatomy of *K. parviflora*

4.4.2.1 Epidermal characters of K. parviflora

Microscopic observations of both the adaxial and abaxial surface of leaves of *K. parviflora* are presented in plate 17 and table 95.

Table 95: Stomatal characters of *K. parviflora*

Type of stomata	Length of stomata (µm)	Breadth of stomata(µm)	Stomatal index (%)
Hexacytic	(U)58.94	35.77	10.55
	(L)57.32	35.42	15.99

Epidermal cells were irregular or polygonal and usually run perpendicular to veins. Stomata were hexacytic and more in lower surface (abaxial) than the upper surface (adaxial). Stomatal length for upper surface was 58.94 µm, while that of lower surface was 57.32µm. In case of breadth of stomata, it was 35.77 µm for upper surface and 35.42µm for lower surface. Stomatal index was higher (15.99) in upper surface and lower (10.55) in lower surface. Unicellular trichomes were present in the lower epidermal cells (Plate 17).

4.4.2.2 TS of leaf lamina of K. parviflora

TS of leaf lamina of *K. parviflora* is given in plate 18.

A transverse section of leaf consisted of upper and lower epidermis. The epidermis was uniserriate and cells were bigger than *K. galanga* in both the surfaces. The outline of the mesophyll cells was irregular (Plate 18). It had collateral and closed type of vascular bundle. There were prismatic calcium oxalate crystals present along with mesophyll cells. Presence of oil globule was not observed in the TS of leaf.

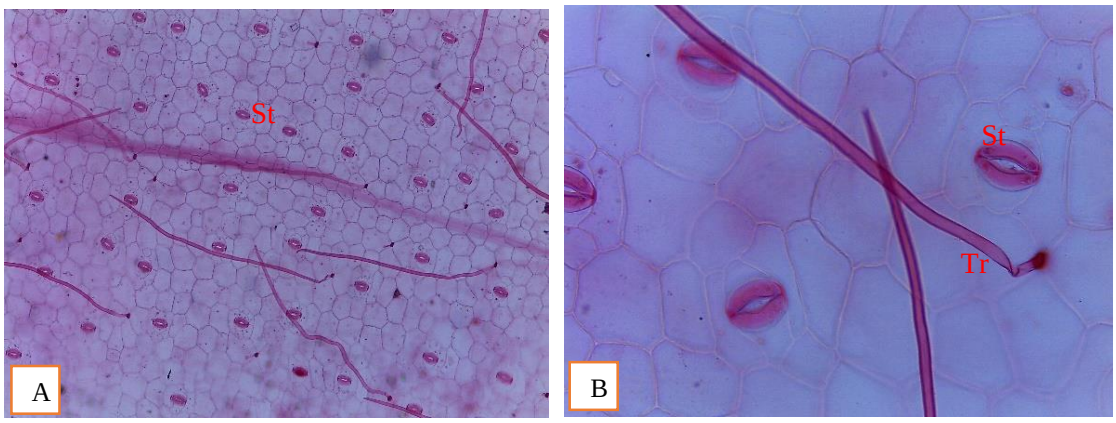


Plate 17: Epidermal characters of *K. parviflora*. (A)- Leaf epidermis (10X), (B)- Stomata, epidermal cells and trichomes (40X). (St-Stomata, Tr-Trichome)

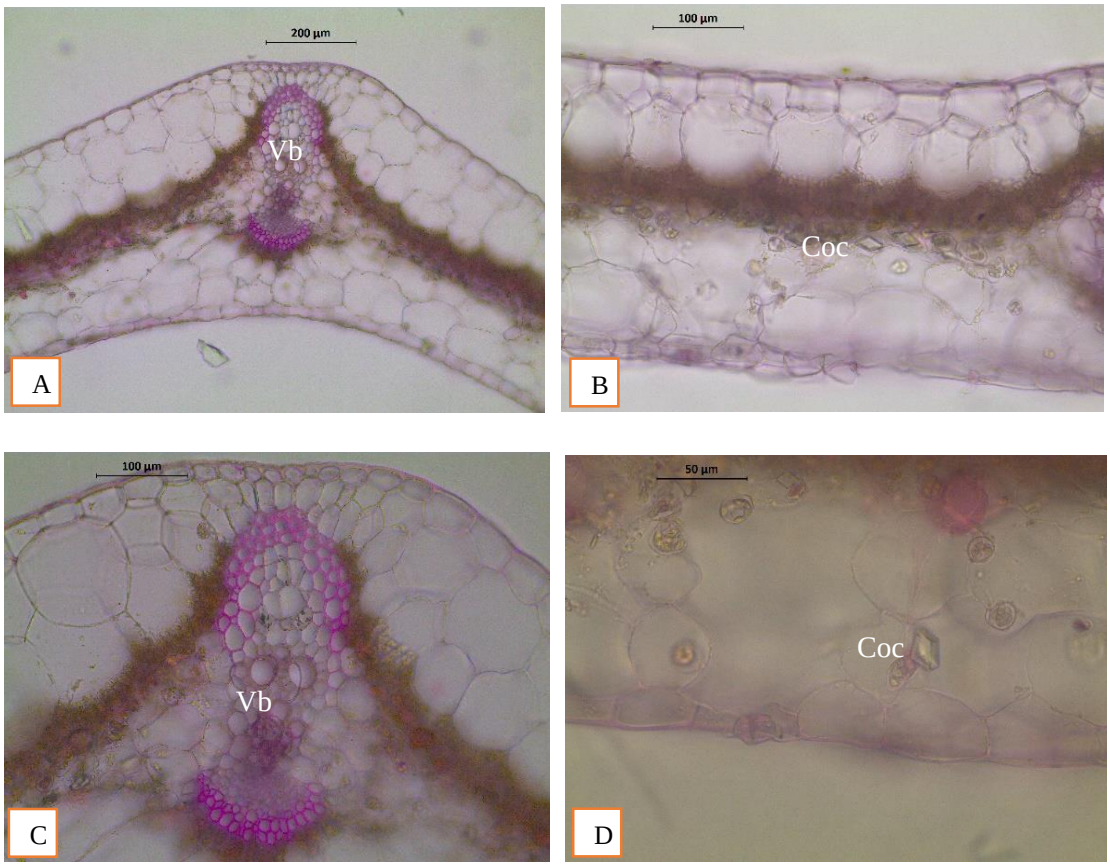


Plate 18: TS of leaf lamina of *K. parviflora.* *(*A) TS of leaf lamina at (10X), (B)- TS of leaf lamina at (20X), (C)- TS of leaf lamina at 20X, (D)- TS of leaf lamina at 40X. (Vb-Vascular bundles, Coc-Calcium oxalate crystal)

4.4.2.3 TS of stem K. parviflora

TS of stem *K. parviflora* is presented in plate 19.

Epidermis was single layered with bigger cells when compared with *K. galanga*. In the hypodermis cells were irregular in outline (Plate 19). The vascular bundles near the epidermal layer was small and the size of vascular bundle increased towards centre. Presence of oil globules was not noticed in the TS of stem.

4.2.2.4 TS of rhizome of K. parviflora

TS of rhizome of *K. parviflora* is presented in plate 20.

The TS of rhizome showed outer brown coloured cork cells with 8-9 layers followed by parenchymatous tissue. Endodermis were thin, single layered with elongated cells. It had collateral and closed vascular bundles and were scattered in cortical and ground tissues as well as embedded in endodermis layer. Starch granules were present throughout the section however more concentrated in endodermis region and were oval to spherical in shape (Plate 20). Dark orange coloured oil globules were abundant along with light orange coloured oleoresin mass. Both the oil and oleoresin globules were abundant near the epidermal layer.

4.2.2.5 TS of root tuber of K. parviflora

TS of root tuber of *K. parviflora* is presented in plate 21.

The transverse section of root tuber was circular in outline. Exodermis was single layered and had thin wall (Plate 21). Inner cortex was marked by one layer of endodermis. Vascular tissues were polyarch in arrangement. In the inner cortex region there were 1-3 radial cell layers seen radiating from the endodermis. Starch granules were oval shaped and were present abundantly near the endodermal layer and in the pith.

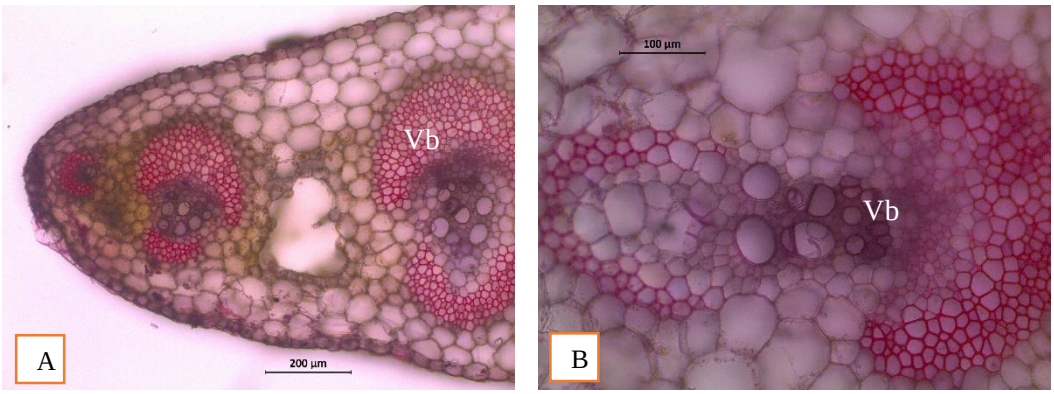


Plate 19: TS of stem *K. parviflora* (A)-TS of stem showing alternate arrangement of air chamber and vascular bundles, (B)- Vascular bundles of stem (20X), (Vb-Vascular bundles)

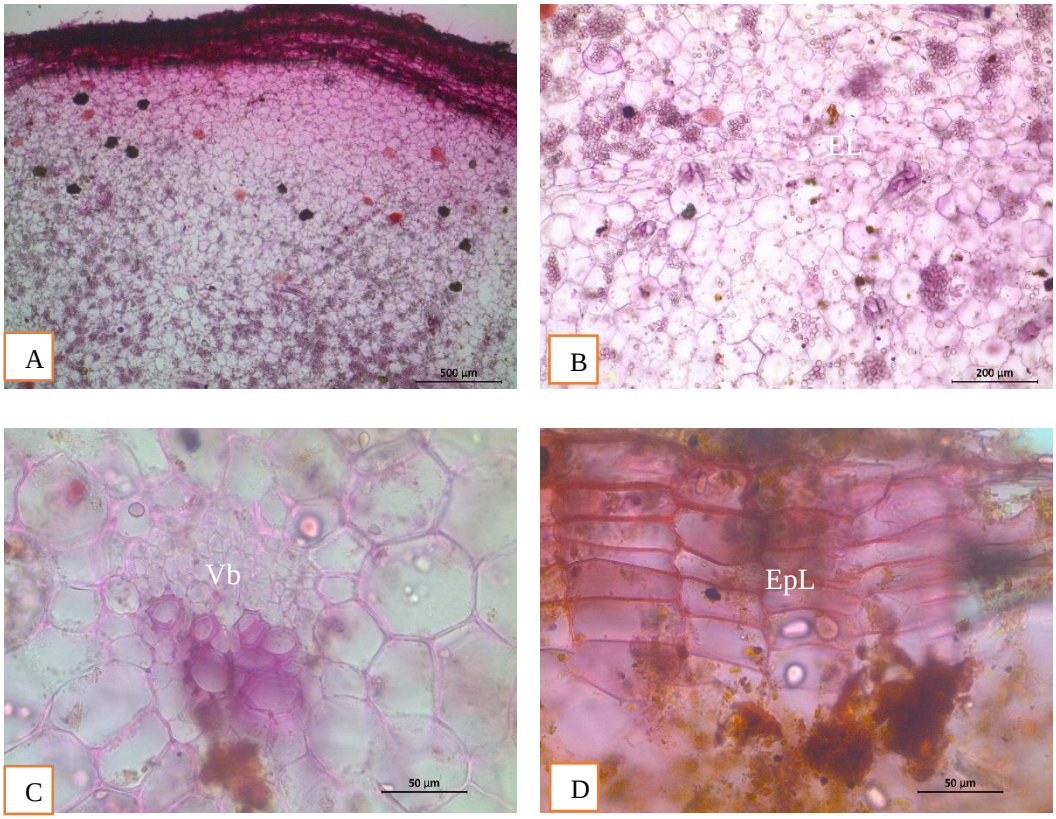


Plate 20: TS of rhizome of *K. parviflora* (A)-TS of rhizome with oil globules, oleoresin mass and flavonoids vacuoles (4X), (B)- Endodermal layer with scattered starch grains (10X), (C)-Vascular bundles (40X), (D)-Epidermal layer of rhizome (40X). (Vb-Vascular bundles, EpL-Epidermal layer)

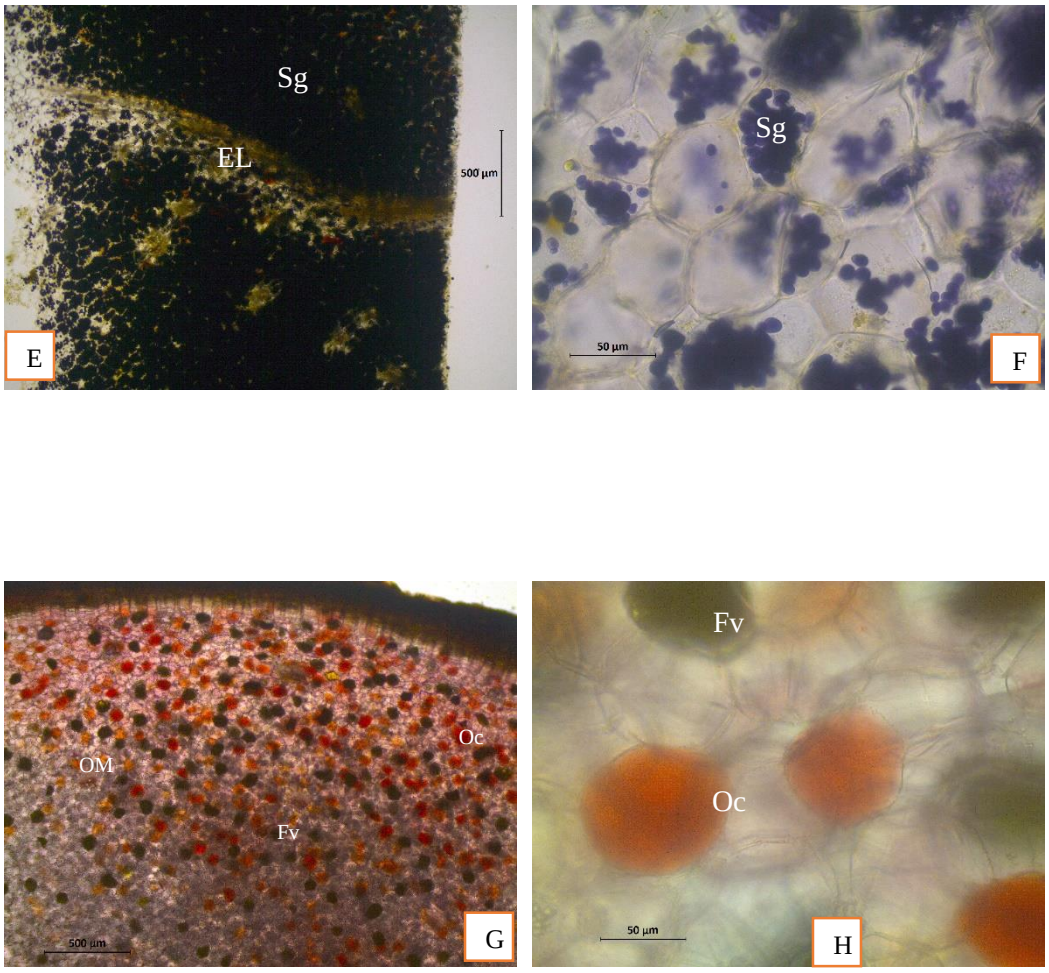
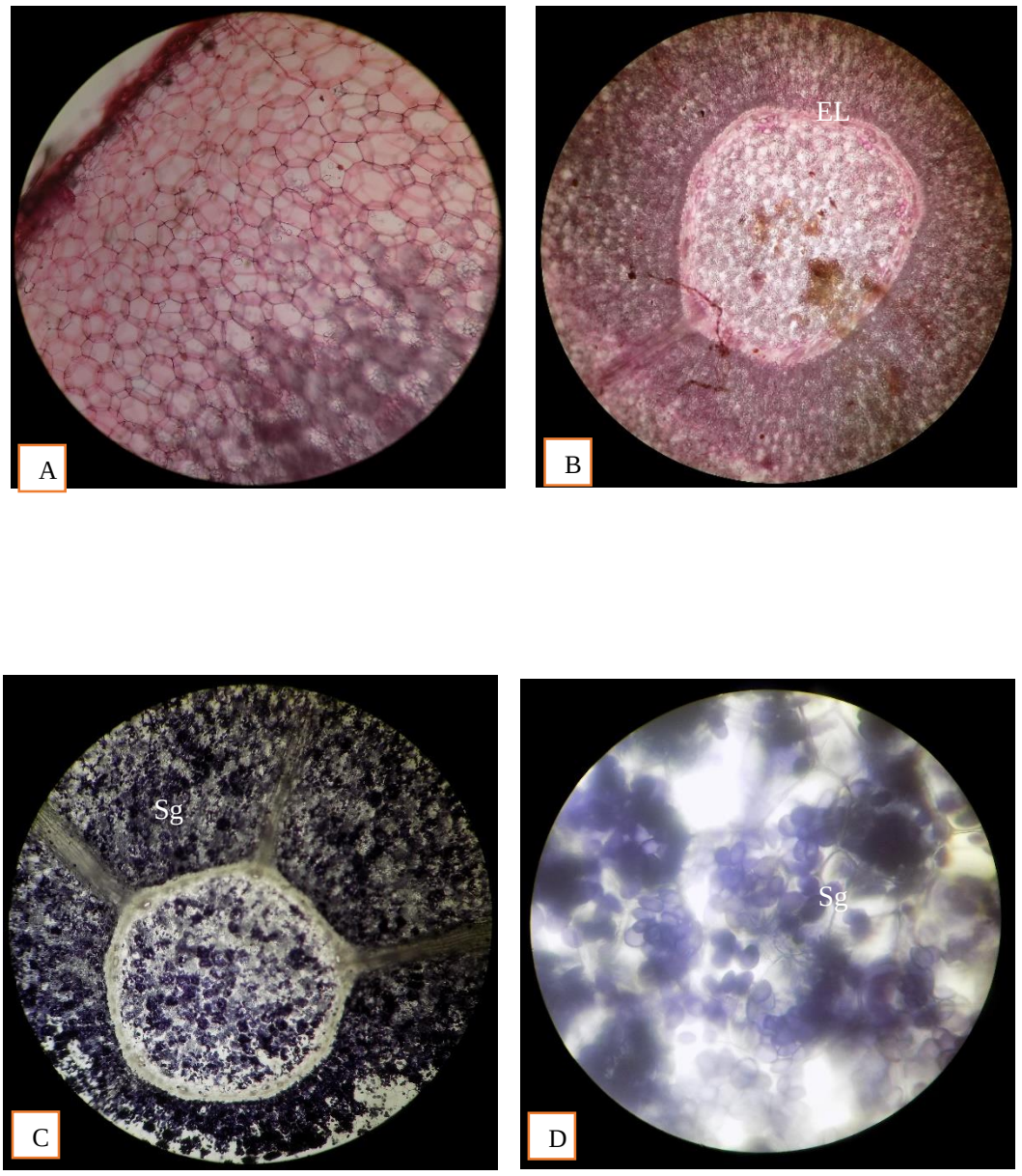


Plate 20: TS of rhizome of *K. parviflora* (E)- Starch grains concentrating near endodermal layer, (F)-Starch grains (40X), (G)-Oil globules, oleoresin mass and flavonoids vacuoles in the cortical region (4X), (H)-Oil globules and flavonoids vacuoles (40X). (Sg-Starch granules, EL- Endodermal layer, OM-Oleoresin mass, Oc-Oil globules, Fv-Flavonoid vacuole)



Plates 21: TS of root tuber of *K. parviflora* (A)-Epidermis and cortical region (10X), (B)-Cortical region and endodermal layer with visible polyarch arrangement of vascular bundles, (C)-Distribution of starch granules near the endodermal layer and pith region (10X), (D)-Starch granules (40X). (EL-Endodermal layer, Sg-Starch granules)

4.2.3 Anatomy of *K. rotunda*

4.2.3.1 Epidermal characters of K. rotunda

Microscopic observations of both the adaxial and abaxial surface of leaves of *K. rotunda* is presented in plates 22 and table 96.

Table 96: Stomatal characters of *K. rotunda*

Type of stomata	Length of stomata (µm)	Breadth of stomata(µm)	Stomatal index (%)
Hexacytic	(U)57.82	39.45	16.15
	(L)72.3	46.36	6.79

Epidermal cells were irregular or polygonal and usually run perpendicular to veins. Stomata were hexacytic and more in lower surface (abaxial) than the upper surface (adaxial). Stomatal length for upper surface was 57.82µm, while that of lower surface was 72.3µm. In case of breadth of stomata, it was 39.45µm for upper surface and 46.36 µm for lower surface. Stomatal index was higher (16.15) for lower surface and lower (6.79) for upper surface. Unicellular trichomes were present in the epidermal cells. Presence of calcium oxalate crystal was observed in the epidermal cell (Plate 22).

4.2.3.2 TS of leaf lamina of K. rotunda

TS of leaf lamina of *K. rotunda* is given in plate 23. As seen in the plate 23, transverse section of leaf consists of upper and lower epidermis. The epidermis were uniserriate and cells were thicker walled than *K. galanga*. The lower epidermis was thicker when compared to the upper epidermis. The outline of the mesophyll cells was irregular. It had collateral and closed type of vascular bundle. Presence of oil globule and calcium oxalate crystals were not observed.

4.2.3.3 TS of stem of K. rotunda

TS of stem of *K. rotunda* is given in plate 24. Epidermis was single layered with small cells. Cells in the hypodermal region were irregular in outline (Plate 24).

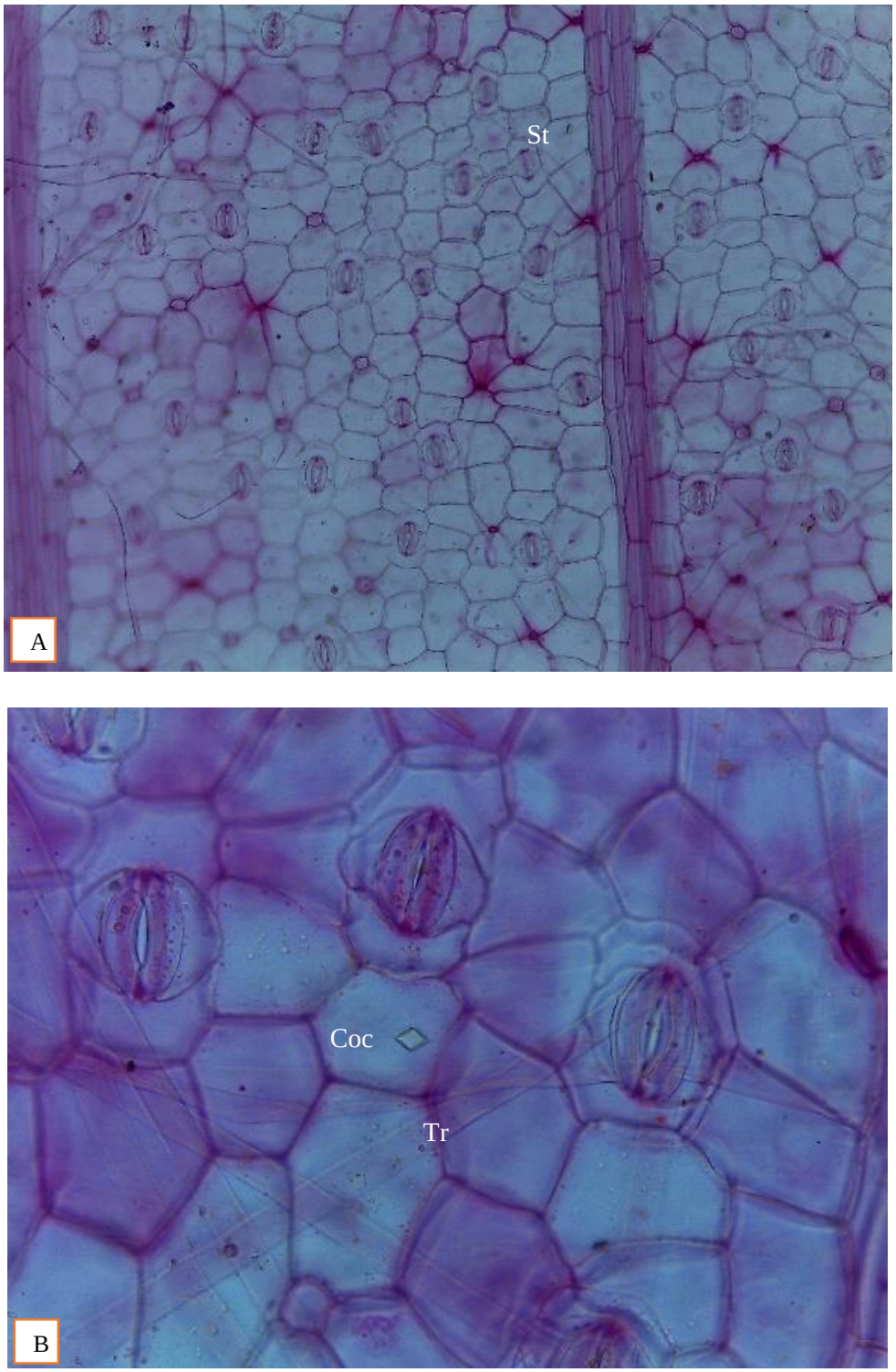


Plate 22: Epidermal leaf characters of *K. rotunda*. (A)- leaf epidermis (10X), (B)-Stomata, epidermal cells, calcium oxalate crystals and lightly visible trichomes. (St-Stomata, Coc-Calcium oxalate crystal, Tr-Trichome)

The vascular bundles near the epidermis were smaller and the size increases as it goes down. Presence of oil globules was not noticed.

4.2.3.4 TS of rhizome of K. rotunda

TS of rhizome of *K. rotunda* is given in plate 25. Transverse section of rhizome showed outer brown coloured cork cells of 6-8 layers followed by parenchymatous tissue. Endodermis was single layered and was conspicuous with tangentially elongated cells. It had collateral and closed vascular bundles scattered in cortex as well as ground tissues. Small bundles were present in the endodermis layer. Starch were oval in shape and were available in all the regions, more concentrated in endodermis region. Dark orange coloured oil globules were abundant along with light orange coloured oleoresin mass. Both oil and oleoresin globules were concentrated near the epidermis.

4.2.3.5 TS of root tuber of K. rotunda

TS of root tuber of *K. rotunda* is presented in plate 26. The transverse section of root tuber was circular in outline. Exodermis was single layered and has thin wall. Inner cortex was marked by one layer of endodermis. Vascular tissues were polyarch in arrangement. Starch granules were elongated oval shaped and present abundantly near the endodermal layer and in the pith.

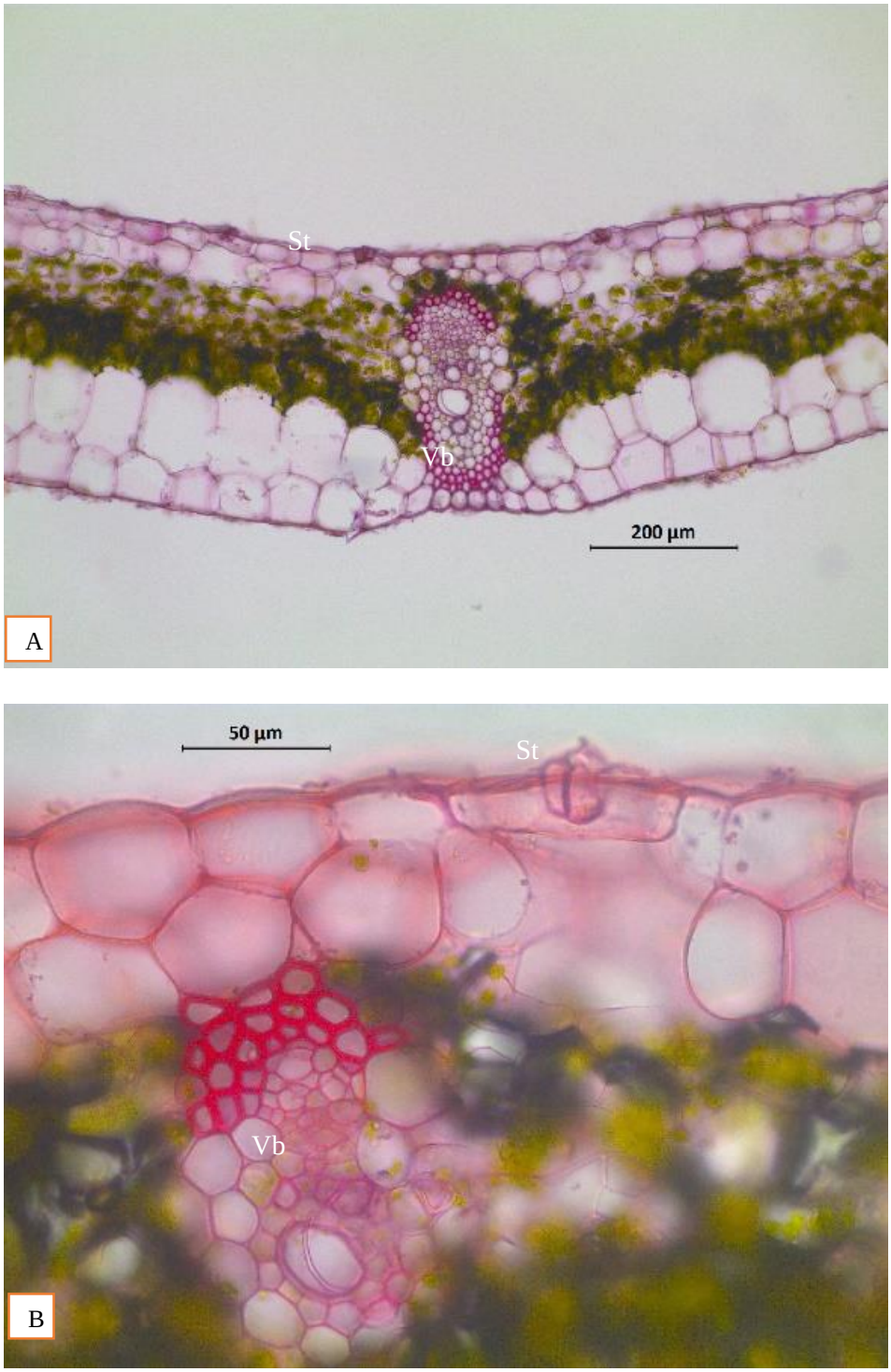


Plate 23: TS of leaf lamina of *K. rotunda* (A)-TS of leaf lamina (10X), (B)-TS of leaf lamina with stomata (40X). (St-Stomata, Vb-Vascular bundles)

Plate 24: TS of stem of *K. rotunda*. (A)-Outer cells, vascular bundles alternately arranged with air chamber (10X), (B)- Smaller and larger vascular bundles (40X)

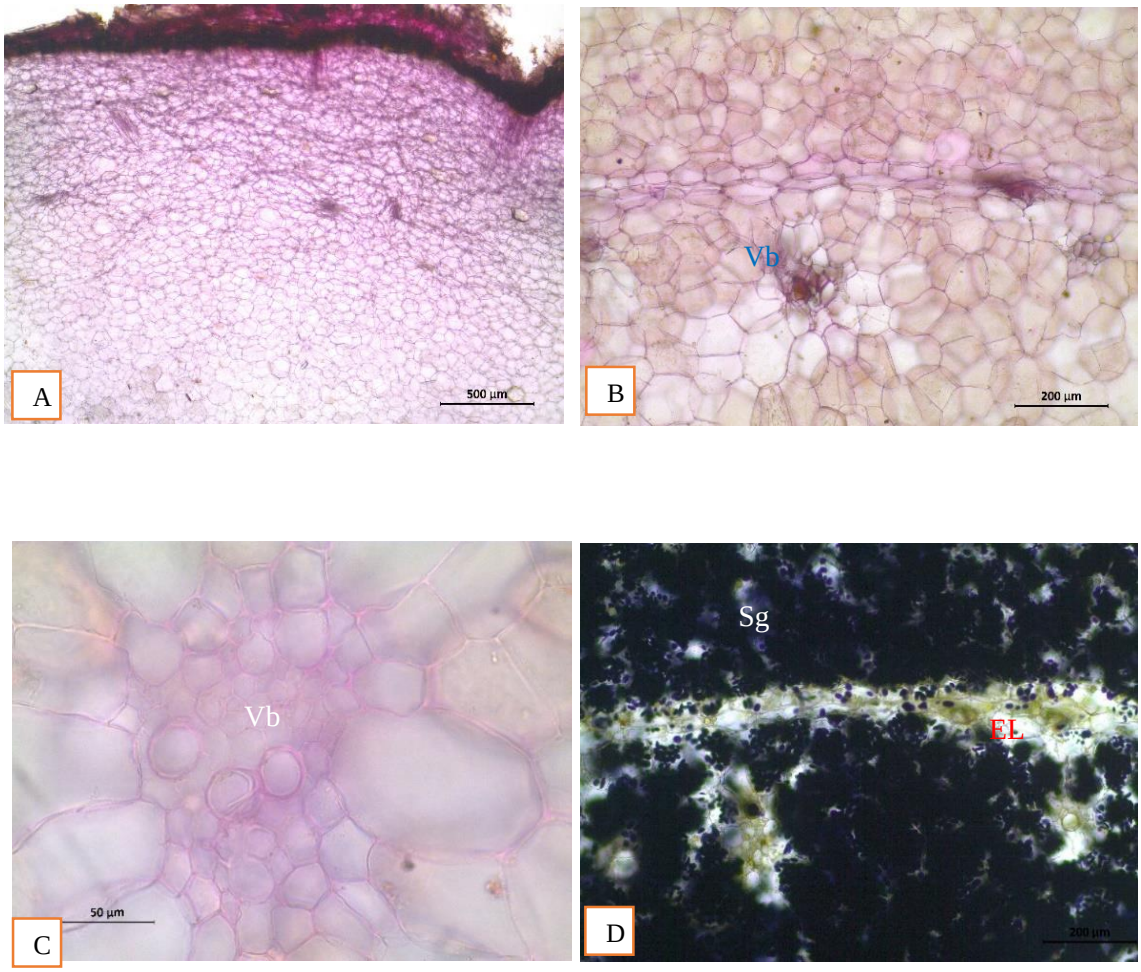


Plate 25: TS of rhizome of *K. rotunda*. (A)- TS of rhizome (4X), (B)-Endodermal layer with vascular bundles (10X), (C)-Vascular bundles (40X), (D)-Starch granules concentrating near endodermal layer. (Vb-Vascular bundles, Sg-Starch granules, EL-Endodermal layer,)

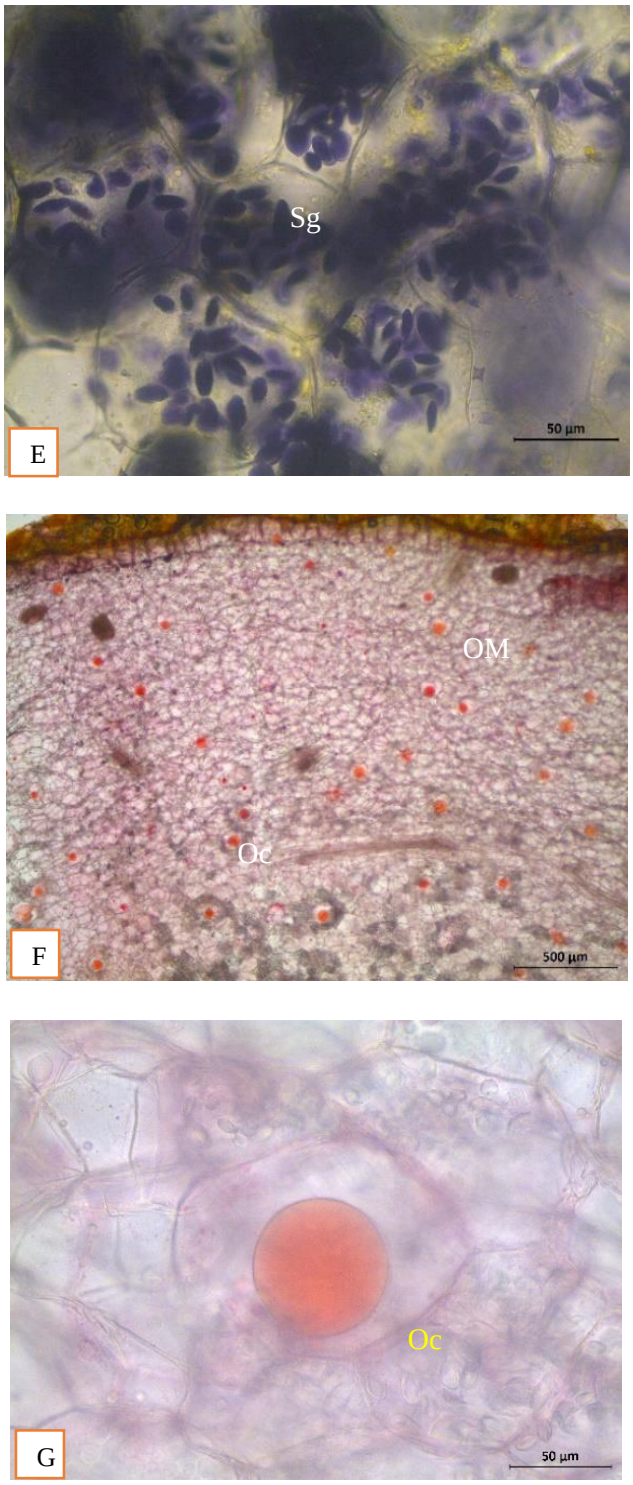


Plate 25: TS of rhizome of *K. rotunda*. (E)-Starch granules (40X), (F)- Oil globules and oleoresin mass in cortical region (4X), (G)- Oil globules (40X). (OM-Oleoresin mass, Oc-Oil globules)

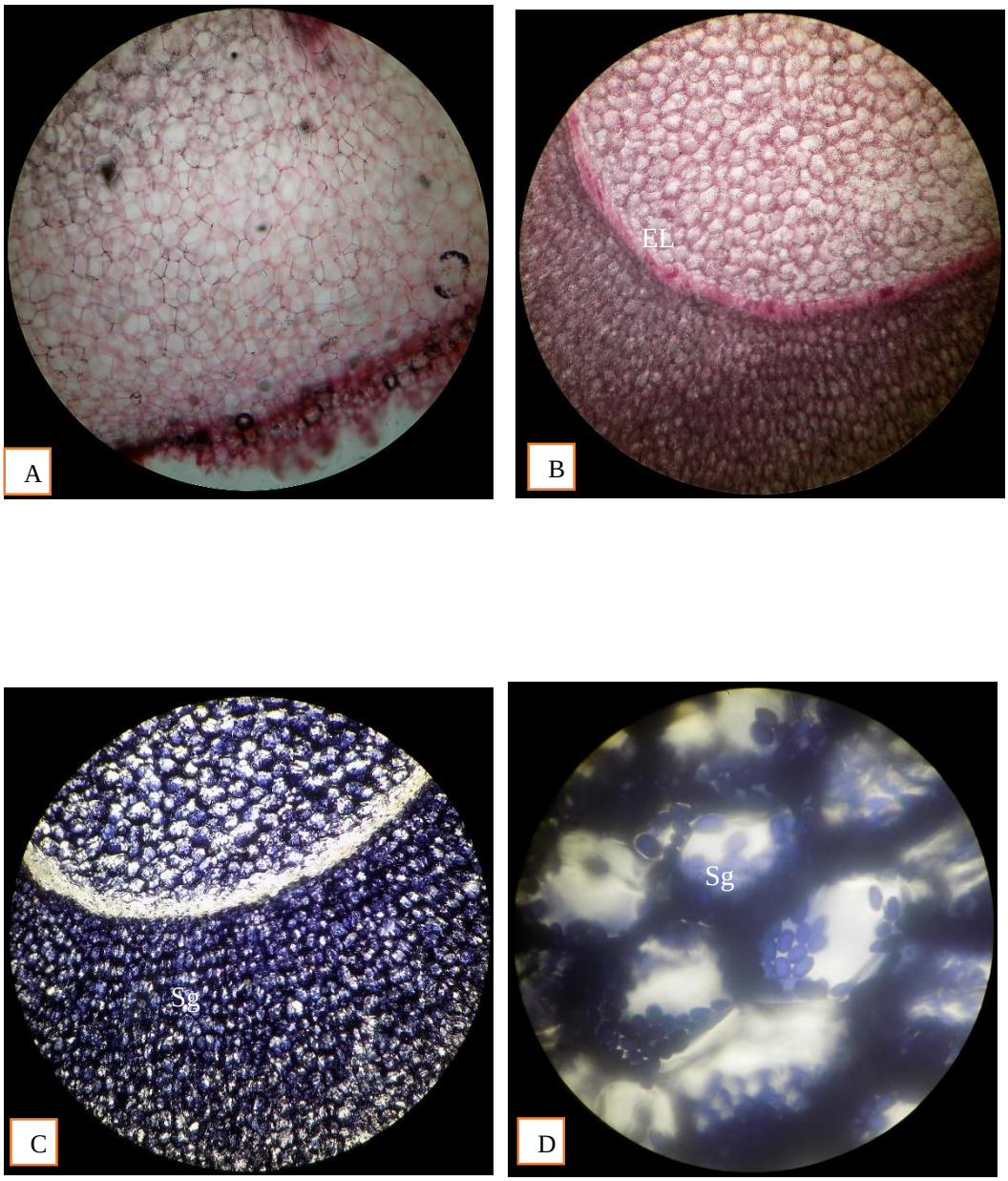


Plate 26: TS of root tuber of *K. rotunda* (A) outer cortex and cortical region (10X), (B)-Endodermal layer (10X), (C)-Starch granules near endodermal layer (10X), (D)- Starch granules (40X). (EL-Endodermal layer, Sg-Starch granules)

4.5 PHARMACOLOGICAL EVALUATION

Ethanolic extract of rhizome of *K. rotunda* and *K. parviflora* were subjected to detailed *in vitro* as well as *in vivo* pharmacological studies and the results are presented here under different sub-heads.

4.5.1 *In vivo* studies

4.5.1.1 Acute toxicity study of ethanolic rhizome extract of K. rotunda and K. parviflora

Acute toxicity test was conducted in Swiss albino mice with dose of 2000 mg/kg b.w. of ethanolic extract of *K. rotunda* and *K. parviflora*. Test animal was observed with special attention for first 30 min and then for 4 h. Observations were noted at regular time intervals throughout the study period (14 days) for behavioural change, mortality and finally post mortem examination was done. Body weight recorded at 0^{th}, 7^{th} and 14^{th} day of experiment is presented in table 97.

Table 97: Effect of ethanolic extract of *K. parviflora* and *K. rotunda* on body weight of mice in acute toxicity study

Day / **Body weight (g)**	***K. rotunda***	***K. parviflora***
0^{th} Day	20.15±0.1	20.75±1.49
7^{th} Day	22±0.70	24.5±1.19
14^{th} Day	24±1.41	28.25±1.18

Values are expressed in mean ± SE. N=6 Swiss albino mice, per group

The body weight of the test animal increased progressively throughout the study period after the administration of ethanolic extract of both *K. rotunda* and *K. parviflora* (table 108). Mean body weight of *K. rotunda* treated animals at 0^{th} day was 20.15±0.1 g and it was 24±1.41 g at 14^{th} day. In case of *K. parviflora* treated animals, mean body weight was 20.75±1.49 g at 0^{th} day which increased to 28.25±1.18 g at 14^{th} day of the experiment.

4.5.1.1.1 Behavioural pattern and post mortem examination

No mortality as well as no behavioural change was observed in the test animals during the experimental period. On 14th day, all the animals were sacrificed and post mortem examination was conducted. No gross lesions were observed in all the organs *i.e*. kidney, liver, heart and spleen of the test animals**.**

4.5.1.2 In vivo immunomodulatory study

The *in vivo* immunomodulatory study was carried out in Swiss albino mice using cyclophosphamide with the following treatments:

Group I: Vehicle control group

Group II: Cyclophosphamide immunosuppressed group

Group III: *K. rotunda* alone treated group

Group IV: *K. rotunda* + cyclophosphamide treated group

Group V: *K. parviflora* alone treated group

Group VI: *K. parviflora* + cyclophosphamide treated group

Results of various immunomodulatory parameters are presented in the following tables.

4.5.1.2.1 Physiological parameters

The weight of individual mouse was recorded before, during (on 12th day) and at the end (19th day) of the experiment. The weight of the organs like spleen and liver were conjointly recorded at the time of sacrifice.

Table 98: Effect of ethanol extract of *K. parviflora* and *K. rotunda* in relative change in body weight in cyclophosphamide immunosuppressed Swiss albino mice, g.

Group	Day	A_I	A_{II}	A_{III}	A_{IV}	A_V	A_{VI}
First six animals	12^{th} day	4.52± 0.61^b	-6.28± 0.41^c	9.65± 0.91^a	2.49± 0.13^b	9.07± 0.66^a	3.28± 0.23^b
Second six animals	12^{th} day	7.20± 0.55^a	-4.53± 0.54^c	4.76± 0.55^a	4.67± 0.53^a	5.45± 0.36^a	1.35± 0.20^b
	19^{th} day	8.26± 0.73^a	-2.66± 0.29^d	4.59± 0.71^b	-5.46± 0.31^e	2.11± 0.28^c	1.39± 0.18^c

Values are expressed in mean ± SE. N=6 Swiss albino mice, per group

Relative change in body weight of the groups A_I to A_{VI} from 0^{th} to 12^{th} day for first six animals and 0^{th} to 12^{th} days, 12^{th} to 19^{th} days for second six animals of the experiment are presented in Table 98.

On 12^{th} day of the experiment, relative change in body weight for first six animals showed significantly increased body weight in *K. rotunda* alone treated group (A_{III}) which was on par with *K. parviflora* alone treated group (A_V) when compared with cyclophosphamide immunosuppressed group (A_{II}). In case of second six animals at 12^{th} day of experiment, increase in body weight was significantly highest in group A_{III}, which was on par with A_{IV}, and A_V when compared with cyclophosphamide alone treated group (A_{II}).

On 19^{th} day, the body weight of normal control showed significantly highest value followed by *K. rotunda* alone treated group.

On all the three stages of observation, cyclophosphamide alone treated group (A_{II}) showed significant reduction in body weight when compared with normal control. *K. rotunda* treated cyclophosphamide immunosuppressed group (A_{IV}) showed significant reduction in body weight on 19^{th} day.

Table 99: Effect of ethanol extract of *K. rotunda* and *K. parviflora* on the liver weight in cyclophosphamide immunosuppressed Swiss albino mice, g/100

Day / Group	A_I	A_{II}	A_{III}	A_{IV}	A_V	A_{VI}
12th day	5.65±0.44	4.76±0.23	6.96±0.62	5.55±0.12	6.82±0.38	5.90±0.37
19th day	5.09±0.31	4.58±0.10	5.85±0.39	5.61±0.15	5.47±0.23	6.40±0.52

Values are expressed in mean ± SE. N=6 Swiss albino mice, per group

The weight of internal organs like spleen and liver was taken on 12th and 19th day of the experiment and expressed as relative organ weights (Table 99). There was no significant difference in liver weight on both 12th and 19th days.

Table 100: Effect of ethanol extract of *K. rotunda* and *K. parviflora* on spleen weight in cyclophosphamide immunosuppressed Swiss albino mice, g/100

Day / Group	A_I	A_{II}	A_{III}	A_{IV}	A_V	A_{VI}
12th day	0.43±0.05	0.42±0.03	1.24±0.03	0.59±0.04	0.66±0.03	0.59±0.03
19th day	0.46±0.04	0.45±0.01	0.60±0.05	0.58±0.02	0.49±0.03	0.60±0.03

Values are express in mean ± SE. N=6 Swiss albino mice, per group

No significant difference was observed in spleen weight on both 12th and 19th days. *K. rotunda* alone treated group (A_{III}) followed by *K. parviflora* alone treated group (A_V) showed higher spleen weight when compared with A_{II} on 12th day of experiment (table 100). On 19th day, *K. rotunda* alone treated group (A_{III}) showed higher spleen weight when compared with A_{II}.

4.5.1.2.2 Haematological parameters

The data on parameters namely total leukocyte count, lymphocyte, monocyte and neutrophil count is presented in table 101.

Table 101: Effect of ethanol extract of *K. rotunda* and *K. parviflora* on the total leukocyte count in cyclophosphamide immunosuppressed Swiss albino mice, 10^3/µl

Group / Day	12th Day	19th Day
A_I	5.56±0.48^b	5.38±0.35^c
A_{II}	4.48±0.48^c	3.45±0.22^d
A_{III}	8.04±0.46^a	6.75±0.38^b
A_{IV}	4.25±0.15^c	4.77±0.19^c
A_V	4.60±0.18^c	10.31±0.51^a
A_{VI}	7.96±0.36^a	6.11±0.37^b

Values are expressed in mean ± SE. N=6 Swiss albino mice, per group

Total leukocyte count recorded on 12th and 19th day of the experiment is presented in the Table 101. Cyclophosphamide control group (A_{II}) showed significant reduction in total leukocyte count when compared with normal control on both 12th and 19th days. On 12th day, the value of A_{II} was on par with A_{IV} and A_V.

On 12th day, both *K. rotunda* alone treated group (A_{III}) and *K. parviflora* treated cyclophosphamide immunosuppressed group (A_V) showed significantly higher leukocyte count when compared with cyclophosphamide control.

On 19th day, significantly higher leucocyte count was noted in *K. rotunda* alone and *K. parviflora* alone treated animals (A_{III} and A_V) when compared with cyclophosphamide control group. Among the immunosuppressed animals, both *K. rotunda* and *K. parviflora* treated mice (A_{IV} and A_{VI}) showed significant increase in leukocyte count when compared with cyclophosphamide control. *K. parviflora* treated cyclophosphamide immunosuppressed mice (A_{VI}) showed significantly higher value when compared with *K. rotunda* treated cyclophosphamide immunosuppressed mice (A_{III}). The highest (10.31±0.51 10^3/µl) total leukocyte count was observed in the *K. parviflora* alone treated mice on 19th day of the experiment.

Table 102: Effect of ethanol extract of *K. parviflora* on the lymphocyte count in cyclophosphamide immunosuppressed Swiss albino mice (%)

Group / Day	12th Day	19th Day
A_I	5.30±0.25[a]	5.14±0.11[b]
A_{II}	3.47±0.31[b]	3.57±0.19[c]
A_{III}	7.72±0.39[a]	5.71±0.08[b]
A_{IV}	3.80±0.14[b]	4.81±0.32[b]
A_V	4.10±0.11[b]	8.08±0.56[a]
A_{VI}	6.68±0.29[a]	5.66±0.16[b]

Values are expressed in mean ± SE. N=6 Swiss albino mice, per group

The data on lymphocyte count in the groups are given in table 102.

On 12th day, cyclophosphamide control group showed significantly lower lymphocyte count when compared with normal control and it was on par with A_{IV} and A_V. *K. rotunda* alone treated mice (A_{III}) showed significantly higher value when compared with cyclophosphamide control (A_{II}). *K. parviflora* treated cyclophosphamide immunosuppressed mice (A_{VI}) showed significantly higher lymphocyte count when compared with cyclophosphamide control (A_{II}).

On 19th day, both *K. rotunda* alone and *K. parviflora* alone treated animals (A_{III} and A_V) showed significant increase in lymphocyte count when compared with cyclophosphamide control group. In case of immunosuppressed mice, both *K. rotunda* and *K. parviflora* treated animals (A_{IV} and A_{VI}) showed significantly higher lymphocyte count when compared with cyclophosphamide control group (A_{II}).

The highest lymphocyte count of 8.08±0.56 per cent was obtained on day 19 in *K. parviflora* alone treated mice (A_V).

Table 103: Effect of ethanol extract of *K. parviflora* on the monocyte count in cyclophosphamide immunosuppressed Swiss albino mice (10^3/µl)

Group / Day	12th Day	19th Day
A_I	0.47±0.04	0.40±0.05
A_{II}	0.42±0.04	0.36±0.03
A_{III}	0.72±0.04	0.67±0.04
A_{IV}	0.15±0.02	0.45±0.02
A_V	0.59±0.05	0.64±0.05
A_{VI}	0.42±0.03	0.59±0.03

Values are expressed in mean ± SE. N=6 Swiss albino mice, per group

The data on monocyte count of six groups of animals are presented in the table 103.

No significant difference was observed among the treatments in case of monocyte count. However, higher monocyte count was noticed in *K. rotunda* alone treated group (A_{III}) and its value was 0.72±0.04 10^3/µl.

Table 104: Effect of ethanol extract of *K. rotunda* and *K. parviflora* on the neutrophil count in cyclophosphamide immunosuppressed Swiss albino mice (%)

Group / Day	12th Day	19th Day
A_I	0.48±0.04[a]	0.51±0.05
A_{II}	0.55±0.06[a]	0.70±0.04
A_{III}	0.46±0.02[a]	0.41±0.04
A_{IV}	0.27±0.03[b]	0.38±0.03
A_V	0.25±0.02[b]	0.44±0.04
A_{VI}	0.34±0.03[b]	0.45±0.04

Values are expressed in mean ± SE. N=6 Swiss albino mice, per group

On 12th day, *K. parviflora* treated cyclophosphamide immunosuppressed group (A_{VI}) showed significant reduction in neutrophil count when compared with cyclophosphamide control (A_{II}) and it was on par with *K. rotunda* treated

cyclophosphamide immunosuppressed group (A_{IV}) and *K. parviflora* alone treated group (A_V) as given in table 104.

No significant difference in neutrophil count was noticed on day 19. The highest neutrophil count was observed in cyclophosphamide control (A_{II}) on both 12^{th} and 19^{th} days.

4.5.1.2.3 Immunological parameters

The data on immunological parameters *viz*. haemaglutination test and bone marrow cellularity is given in table 105 and table 106.

Table 105: Effect of ethanol extract of *K. rotunda* and *K. parviflora* on the haemaglutination titre in cyclophosphamide immunosuppressed Swiss albino mice.

Day / Group	A_I	A_{II}	A_{III}	A_{IV}	A_V	A_{VI}
0^{th} day	14.67± 1.33	12.00± 1.79	14.67± 1.33	12.00± 1.79	12.00± 1.79	12.00± 1.79
12^{th} day	48.00± 7.16^c	6.67± 0.84^c	234.67± 21.33^b	234.67± 21.33^b	938.67± 85.33^a	938.67± 85.33^a
19^{th} day	53.33± 6.76^c	8.00± 1.79^c	256.00± 21.33^a	117.33± 10.67^b	298.67± 42.67^a	149.33± 21.33^b

Values are expressed in mean ± SE. N=6 Swiss albino mice, per group

The data on haemagglutination titre recorded at 0^{th}, 12^{th} and 19^{th} day is presented in table 105.

There was no significant difference among the groups on 0^{th} day. On both 12^{th} day and 19^{th} day, both *Kaempferia* alone treated group (A_{III} and A_V) and *Kaempferia* treated cyclophosphamide immunosuppressed group (A_{IV} and A_{VI}) showed significantly higher value when compared with cyclophosphamide treated group. In immunosuppressed groups on 12^{th} day, *K. parviflora* treated cyclophosphamide immunosuppressed group showed significantly higher value when compared with *K. rotunda* treated cyclophosphamide immunosuppressed group while they were on par on 19^{th} day.

Table 106: Effect of ethanol extract of *K. rotunda* and *K. parviflora* on the bone marrow cellularity in cyclophosphamide immunosuppressed Swiss albino mice, millions

Day / Group	A_I	A_{II}	A_{III}	A_{IV}	A_V	A_{VI}
12th day	4.300[c]	3.390[d]	12.300[b]	4.200[c]	13.900[a]	4.467[c]
19th day	3.130[d]	2.165[c]	8.700[a]	6.800[b]	9.250[a]	7.200[b]

Values are expressed in mean ± SE. N=6 Swiss albino mice, per group

The data on total bone marrow cellularity recorded on 12th and 19th days of the experiment is presented in table 106.

Cyclophosphamide control group showed significantly lower bone marrow cellularity on 12 and 19 days when compared with normal control. Both *K. rotunda* alone and *K. parviflora* alone treated animals showed increase in bone marrow cellularity on days 12 and 19 when compared with normal control. Both *K. rotunda* and *K. parviflora* immunosuppressed mice of A_{IV} and A_{VI} groups showed significant increase in bone marrow cellularity on both 12th and 19th days when compared with cyclophosphamide control. On 12th day, *K. parviflora* alone treated group showed significantly higher cellular count when compared with *K. rotunda* alone treated group while on 19th day the values were statistically on par. In case of immunosuppressed mice, both *K. rotunda* and *K. parviflora* showed statistically on par values on both 12th and 19th days.

The highest bone marrow cellularity was noticed (13.900 millions) in *K. parviflora* alone treated group on 12th day.

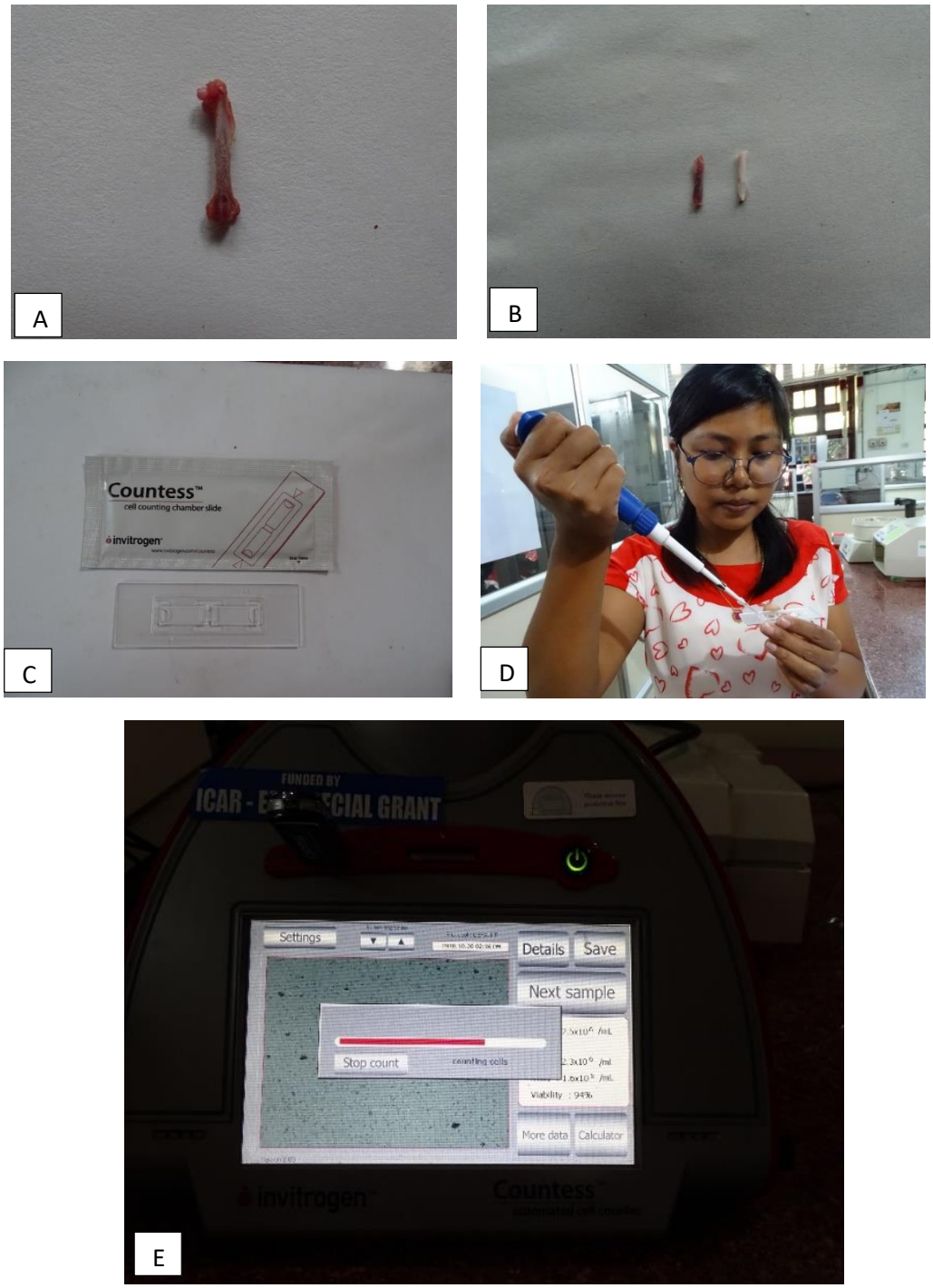


Plate 27: Steps for bone marrow cellularity test. (A) Femur of Swiss albino mice before flushing, (B) Femur of Swiss albino mice after flushing, (C) Cell counting chamber slide, (D) Pipetting out the flushed cells (E) Counting of cells in mammalian cell counter

4.5.1.2.4 Biochemical parameters

The data on mean values of serum protein on 12th and 19th days of the experiment were recorded and presented in table 107.

Table 107: Effect of ethanol extract of *K. rotunda* and *K. parviflora* on the total protein and globulin in cyclophosphamide immunosuppressed Swiss albino mice

Biochemical parameters	Total protein (g/dl)		Globulin (g/dl)	
Groups / Days	12th Day	19th Day	12th Day	19th Day
A_I	5.61±0.20	5.38±0.08[b]	2.48±0.04	2.54±0.16
A_{II}	5.41±0.08	4.66±0.11[c]	2.12±0.08	1.84±0.10
A_{III}	5.61±0.20	5.38±0.08[b]	2.94±0.23	2.43±0.09
A_{IV}	5.34±0.08	5.26±0.08[b]	2.83±0.23	2.20±0.07
A_V	6.37±0.27	5.78±0.13[a]	2.92±0.22	2.45±0.14
A_{VI}	5.80±0.07	5.53±0.11[b]	2.30±0.07	2.15±0.07

Values are expressed in mean ± SE. N=6 Swiss albino mice, per group

On 12th day, no significant difference in serum protein concentration was observed.

There was a significant difference in total protein concentration among the groups on 19th day. Significantly highest value was noticed in *K. parviflora* alone treated group when compared with cyclophosphamide control. In case of immunosuppressed group, both *K. parviflora* and *K. rotunda* showed significantly higher values when compared with cyclophosphamide control.

Though not significant, the highest total protein concentration was observed in *K. parviflora* alone treated group (6.37±0.27 g/dl) on 12th day.

The data on mean values of globulin concentration of six groups on 12th and 19th days is presented in the table 107.

On both 12th and 19th days, no significant difference in globulin concentration was observed.

Table 108: Effect of ethanol extract of *K. rotunda* and *K. parviflora* on the foot pad thickness, mm

Day / Group	B_I	B_{II}	B_{III}	B_{IV}	B_V	B_{VI}
12th day	0.37± 0.02[d]	0.34± 0.02[d]	2.49± 0.16[a]	0.77± 0.04[c]	1.01± 0.05[b]	0.54± 0.04[d]
19th day	0.33± 0.04[b]	0.28± 0.03[b]	0.61± 0.05[a]	0.53± 0.09[a]	0.50± 0.05[a]	0.69± 0.06[a]

Values are expressed in mean ± SE. N=6 Swiss albino mice, per group

The data on foot pad thickness of all groups of animals on 12th and 19th days are depicted in table 108.

Both *K. rotunda* alone and *K. parviflora* alone treated mice (B_{III} and B_V) as well as immunosuppressed mice (B_{IV} and B_{VI}) showed significant increase in foot pad thickness on 12th and 19th days when compared with normal control. In case of immunosuppressed mice, *K. rotunda* treated mice showed significantly higher foot pad thickness when compared with *K. parviflora* treated mice on 12th day. On 19th day, *K. parviflora* treated cyclophosphamide treated group recorded significantly highest value which was on par with B_{III}, B_{IV} and B_V (Plate 28).

The highest foot pad thickness was noticed in *K. rotunda* alone treated group (2.49±0.16 mm) on 12th day. The lowest was observed in cyclophosphamide control on both 12th and 19th days and it was on par with vehicle control group.

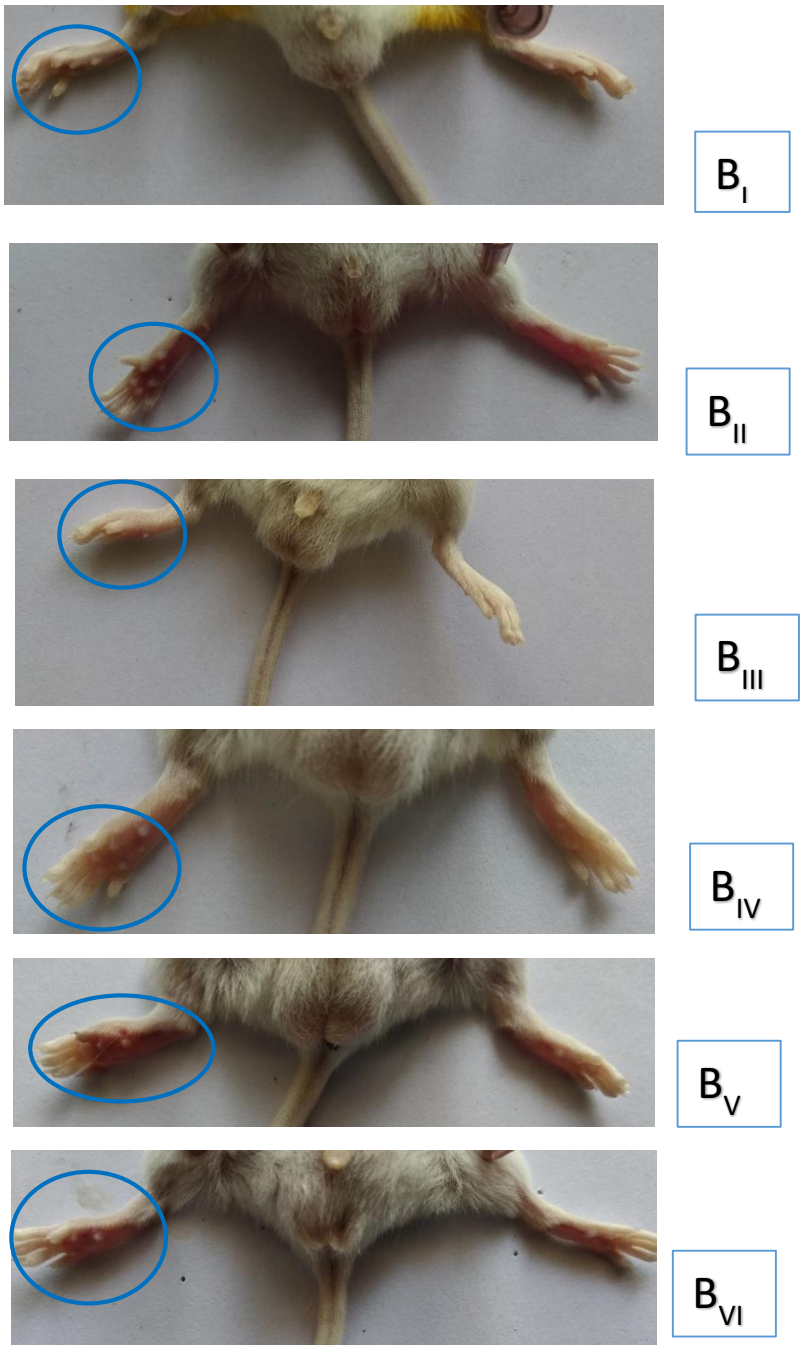


Plate 28: Delayed type hypersensitivity reaction on foot pad of swiss albino mice. B_I-normal control, B_{II}-Cyclophosphamide control, B_{III}-K. rotunda alone, B_{IV}-*K. rotunda* + Cyclophosphamide, B_V-*K. parviflora* alone, B_{VI}-*K. parviflora* + Cyclophosphamide

4.5.1.2.5 Histopathology of spleen

The histopathology of spleen is presented in Plates 29 to 30.

The vehicle control group revealed normal splenic architecture with white pulp and red pulp throughout the study (Plate 29A), whereas, marked lymphoid depletion was observed in white pulp region in cyclophosphamide induced mice as compared to normal control animals (Plate 29 B).

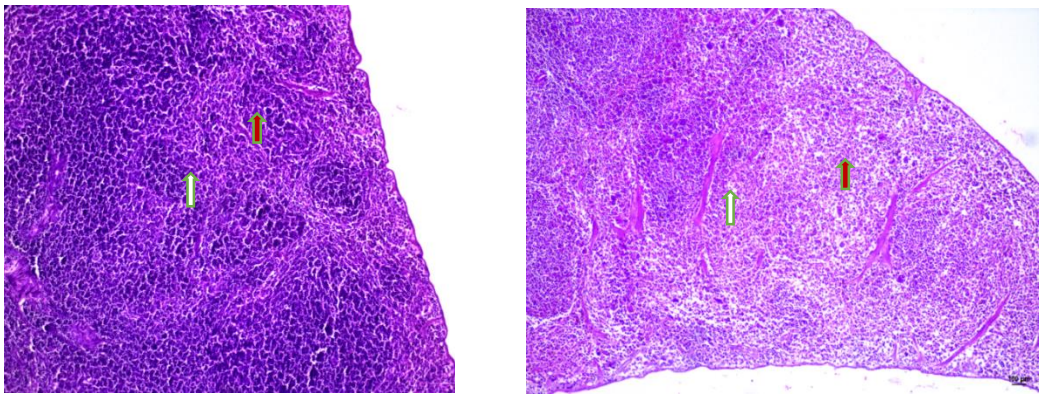
Administration of *K. rotunda* induced hyperplasia of lymphocyte in white and red pulps (Plate 29 C), while there was proliferation of lymphocytes in white pulp and mild depletion was also observed in the marginal zone on 12^{th} day (Plate 29 D) of *K. rotunda* treated cyclophosphamide immunosuppressed mice.

K. parviflora alone treated group revealed that both white pulp and red pulp had proliferation of lymphocytes (Plate 29 E). However, *K. parviflora* treated cyclophosphamide immunosuppressed group attenuated the cyclophosphamide induced lymphocyte depletions in white pulp and red pulp region (Plate 29 F).

On 19^{th} day also vehicle control group showed both red pulp and white pulp packed with lymphocytes (Plate 30 A), while cyclophosphamide group showed severe depletion of lymphocytes in white pulps and marginal zones (Plate 29 B).

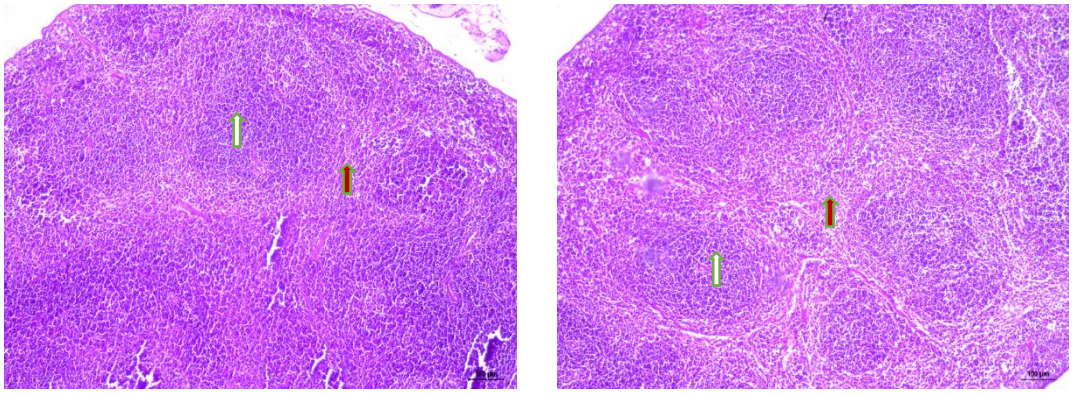
K. rotunda alone treated group showed hyperplasia of lymphocyte with new germinal centre formation on 19^{th} day (Plate 30 C) In case of *K. rotunda* treated cyclophosphamide immunosuppressed mice, moderate attenuation of lymphocyte depletion was noticed on 19^{th} day (Plate 30 D).

Administration of *K. parviflora* induced hyperplasia in white pulp region in 19^{th} day (Plate 30 E). However, administration of *K. parviflora* along with cyclophosphamide exhibited marked attenuation of lymphocyte depletions on 19^{th} day (Plate 30 F).



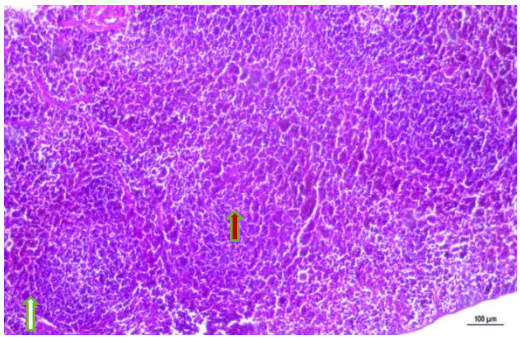
(A) (B)

Plate 29: Light microscopic images of spleen from different groups of animals at 12th day. (A): Control group- Red pulp and white pulp packed with lymphocytes (H &E 100X). (B): Cyclophosphamide group- Depletion of lymphocytes in both white pulp and marginal zone (H & E 100X)

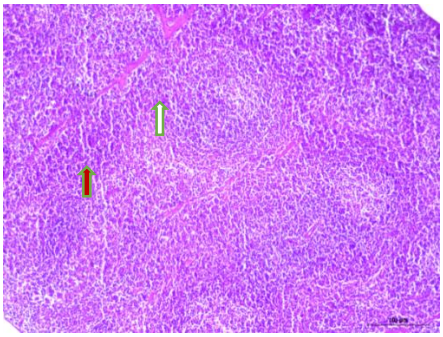


(C) (D)

Plate 29: Light microscopic images of spleen with different groups of animals at 12th day. (C): *K. rotunda* alone treated group – Hyperplasia of lymphocytes in white pulp and red pulp (H &E 100X). (D): *K. rotunda* treated cyclophosphamide immunosuppressed group- Proliferation of lymphocytes in white pulp, mild depletion was also observed in marginal zone (H &E 100 X)

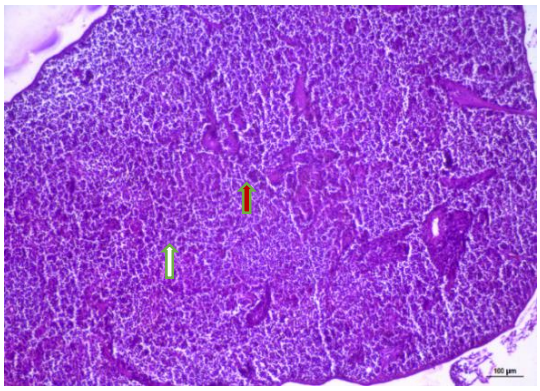


(E)

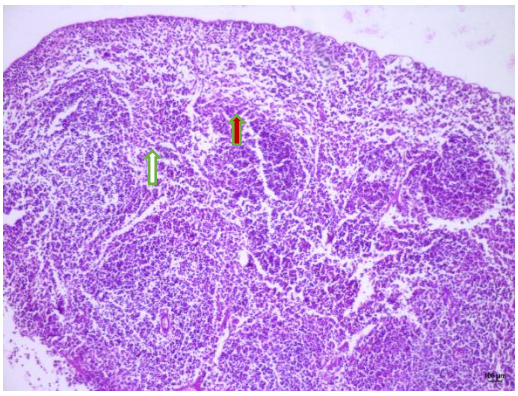


(F)

Plate 29: Light microscopic images of spleen with different groups of animals at 12th day. (E): *K. parviflora* alone treated group- Revealed white pulp and red pulp contained proliferation of lymphocytes (H & E 100X). (F): *K. parviflora* treated cyclophosphamide immunosuppressed group- Attenuated the cyclophosphamide induced lymphocyte depletions in white pulp and red pulp region (H & E 100X)

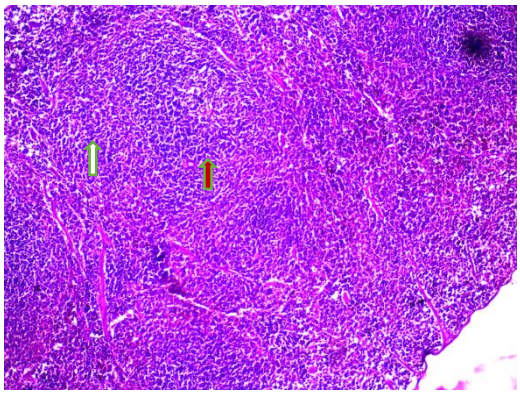


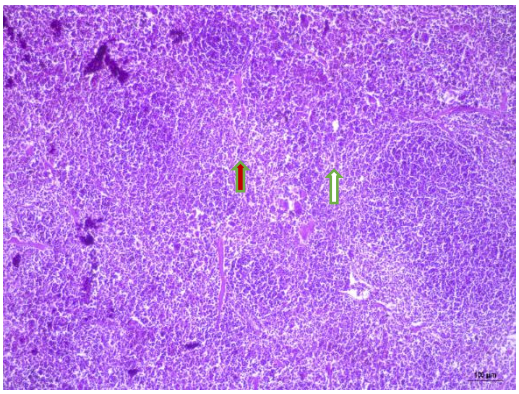
(A)



(B)

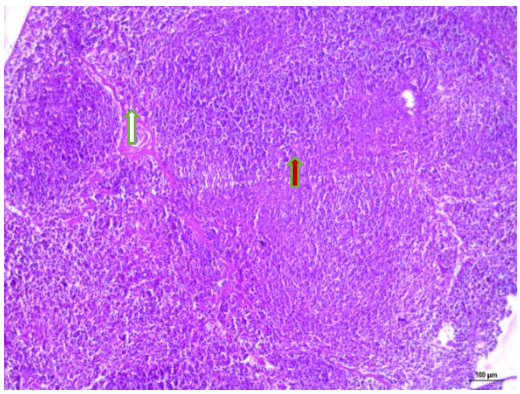
Plate 30: Light microscopic image of spleen with different groups of animals at 19th day. (A): Control group- Red pulp and white pulp packed with lymphocytes (H & E 100X). (B): Cyclophosphamide group- Severe depletion of lymphocytes in white pulp and marginal zone (H & E100X)

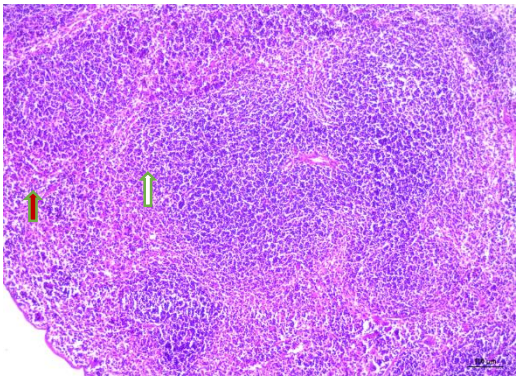




(C) (D)

Plate 30: Light microscopic image of spleen with different groups of animals at 19th day. (C): *K. rotunda* alone treated group – Hyperplasia of lymphocyte with new germinal centre formation (H & E 100X). (D): *K. rotunda* treated cyclophosphamide immunosuppressed group- Ameliorated effect of *K. rotunda* was appeared as proliferation of lymphocytes in white pulp. (H & E 100X)





(E) (F)

Plate 30: Light microscopic image of spleen with different groups of animals at 19th day. (K): *K. parviflora* treated group revealed hyperplasia of lymphocyte with new germinal centre (H &E100X). (L): *K. parviflora* treated cyclophosphamide immunosuppressed group - Attenuation of cyclophosphamide induced lymphocyte depletion (H & E 100X).

(Red arrow indicate white pulp and white arrow indicate white pulp)

4.5.3 *In vitro* studies

4.5.3.1 In vitro antioxidant activity

The per cent inhibition of DPPH radical by ethanolic rhizome extract *K. rotunda* and *K. parviflora* at different concentrations is given in the table 109.

Table 109: DPPH free radical scavenging assay: the per cent inhibition of DPPH free radical by *K. rotunda* and *K. parviflora*

Concentration (µg/ml)	% inhibition of DPPH free radical		
	K. rotunda	***K. parviflora***	**Vitamin C standard**
500 µg/ml	78.95±1.37 [a]	68.42±0.73 [a]	93.80±0.88 [a]
250 µg /ml	72.01±1.53 [b]	52.27±0.53 [b]	85.51±0.80 [b]
125 µg /ml	55.61±2.04 [c]	43.21±0.41 [c]	79.21±0.80 [c]
62.5 µg /ml	35.20±1.39 [d]	31.61±0.36 [d]	46.09±2.28 [d]

The per cent inhibition of DPPH radical by ethanolic extract of *K. rotunda* at concentrations ranging from 62.5 to 500 µg/ml is given in Table. The per cent inhibition was 78.95±1.37 at 500 µg/ml, and 35.20±1.39 at 62.5 µg/ml. The per cent inhibition was comparable to Vitamin C. The Inhibitory concentrations 50 (IC_{50}) calculated for *K. rotunda* was 131.15±4.83 µg/ml, and that for Vitamin C was 116.1±5.05 µg/ml.

The per cent inhibition for *K. parviflora* was 68.427±0.73 at 500 µg/ml, and 31.61±0.36 at 62.5 µg/ml. The per cent inhibition was comparable to Vitamin C. The Inhibitory concentrations 50 (IC_{50}) calculated for *K. parviflora* was 198.68±7.62 µg/ml.

4.5.3.2 In vitro anticancer activity

The cytotoxicity of ethanol extract of *K. rotunda* and *K. parviflora* assessed in MDA-MB-231 and MCF-7 cell lines using MTT assay is presented in table 110 and table 111. The doses taken for pilot study were 2.5, 5, 10, 20, 40, 80, 160, 320 and 500 μgmL^{-1} from which the dose for main study was selected in range of 5 to 500 μgmL^{-1}.

Table 110: The per cent cell viability of MDA-MB-231 and MCF-7 cells after 48 h treatment with *K. rotunda* determined by MTT reduction assay

Concentration (µg/ml)	% inhibition of *K. rotunda*	
	MCF cells	MDA-MB231 cells
500	76.70±1.18 [a]	62.82±1.45 [a]
320	68.77±1.27 [b]	46.78±1.73 [b]
160	52.63±1.47 [c]	35.77±1.41 [c]
80	27.82±1.68 [d]	12.77±0.73 [d]

Positive results were obtained at concentrations of 80 to 500 µg/ml. The results of MTT reduction assay after 48 h treatment with *K. rotunda* in MDA-MB-231 and MCF-7 tumour cell lines are presented in Table 110. The per cent inhibition for MCF-7 cells was 76.70±1.18 at 500 µg/ml, and 27.82±1.69 at 80 µg/ml while for MDA-MB-231 cells, per cent inhibition were 62.82±1.46 at 500 µg/ml and 12.77±0.73 at 80 µg/ml. The half maximal inhibitory concentration (IC_{50}) for *K. rotunda* was found to be 194.80±8.97 µg/ml for MDA-MB-231 cells and 167.1±5.60 µg/ml for MCF-7 cells.

Table 111: The per cent cell viability of MDA-MB-231 and MCF-7 cells after 48 h treatment with *K. parviflora* determined by MTT reduction assay

Concentration (µg/ml)	% inhibition of *K. parviflora*	
	MCF cells	MDA-MB231 cells
500	92.51±0.25 [a]	69.85±0.39 [a]
320	74.01±0.31 [b]	60.04±0.63 [b]
160	55.12±0.63 [c]	42.41±0.48 [c]
80	36.66±0.61 [d]	34.01±0.55 [d]
40	27.20±0.19 [e]	29.60±0.38 [e]
20	21.05±0.14 [f]	26.21±0.78 [f]
10	18.46±0.41 [g]	22.89±0.18 [g]
5	15.99±0.18 [h]	17.97±0.41 [h]

Ethanolic extract of *K. parviflora* was assessed in MDA-MB-231 and MCF-7 cell lines using MTT assay for cytotoxicity. Positive results were obtained at concentrations of 5 to 500 µg/ml. The results of MTT reduction assay after 48 h treatment with *K. parviflora* in MDA-MB-231 and MCF-7 tumour cell lines are given in Table. The per cent inhibition for MCF-7 cells was 92.52±0.25 at 500 µg/ml, and 15.99±0.18 at 5 µg/ml while for MDA-MB-231 cells, per cent inhibition was 69.85±0.39 at 500 µg/ml and 17.98±0.41 at 5 µg/ml. The half maximal inhibitory concentration (IC_{50}) for *K. parviflora* was found to be 126.35±2.53 µg/ml for MDA-MB-231 cells and 143.03±2.70 µg/ml for MCF-7 cells.

4.5.3.3 In vitro antimicrobial studies

The antimicrobial activity of ethanolic extract of *K. rotunda* and *K. parviflora* were tested against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enterica* and *Pseudomonas aeruginosa* and the data are furnished in table 112 and 113.

Table 112: Zone of inhibition for the ethanolic extract of *K. rotunda* at different concentrations

Concentration (mg/ml)	*E. coli*	*S. enterica*	*S. aureus*	*P. aeruginosa*
150	8.17±0.31 [d]	7.17±0.31 [d]	11.62±0.20 [d]	7.85±0.30 [d]
200	9.33±0.21 [c]	10.42±0.20 [c]	12.62±0.20 [c]	9.73±0.48 [c]
250	11.13±0.16 [b]	11.47±0.29 [b]	14.18±0.32 [b]	11.52±0.38 [b]
-ve control	0.00	0.00	0.00	0.00
+ve control	22.58±0.21 [a]	22.00±0.37 [a]	27.83±0.48 [a]	26.33±0.49 [a]

Kaempferia rotunda exhibited significant inhibition of all microorganisms used in the present experiment. The data showed that *K. rotunda* extract at the concentration of 250 mg/ml had significantly highest zone of inhibition (11.13±0.16 mm) against *E. coli*. The zone of inhibition reduced with reduction in the concentration of plant extract. In case of *Salmonella*, the diameter of the zone of inhibition was significantly highest (11.47±0.29 mm) in the extract concentration of 250 mg/ml. The significantly highest antimicrobial activity with the zone of inhibition 14.18±0.32mm was observed against *Staphylococcus* in the present experiment. The study also showed antimicrobial activity of *K. rotunda* at the extract concentration of 150 to 250mg/ml against *Pseudomonas*. In case of standard drugs used, the zone of inhibition were 22.58±0.201, 22.00±0.37, 27.83±0.48 and 26.33±0.49 respectively against *E. coli*, *Salmonella*, *Staphylococcus* and *Pseudomonas.* The negative control was DMSO which showed no inhibition at all.

Table 113: Zone of inhibition for the ethanolic extract of *K. parviflora* at different concentrations

Concentration (mg/ml)	*E. coli*	*S. enterica*	*S. aureus*	*P. aeruginosa*
150	8.83±0.26 [d]	10.45±0.14 [d]	12.92±0.05 [d]	7.15±0.06 [d]
200	10.23±0.16 [c]	12.22±0.09 [c]	13.85±0.17 [c]	8.35±0.21 [c]
250	12.32±0.12 [b]	13.8±0.16 [b]	15.48±0.23 [b]	11.17±0.31 [b]
-ve control	0.00	0.00	0.00	0.00
+ve control	23.67±0.21 [a]	28.83±0.31 [a]	27.83±0.31 [a]	23.17±0.48 [a]

Kaempferia parviflora showed significant inhibition of all microorganisms used in the present experiment. The data showed that *K. parviflora* extract at the concentration of 250 mg/ml had significantly highest zone of inhibition (12.32±0.12 mm) against *E. coli*. The zone of inhibition reduced with reduction in the concentration of plant extract. In case of *S. enterica*, the diameter of the zone of inhibition was significantly highest (13.8±0.16 mm) in the extract concentration of 250 mg/ml. The significantly highest antimicrobial activity with the zone of inhibition 15.48±0.23mm was observed against *S. aureus* in the present experiment. The study also showed antimicrobial activity of *K. parviflora* against *P. aeruginosa* with zone of inhibition 11.17±0.31 mm at 250 mg/ml. In case of standard drugs used, the zone of inhibition was 23.67±0.21, 28.83±0.31, 27.83±0.31and 23.17±0.48 respectively against *E. coli*, *Salmonella*, *Staphylococcus* and *Pseudomonas.* Dimethyl Sulphoxide (DMSO) which was used as negative control showed no zone of inhibition.

Discussion

5. DISCUSSION

The results of the study entitled "Performance analysis of medicinal *Kaempferia* species" carried out in the department of Plantation crops and Spices, College of Horticulture is discussed in this chapter.

Three species of *Kaempferia* were used in the study *viz*. *K. rotunda*, *K. parviflora* and *K. galanga*. *Kaempferia rotunda* and *K. galanga* are commonly found in India, whereas *K. parviflora* is of rare occurrence in India. *K. rotunda* is commonly known as Peacock ginger, green ginger, *Bhoomi champa* (Hindi), *Chenghanir Khirangu* (Malayalam) and *Leipaklei* (Manipur). *K. galanga* is popularly known as aromatic ginger, *Kacholam*, *Kachhuram* (Malayalam), *Chandramoolika* (Sanskrit), *Chandramula* (Hindi). *K parviflora* is known as Black ginger, Thai ginseng or *Krachai dum* (Thailand), *Khongban takhellei* (Manipur).

5.1 MORPHOLOGICAL EVALUATION

There were thirteen collections of *K. rotunda* and three collections of *K. parviflora*. Two collections of *K. galanga*, an already exploited medicinal crop were also added in the evaluation as a reference species.

5.1.1 *Kaempferia rotunda*

5.1.1.1 Qualitative parameters

All the *K. rotunda* genotypes evaluated in the present study possessed similar qualitative characters. The fleshy rhizomes possessed brownish outer skin and off-white inner core. Leaves were erect/lanceolate with entire margin. Root tubers were seen arising from the rhizome in abundance.

In her study, Sereena *et al*. (2011) also reported *Kaempferia rotunda* rhizomes as very fleshy, 3 - 3.5 cm in length and 1.5 - 1.75 cm in diameter with several root tubers. Each tuber had the shape of club with a bulged lower portion and a stalk like cylindrical upper portion. Phokham *et al*. (2013) observed hairy petioles and elliptic to lanceolate-oblong leaves in *K. rotunda*.

5.1.1.2 Days to spout

From the two year data it was evident that *K. rotunda* took 5.67 to 17.92 days to sprout (Fig.1). The Kerala collection KCR-2 was earliest to sprout, whereas the Manipur collection MCR-1 took the highest days to sprout. Variation in number of days to sprout noticed in the present study is supported by findings of Divya (2008) in *K. galanga*. In the present study, planting was done during 15th May which coincided with summer rains which is favourable for the sprouting of rhizomatous crops. In *Curcuma alismatifolia*, rhizomes sprouted 2-3 weeks after planting during rainy season (April to May) depending on the size and environmental condition (Ruamrungsri, 2015). In the same species, rhizome with greater number of root tubers sprouted faster than those with fewer number of root tubers (Paz, 2003). In the present study also rhizomes along with root tubers were used for planting which might have speeded up the sprouting.

5.1.1.3 Number of tillers per plant

Significant variation was observed in tiller production per plant among *K. rotunda* genotypes (Fig. 2). Tiller production was not observed upto 60 DAP in any of the genotypes and at 90 DAP, tillers were observed only in seven genotypes during 2017-18, whereas it was noticed in 12 genotypes during 2018-19. Highest tiller production was obtained at 180 DAP in all the genotypes and thereafter it declined. Genotype KCR-3 produced the highest number of tillers and lowest was recorded in the genotype KCR-6. Variation in tiller production might be due to genetic makeup of the types. Highest tiller production at 180 DAP indicate that the species reaches maximum growth at this stage. Variation in number of tillers was reported by Latha (1994) and Divya (2008) in *K. galanga*. In *Curcuma amada*, tillers produced per plant ranged from 1 to 4 among the nine accessions studied at Tsukuba, Japan (Jatoi *et al*., 2015). Number of tillers per plant in turmeric ranged from 1.6 to 3.4 as reported by Naidu and Murthy, (2013).

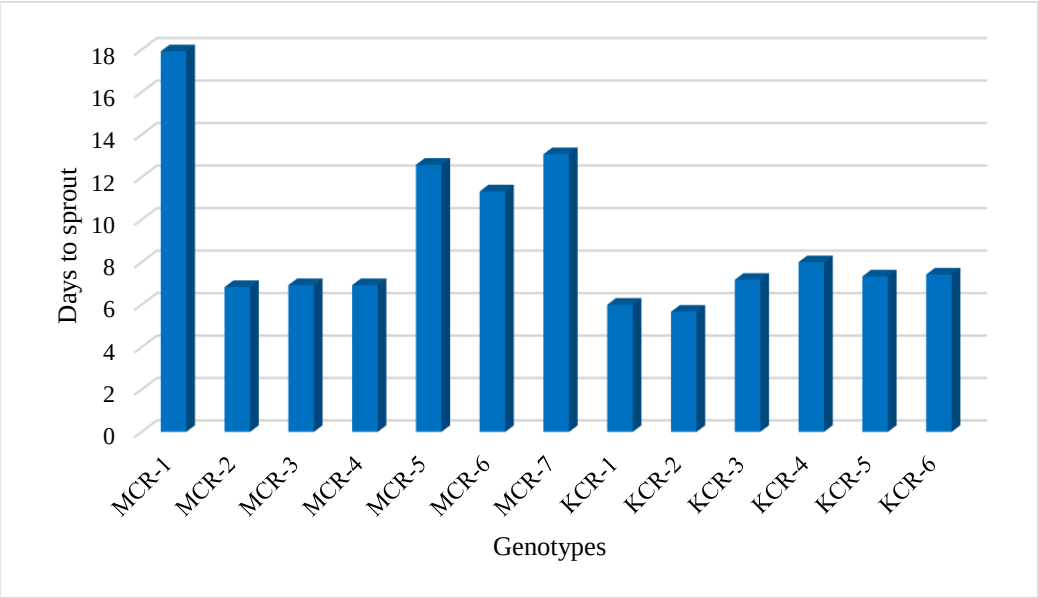


Figure 1: Days to sprout in *K. rotunda* genotypes

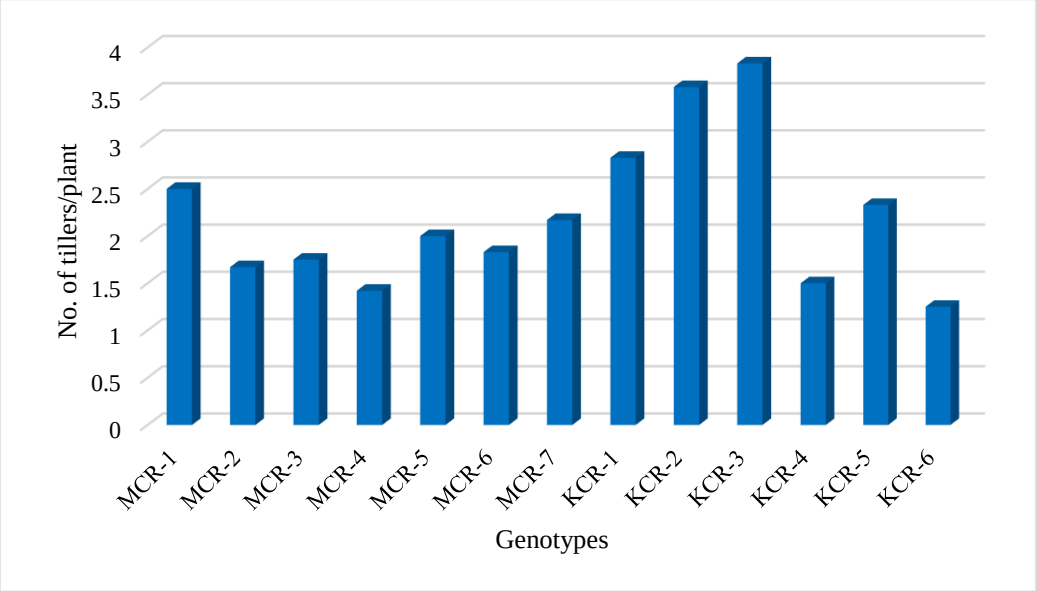


Figure 2: Number of tillers per plant at 180 DAP in *K. rotunda* genotypes

5.1.1.4 Number of leaves per plant

In the present experiment, observations were recorded at one month interval for the number of leaves produced. It could be noticed that during all months there was significant variation in the number of leaves produced (Fig.3). Leaf production increased steadily and reached highest at 180 DAP and declined thereafter. The genotypes KCR-1 and KCR-2 ranked first with respect to number of leaves and the lowest was recorded in genotype KCR-6. Variation in number of leaves may be explained as the result of the indirect influence of plant height and number of tillers as reported by Nybe (1978) in ginger. In ginger, number of leaves per plant ranged from 12.58 to 14.87 at 14 weeks after planting (Egbuchua and Enujeke, 2013). In this study also genotype KCR-1 recorded the higher value for plant height and KCR-2 recorded the higher number of tillers.

5.1.1.5 Leaf length, breadth and Leaf area

As in the case of other growth parameters, leaf length, breadth and leaf area reached the highest value at 180 DAP (Fig. 4 and Fig 5). Significant variation was noticed in length, breadth and leaf area among the genotypes at all growth stages. At 180 DAP, the highest leaf length as well as leaf area was observed in genotype MCR-6, whereas highest leaf breadth was noticed in genotype MCR-3. The variability in length, breadth and leaf area among the genotypes might be due to the inherent character of the genotypes. The present results reveal the better production capacity of vegetative growth by the genotypes MCR-6, MCR-3, and MCR-4. Latha (1994) has reported variation in leaf length, breadth and leaf area in *K. galanga* genotypes. A study carried out in *Kaempferia elegans* accessions collected from south India by Sabu *et al*. (2013) showed variation in leaf length and breadth. Similar variations in leaf length and breadth have been reported in turmeric by Kumar *et al*. (2015) and in leaf area by Krishna *et al*. (2019).

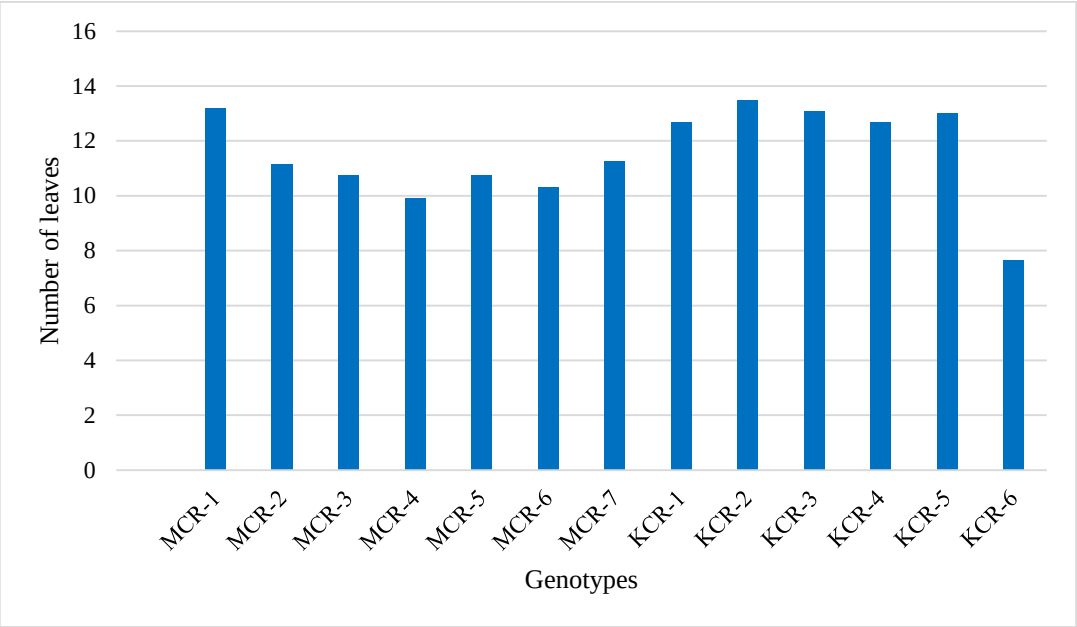


Figure 3: Number of leaves at 180 DAP in *K. rotunda* genotypes

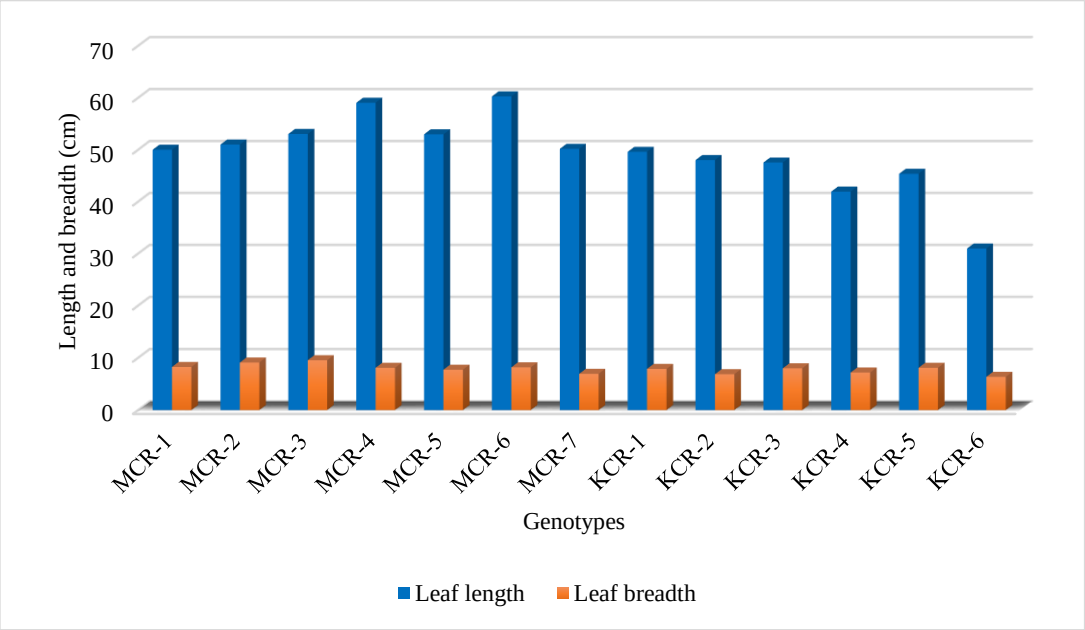


Figure 4: Leaf length and breadth at 180 DAP in *K. rotunda* genotypes

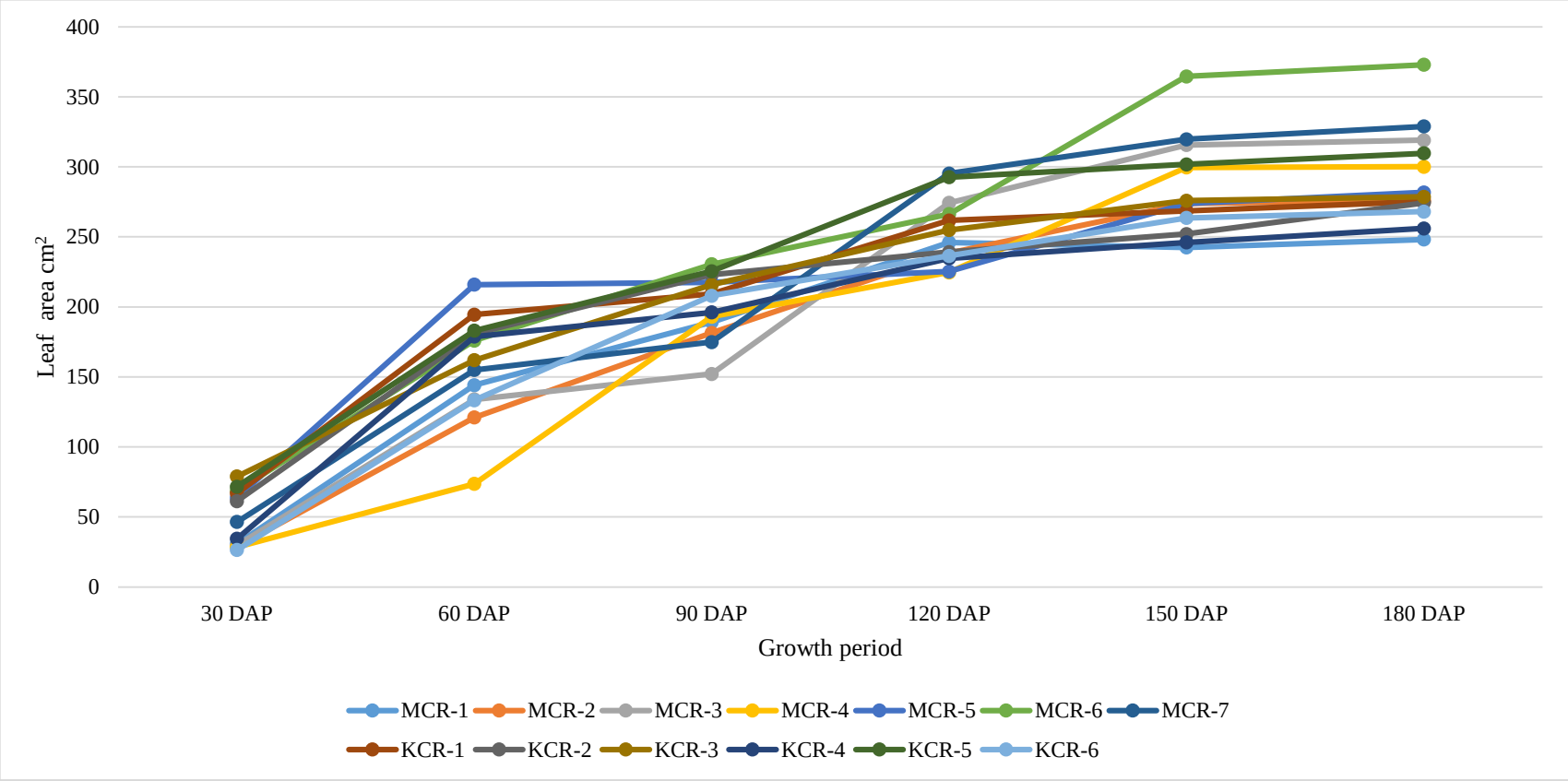


Figure 5: Leaf area at different growth stages in *K. rotunda* genotypes

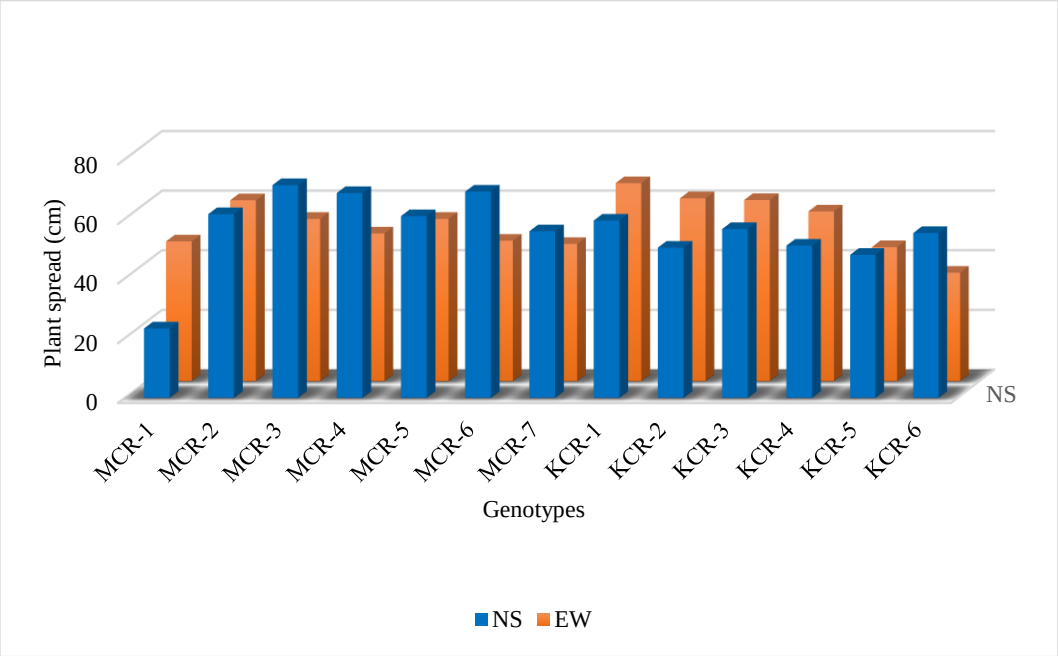


Figure 6: N-S and E-W spread of *K. rotunda* genotypes

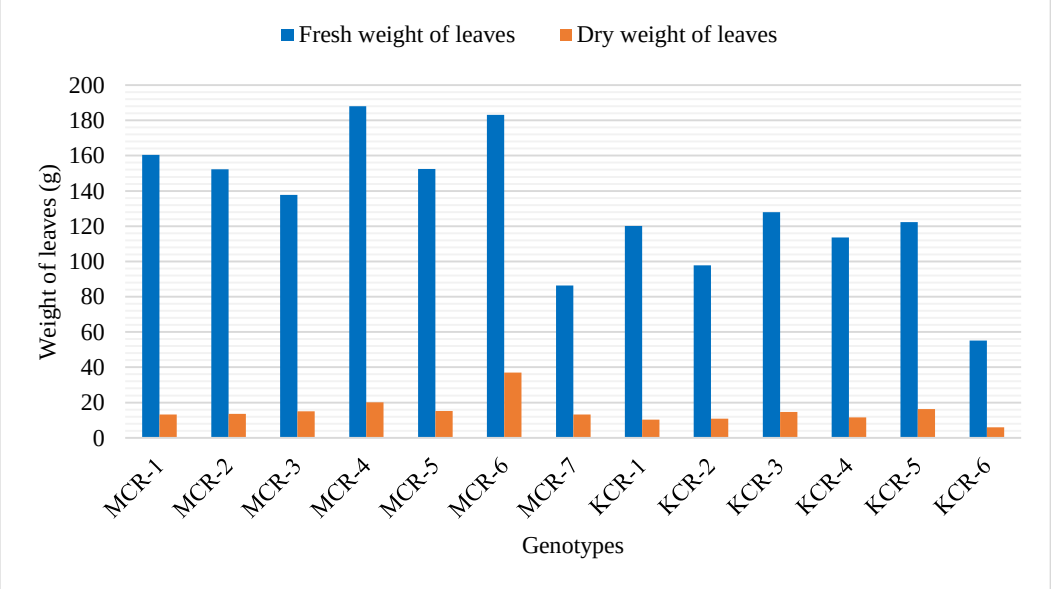


Figure 7: Fresh and dry weight of leaves per plant in *K. rotunda* genotypes

5.1.1.6 Plant spread (NS and EW)

Plant spread was found to be significant among the genotypes (Figure 6). North-south spread was highest in the genotype MCR-3 and it was on par with MCR-6 and MCR-4 (Fig 6.). East-west spread was highest in the genotype KCR-1 and it was on par with KCR-2, KCR-3 and MCR-2. Both the number of tillers as well as leaf area are the contributing factors for higher plant spread. In the present study genotypes having higher number of tillers/leaf area have shown higher plant spread. Similar findings have been reported by Latha (1994) and Divya (2008) in *K. galanga*.

5.1.1.7 Fresh and dry weight of leaves per plant

There was considerable variation among the genotypes in both the fresh and dry weight of leaves at 180 DAP (Fig. 7). The highest fresh weight was recorded in the genotype MCR-4 and it was on par with MCR-6, whereas genotype MCR-6 recorded the highest dry weight of leaves. These genotypes recorded the highest plant spread also, which could be the reason for the highest fresh as well as dry weight of leaves. In *curcuma xanthorhizha*, fresh and dry weight of leaves per plant was 118.07 g and 16.50 g, respectively when strip relay cropping system was followed (Nihayati *et al*., 2017).

5.1.1.8 Plant height

The plant height reached peak at 180 DAP and it ranged from 66.33 to 116.25 cm among the genotypes. The tallest plant was observed in genotype MCR-6 followed by KCR-1 and shortest in KCR-6 (Fig. 8). However, according to Joy *et al*., (2006), the plant height recorded in *K. rotunda* ranged from 40.3 cm to 47.7 cm only. Low light intensity under rain shelter could cause increment in plant height (Nimisha, 2018). Similar result on varying plant height in genotypes has been reported by Basak *et al*. (2019) and Jutoi and Watanabe (2013) in ginger.

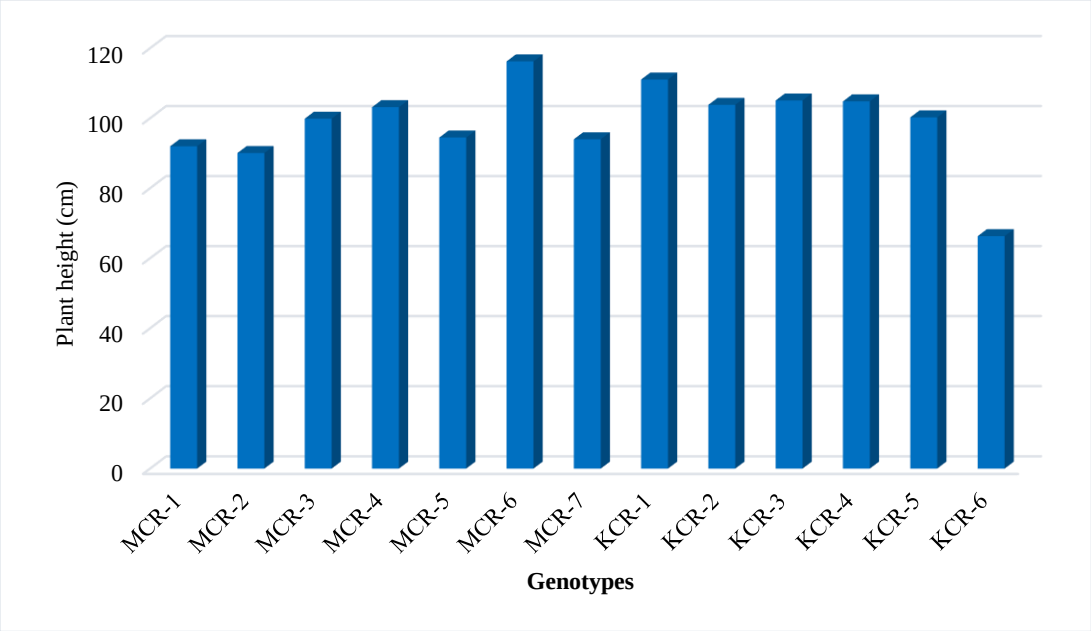


Figure 8: Plant height of *K. rotunda* genotypes

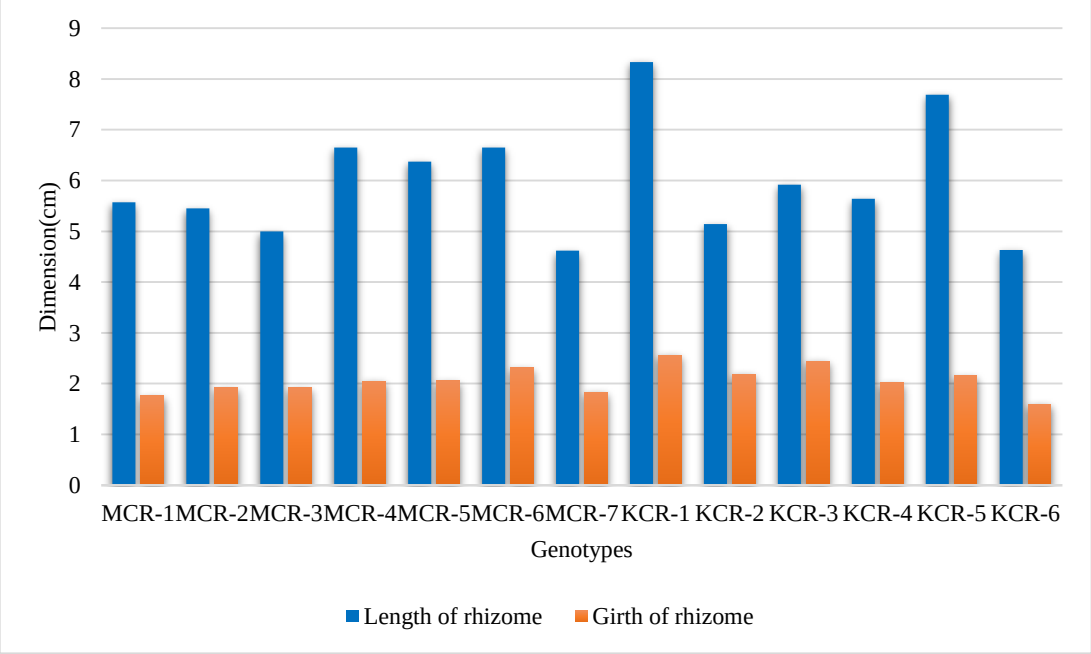


Figure 9: Length and girth of rhizome in *K. rotunda* genotypes

5.1.1.9 Length and girth of rhizome

The genotypes differed significantly in the length and girth of rhizome (Figure 9). Significantly highest value for dimensions rhizome was recorded in the genotype KCR-1. Sereena *et al*. (2011) have recorded fresh rhizome length of *K. rotunda* as 3 - 3.5 cm and the girth as 1.5 - 1.75 cm. Variability in length and girth of rhizome has been reported by Nimisha (2018) in ginger and Latha (1994) in *K. galanga*.

5.1.1.10 Fresh and dry yield of rhizomes

Rhizome yield is the most important economic trait which decides the superiority of a genotype. In the present study, fresh and dry weight of rhizome differed significantly among the genotypes (Fig. 10). The highest fresh as well as dry yield was recorded in the genotype MCR-6 (52.44 and 17.73 g, respectively). The genotype MCR-6 had higher leaf area, plant spread, fresh and dry weight of leaves which might have resulted in higher photosynthesis and contributed to higher fresh and dry weight of rhizome. A direct and positive contribution to dry rhizome yield by fresh rhizome yield was reported by Chandana (2011) in *K. galanga*. Higher yield could be the result of maximum vegetative growth with more number of leaves and maximum foliage spread substantiating the fact that optimum vegetative growth is a pre requisite for realizing high yield. Fresh rhizome yield of *K. galanga* ranged from 29.7 to 48.6 g/plant and dry rhizome yield from 6.5 to 14.5 g/plant among 12 types as reported by Prasannakumari *et al*. (1997). Variation was observed in fresh rhizome yield, dry rhizome yield and oil yield among the 10 genotypes of *K. galanga* evaluated by Latha (1994) evaluated under Kerala conditions. Joy *et al*. (2006) have recorded fresh weight of rhizome as 12.98 t/ha and dry weight of rhizome as 3.90 t/ha in *K. rotunda*. In ginger there are studies where number of tillers, aerial shoots and leaves are positively correlated with fresh weight of rhizome (Devi *et al*., 2015). Difference in varietal character for rhizome yield has also been reported in ginger by Mohanty *et al*. (1981).

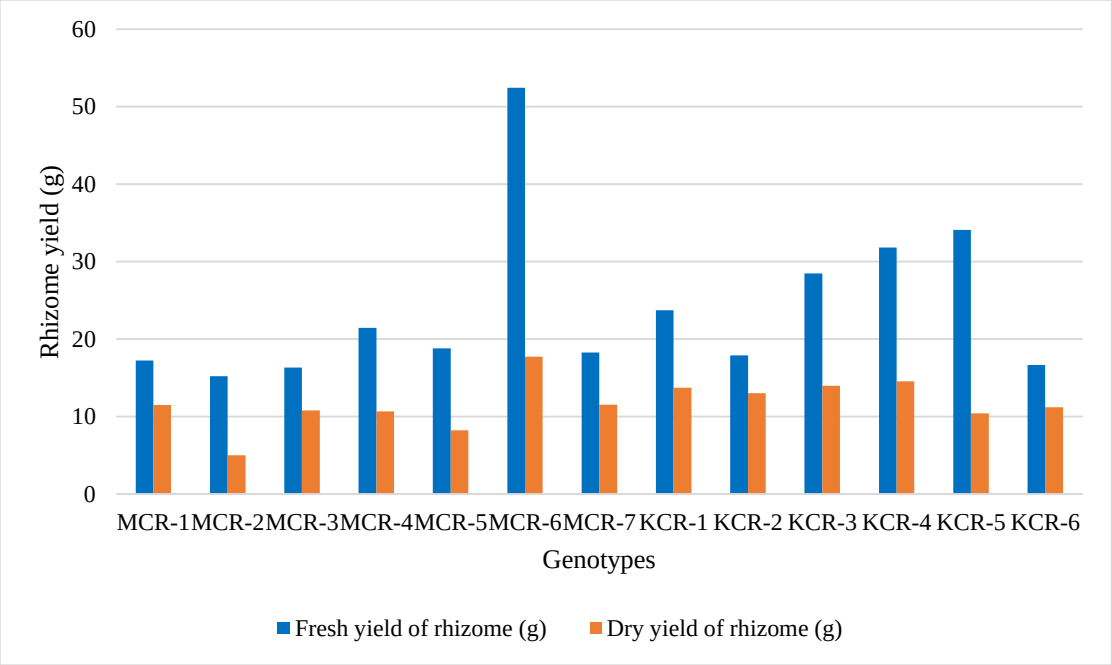


Figure 10: Fresh and dry yield of rhizomes in *K. rotunda* genotypes

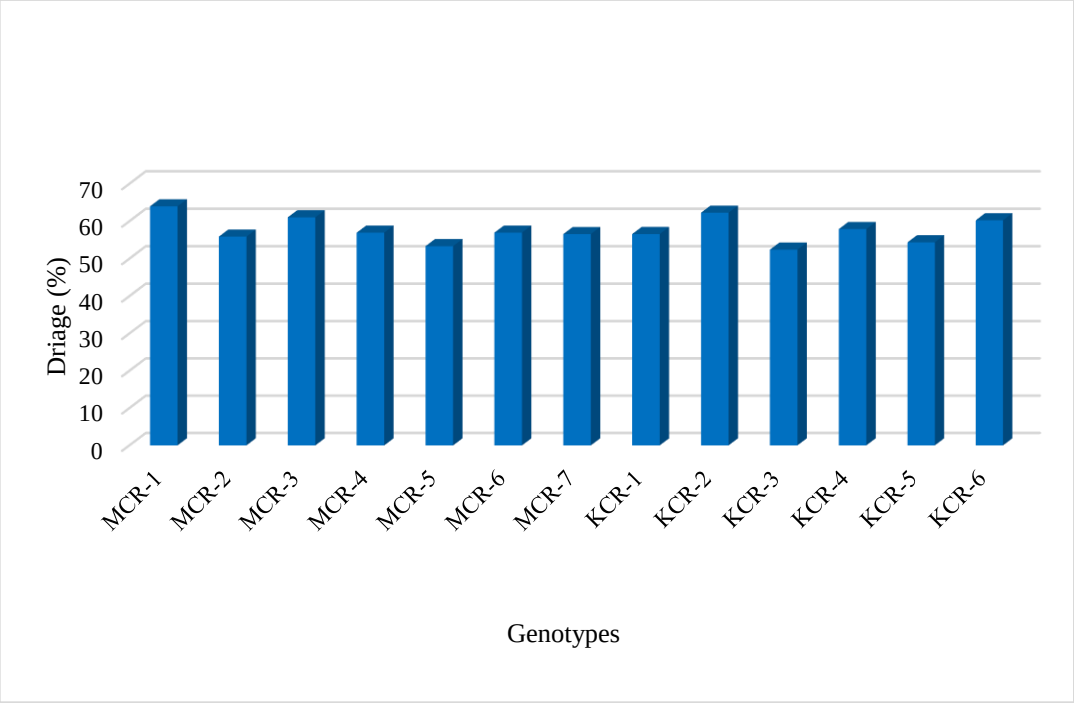


Figure 11: Driage of rhizome in *K. rotunda* genotypes

5.1.1.11 Driage of rhizome

There was huge variation with respect to driage of rhizome and it ranged from 52.41 to 63.98 per cent (Figure 11). Highest driage was recorded in MCR-1 which was on par with KCR-2. Compared to other rhizomatous crops, this is a high recovery which indicates the lower moisture content of the *K. rotunda* rhizome. The moisture content reported by Sereena *et al*. (2011) in *K. rotunda* was 10.41 per cent and this could be the reason for higher drying per cent obtained in the present study. The presence of root tubers which store much of water also justifies the high dry recovery in *K. rotunda*. A dry recovery of 32.78 and 34.48 per cent has been observed in Kasturi and Rajani varieties of *K. galanga* by Preetha *et al*. (2016). Prasannakumari *et al*. (1994) evaluated five local types of *K. galanga* and found that they differed significantly in fresh and dry yield of rhizome as well as dry recovery.

5.1.1.12 Number of root tubers

Root tubers are present in many members of the family Zingiberaceae. In the present study also all the genotypes possessed root tubers. Number of root tubers varied significantly among the genotypes (Plate 10). In the pooled mean of genotypes, KCR-1 showed significantly highest number and lowest was noticed in genotype KCR-6. The tuberous roots are modified form of contractile roots where terminal part is swollen and egg shaped (Ruamrunsgri, 2015). He opined that at least four storage roots attached to rhizome are required for exporting *C. alismatifolia* which stored carbohydrate that would be utilized by the plant for proper growth and development in the next season. The variation in the number of root tubers among the genotypes might be due to the variation in their climate resilient capacity.

In their native habitat, most genera of the Zingiberaceae family grow well during the rainy season, and then go completely dormant during the dry season. Before the onset of dormancy, products of photosynthesis from leaves and

pseudostems are transferred to accumulate in the rhizome and storage roots (Paz, 2003).

In anatomical studies of root tubers by Seema (2015), the sclerenchymatous conjunctive tissue found along with the xylem and the endodermal cells thickening was observed to be totally lost during its transitional development from roots to tuber. She suggested that the tuberisation was mainly due to expansion of the starch filled cortex and pith. However, Vijai (1975), in *C. angustifolia* explained the tuberousness was due the expansion of the ground parenchyma and dilatation of the stelar portion. In the present study also the root tuber anatomy showed the presence of starch granules in the cortex and pith (Plate 25).

5.1.1.13 Length and girth of root tubers

From the data regarding the length and girth of root tubers recorded at harvest (Fig. 13), significant difference was observed among the genotypes. Highest tuber length was recorded in the genotype MCR-4, whereas highest girth was recorded in the genotype KCR-1 and it was on par with MCR-5. Generally elongated tubers were observed in majority of Manipuri collections except MCR-3 and MCR-7 and majority of Kerala collections were observed to have sessile tubers. Plate 10 clearly indicates the spreading/loose nature of the rhizome mass of Manipur genotypes due to the presence of elongated root tubers and the compact nature of rhizome mass of Kerala collections due to the presence of the sessile root tubers. Seema (2015), reported the presence of sessile and stipulated root tubers in 10 *Curcuma* species. Fleshy tubers which are white or watery pearl colour have been reported to be present in many genera of the tribes Globbeae and Zingiberaceae including *Kaempferia* species by Sabu (2006). Sereena *et al*. (2011) have observed the presence of club shaped tubers in *K. galanga*. Rhizome size and the number of storage roots *i.e.* root tubers affected the sprouting, growth, yield and quality of *Cucurma alismatifolia* indicating their important role for storage of food reserves such as the carbohydrates and proteins (Anuwong *et al*., 2014).

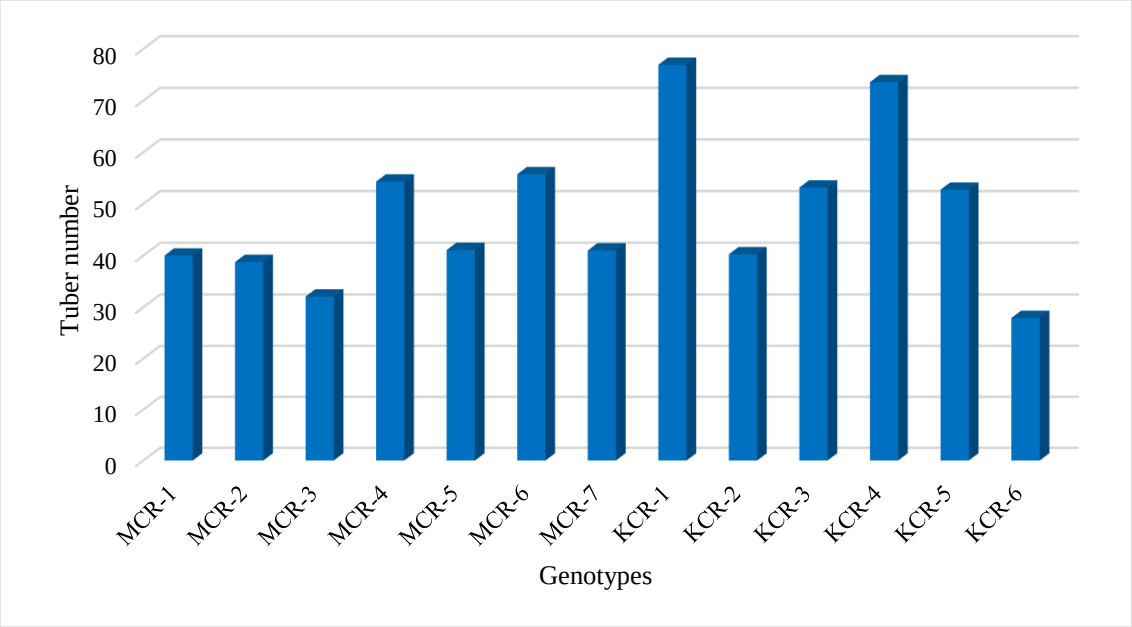


Figure 12: Number of root tubers in *K. rotunda* genotypes

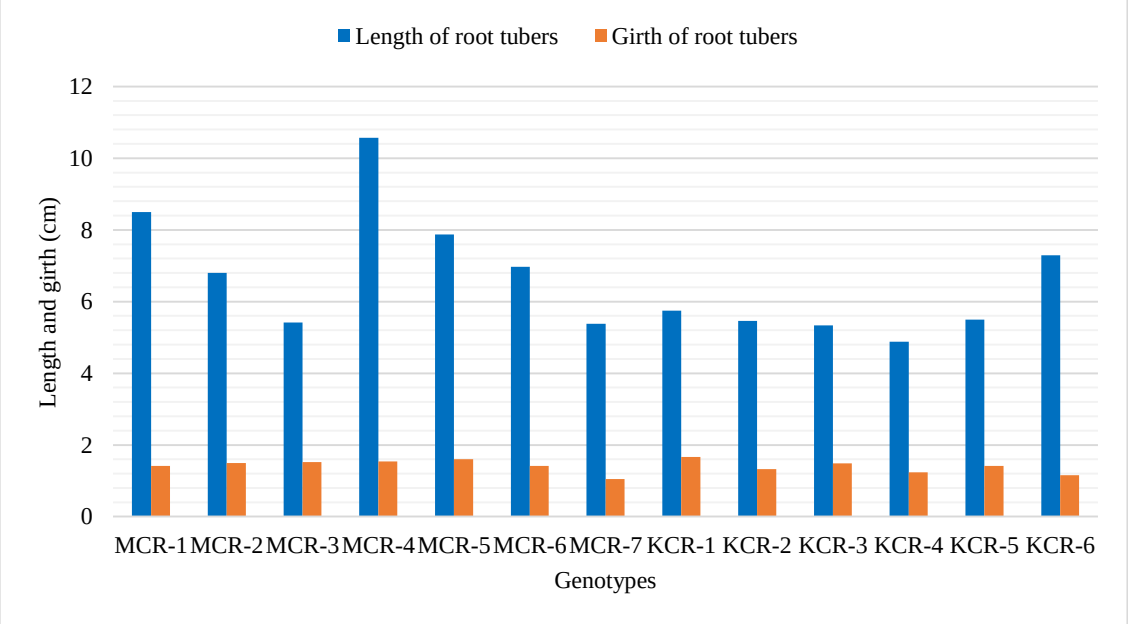


Figure 13: Length and girth of root tubers of *K. rotunda* genotypes

5.1.1.14 Fresh and dry weight of root tubers

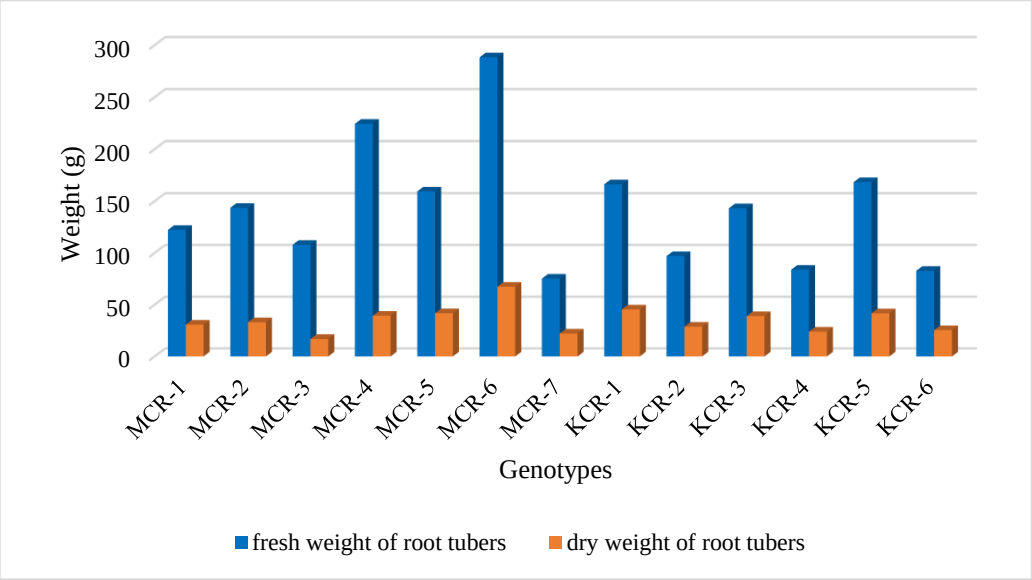


Figure 14: Fresh and dry weight of root tubers in *K. rotunda* genotypes

There was significant variation among the genotypes with respect to the fresh as well as dry weight of root tubers (Fig. 14). Significantly highest fresh weight of root tubers was recorded in the genotype MCR-6 and the lowest was recorded by the genotype MCR-7.

The fresh weight of root tuber was 48.4 ± 4.9 g and dry weight was 13.4 ± 2.2 g in *C. alismatifolia* as reported by Hongpakdee *et al*. (2010) and the mean dry weight of the root tuber was 3.5 ± 0.56 g. In the present experiment, there was huge variation among the genotypes which ranged from 82.71 to 288.3 g on fresh weight basis and 16.89 to 67.38 g on dry weight basis. It could also be observed that genotypes having more number of root tubers *viz.* KCR-4 and KCR-1 had only low tuber weight indicating that the total tuber number and total tuber weight are not correlated.

5.1.1.15 Biological yield

Biological yield is an indication of the total dry matter accumulation of a plant. Significant variation was noticed among the genotypes with regard to biological yield. Significantly highest biological yield was noticed in genotype

MCR-6. Latha (1994) also found variation in biological yield in *K. galanga* genotypes.

5.1.1.16 Harvest index

Significant variation was noticed among the genotypes in the case of harvest index which ranged from 19.44 to 47.43 per cent. Genotype KCR-4 showed significantly highest harvest index. In ginger, harvest index ranged from 31.45 to 55.69 per cent (Sharatbabu *et al*., 2017). Berrocal-Ibarra *et al*. (2002) observed that with increase in economic yield of plant there was increase in harvest index. Improved harvest index represents increased physiological capacity to mobilise photosynthates and translocate them into organs having economic yield. Since economic yield is only a fraction of dry matter produced, the harvest index forms a measure of yield potential (Kumar and Chowdhury, 1986).

Overall performance of *K. rotunda* genotypes

Considering the growth and yield parameters, Manipur collection of *K. rotunda* (MCR-6) emerged as the best performing genotype. It was distantly followed by the Kerala collections KCR-4, KCR-5 and KCR-3.

5.1.2 *Kaempferia parviflora*

5.1.2.1 Qualitative parameters

There was only three genotypes evaluated under *K. parviflora*. The rhizome of *K. parviflora* was brown in colour with dark purple colour inner core. Rhizomes are irregular in shape, branched or palmate. Small tuberous roots with lengthy stems arised from rhizome. Light green colour leaves were semi-erect and lanceolate and had cuspidate leaf tip with wavy margin.

Catherine *et al*. (2014) described *K. parviflora* leaf as ovate or oblong in shape, green colour with red tinted undulated margin. The rhizome were subglobose with brownish colour skin and purple flesh inside. The rhizome possessed many succulent roots.

Two types of *K parviflora* were observed by Labrooy *et al*. (2018) *viz*. Thailand type *K. parviflora* and Malaysian type *K. parviflora*. Thailand type had erect growth habit, yellow green colour ovate shaped leaf with dark red margin and rhizome were dark purple colour while Malaysian type had red colour margin and purple colour rhizome.

5.1.2.2 Days to sprout

There was significant variation among the genotypes for number of days taken for sprouting (Fig. 15). Genotype KCP-2 took least number of days to sprout (15.42) while genotype KCP-1 took more number of days to sprout. According to Catherine *et al*. (2014), *K. parviflora* is sluggish in regeneration and has long dormancy of about six months. Abdullah *et al*. (2014) found that *K. parviflora* required semi shaded condition for proper growth and development which took 30.67 days in 30 per cent shade condition for sprouting. Receipt of summer showers followed by breaking of dormancy and a shade level of 50 per cent might have contributed to early sprouting of *K. parviflora* in the present study.

5.1.2.3 Number of tillers per plant

K. parviflora genotypes started producing tillers after 30 DAP. Tiller production increased gradually reaching a peak at 180 DAP and thereafter it declined in all the genotypes. Number of tillers ranged from 4.92 to 6.67 (Figure 16). Variation among the genotypes with respect to number of tillers was reported by Latha (1994) and Divya (2008) in *K. galanga*. Zulfa, (2012) have reported 4.17 shoots per plant in *K. parviflora* and found that it has no correlation with the yield of rhizome.

5.1.2.4 Plant spread

There was no significant difference among the genotypes regarding the plant spread *i.e*. North-South and East-West spread at 180 DAP (Fig. 17). According to Chandana (2011), plant spread in *K. galanga* is correlated with number of leaves, leaf area and number of tillers. However, in the present study, the contributing parameters for plant spread *i.e.* number of tillers, number of leaves

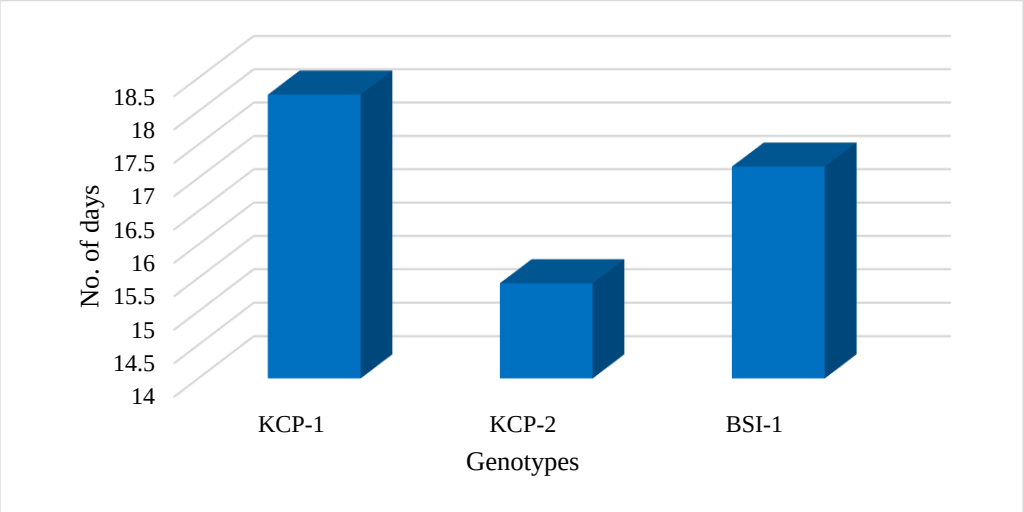


Figure 15: Days to sprout in *K. parviflora* genotypes

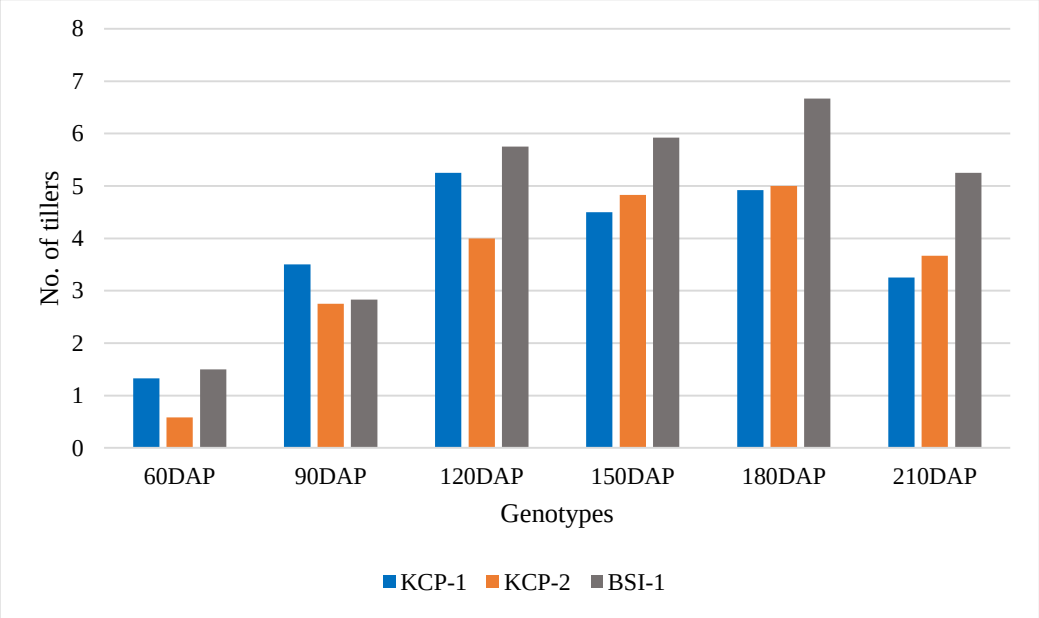


Figure 16: Number of tillers per plant in *K. parviflora* genotypes

and leaf area did not show any pattern among the genotypes, thus making only minimum variation in plant spread.

5.1.2.5 Number of leaves

The number of leaves in *K. parviflora* increased steadily upto 180 DAP after which it declined. There was significant variation among the genotypes for number of leaves at 150 DAP but no significant difference was noticed in the pooled mean of genotypes at 180 DAP. Number of leaves ranged from 16.42 to 17.92 at 180 DAP. Zulfa (2012) also observed similar pattern of leaf production in *K. parviflora*. Abdulla *et al*. (2014) has reported 21 leaves per plant at 30 per cent shade and 10.33 at 50 per cent shade in *K. parviflora*.

5.1.2.6 Plant height

There was no significant difference among the genotypes with respect to plant height which ranged from 50.45 cm to 54.08 cm at harvest. This similarity in plant height might be due to innate quality of genotypes. According to Evi (2012), *K. parviflora* grows taller in low altitude. Few reports on plant height are available in *K. parviflora viz*. 30-40 cm (Catherine *et al*., 2014), 17.41 cm at 13 weeks after planting (Evi, 2012), 29.77cm at 29 weeks after planting (Zulfa, 2012).

5.1.2.7 Leaf area

The leaf area increased rapidly upto 120 DAP and thereafter there was only small increase and reached peak at 180 DAP. The leaf area ranged from 205.21 cm^2 to 249.42 cm^2 at 180 DAP. The highest leaf area was observed in genotype KCP-2 followed by BSI-1. Zulfa (2012) reported difference in leaf area of *K. parviflora* under varying light intensities and he observed that leaves grown under shade becomes bigger for enhancing light absorption to have efficient photosynthesis.

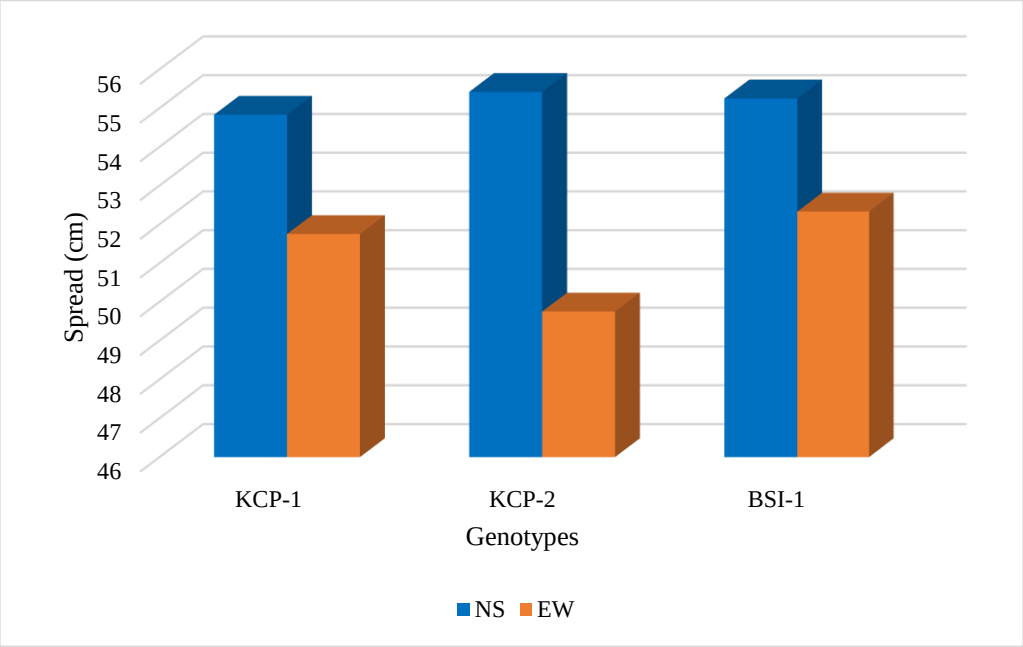


Figure 17: Plant spread of *K. parviflora* genotypes

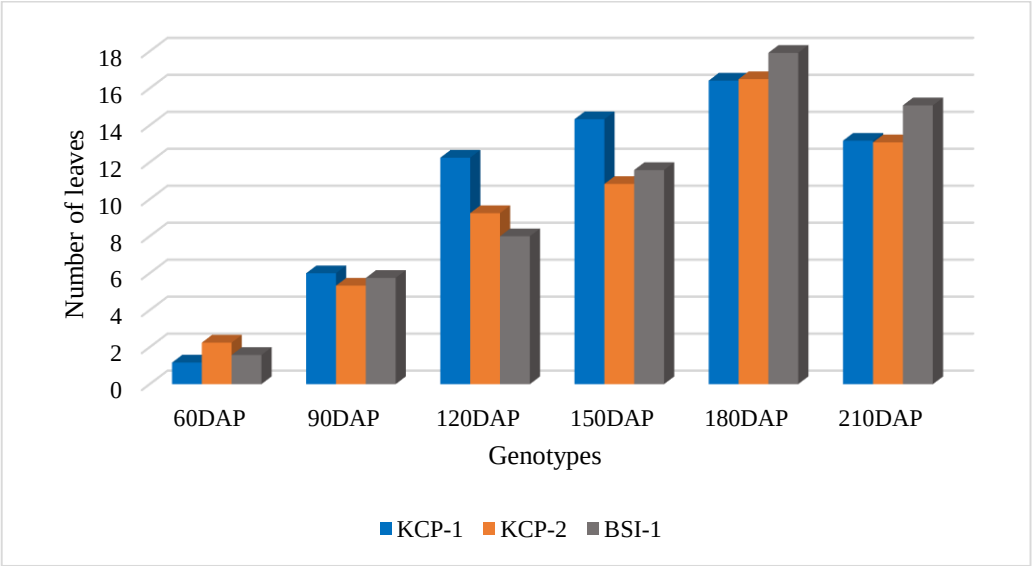


Figure 18: Number of leaves in *K. parviflora* genotypes

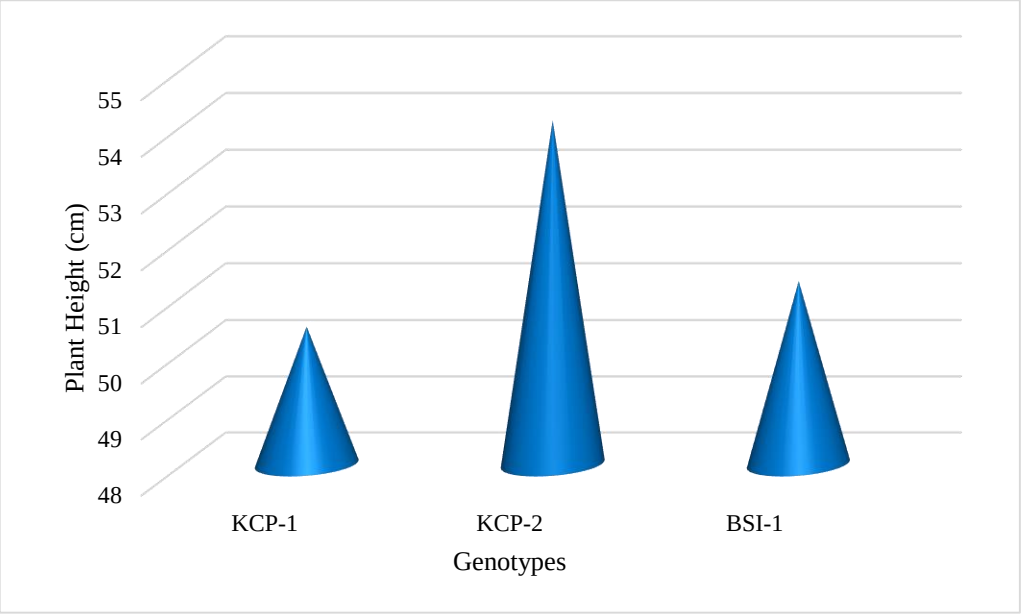


Figure 19: Plant height of *K. parviflora* genotypes

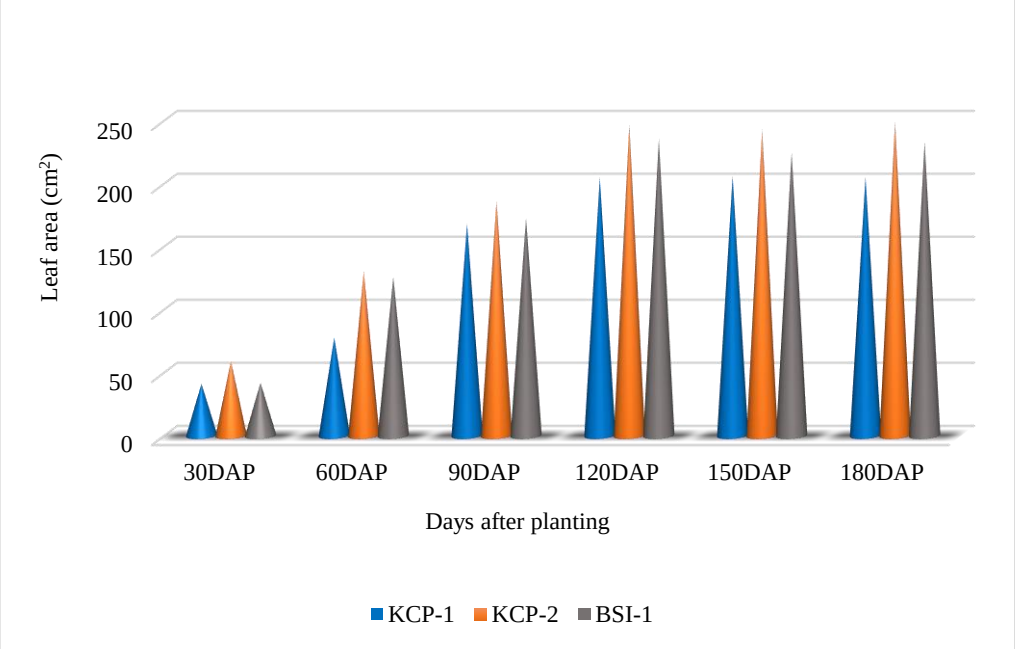


Figure 20: Leaf area of *K. parviflora* genotypes at different growth periods

5.1.2.8 Fresh and dry weight of leaves

There was significant variation among the genotypes with respect to the fresh as well as dry weight of leaves (Fig. 21). Genotype KCP-2 recorded significantly highest fresh weight and dry weight.

The high fresh and dry weights recorded in KCP-2 could be due to higher number of leaves as well as high leaf area recorded in this genotype as evident from figure 21.

In *K. parviflora*, Nurul-Azilla and Wan-Zaliha, (2017) have reported leaf fresh weight ranging from 291 to 1063 g and leaf dry weight ranging from 28.00 to 46.52 g in growing media containing varying biochar substrates.

5.1.2.9 Length and girth of rhizome

There was no significant difference with respect to length as well as the girth of rhizome among the genotypes. According to Zulfa, (2012), *K. parviflora* rhizome falls under small size (5.39 cm), medium size (6.95 cm) and large size (8.18 cm). But in the present study, the three genotypes produced rhizomes of similar dimensions. All the three genotypes in present study had rhizome length above 8.18 cm hence fell under large rhizome group.

5.1.2.10 Fresh and dry yield and drying percentage of rhizome

There was a significant difference in the fresh and dry rhizome yield. Genotype KCP-1 gave highest fresh rhizome yield and highest dry rhizome yield (Fig. 23).

In *K. parviflora*, Nurul-Azilla and Wan-Zaliha, (2017) observed fresh rhizome yield of 147 g per plant when cocopeat was used as growing media, while Zulfa, (2012) reported rhizome yield of 45.66 g per plant with the application of 50% chemical fertilizer and biofertilizer in a field crop. Variation was observed in fresh rhizome yield and dry rhizome yield among the 10 genotypes of *K. galanga* by Latha (1994) under Kerala conditions. A direct and positive contribution to dry

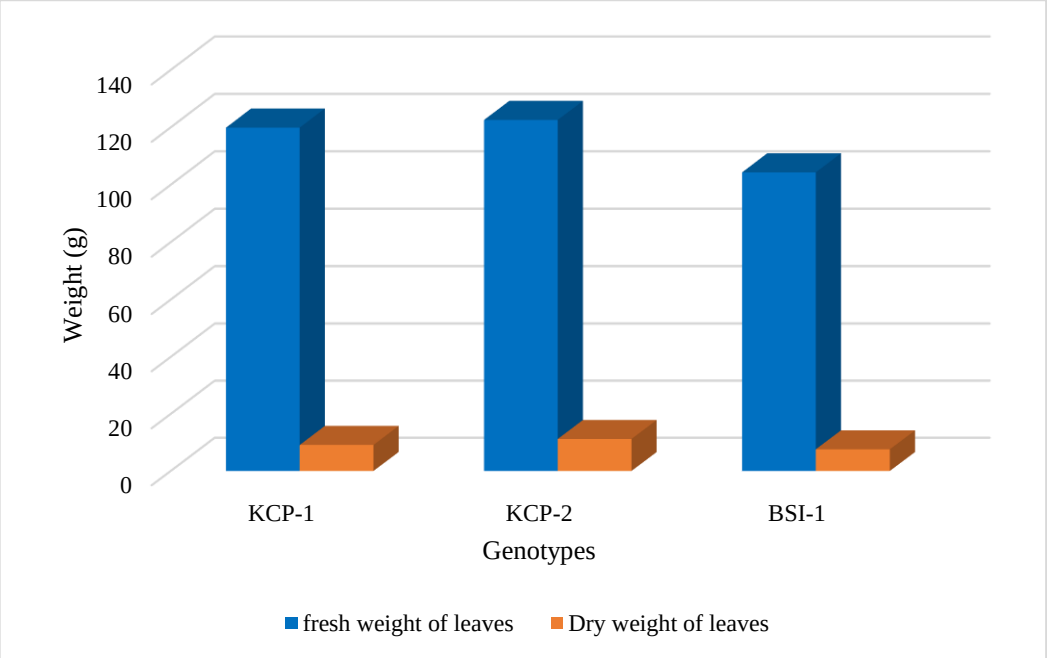


Figure 21: Fresh and dry weight of leaves of *K. parviflora* genotypes

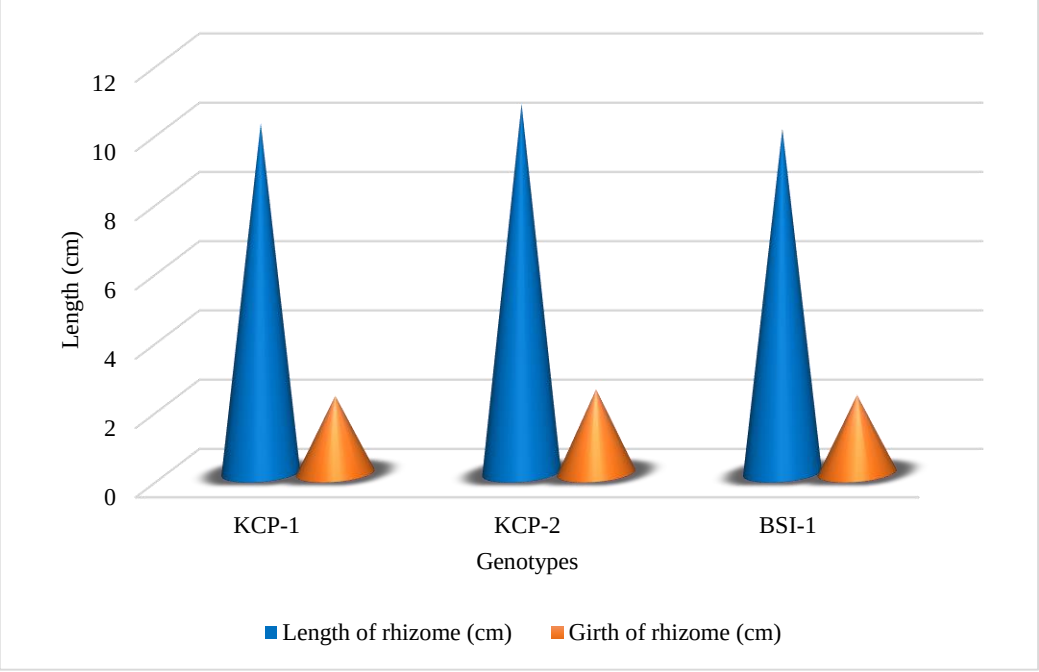


Figure 22: Length and girth of rhizome of *K. parviflora* genotypes

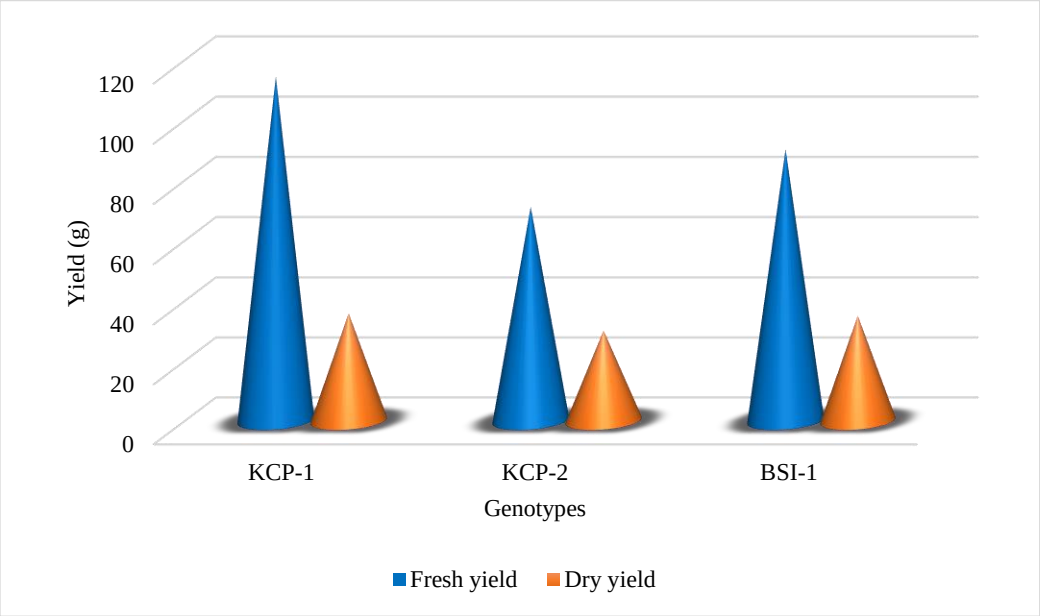


Figure 23: Fresh and dry yield of rhizome of *K. parviflora* genotypes

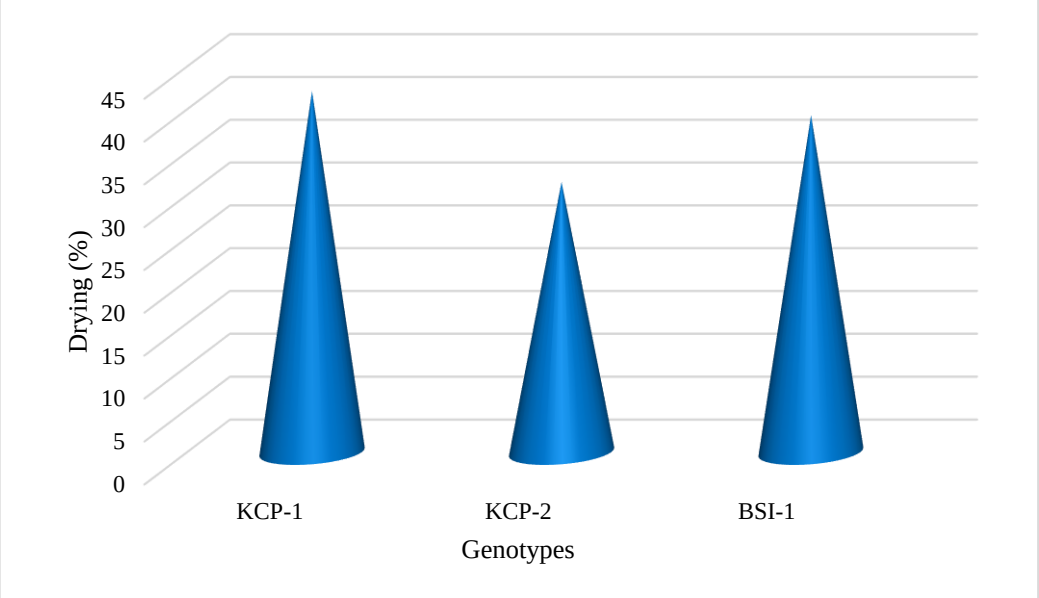


Figure 24: Driage of *K. parviflora* genotypes

rhizome yield by fresh rhizome yield was reported by Chandana (2011) in *K. galanga*.

The driage of rhizome also showed significant variation among the genotypes ranging from 31.51 to 42.15 per cent (Fig. 24). The drying per cent was highest in the genotype KCP-1 which was on par with BSI-1.

Preetha *et al*. (2016) reported dry recovery of two varieties of *K. galanga viz.* Kasturi and Rajani as 32.78 per cent and 34.48 per cent respectively. Prasannanakumari *et al*. (1994) found significant variation in fresh and dry yield of rhizome as well as dry recovery among five local types of *K. galanga*.

5.1.2.11 Number of root tubers

Root tubers were present in all the genotypes of *K. parviflora* also but in lesser numbers (8.58 to 13.67) when compared to *K. rotunda* (27.92 to 76.92) as seen in figure 24. Yearly variation was also noticed, during 2017-18 root tuber production was high.

Variation in number of root tubers was reported in *C. alismatifolia* by Hongpakdee *et al* (2010) when grown under different night temperature and day length conditions. There was 12.6 root tubers in short day condition and 4.6 number of tubers under long day condition. Ruamrungsri *et al* (2004) reported 8.27 root tubers in the *K. parviflora* in a fertilizer trial. The difference observed in the number of root tubers in two years might be the adaptation to different climatic situations. The yearly variation in the root tubers was observed in *K. rotunda* too in the present study.

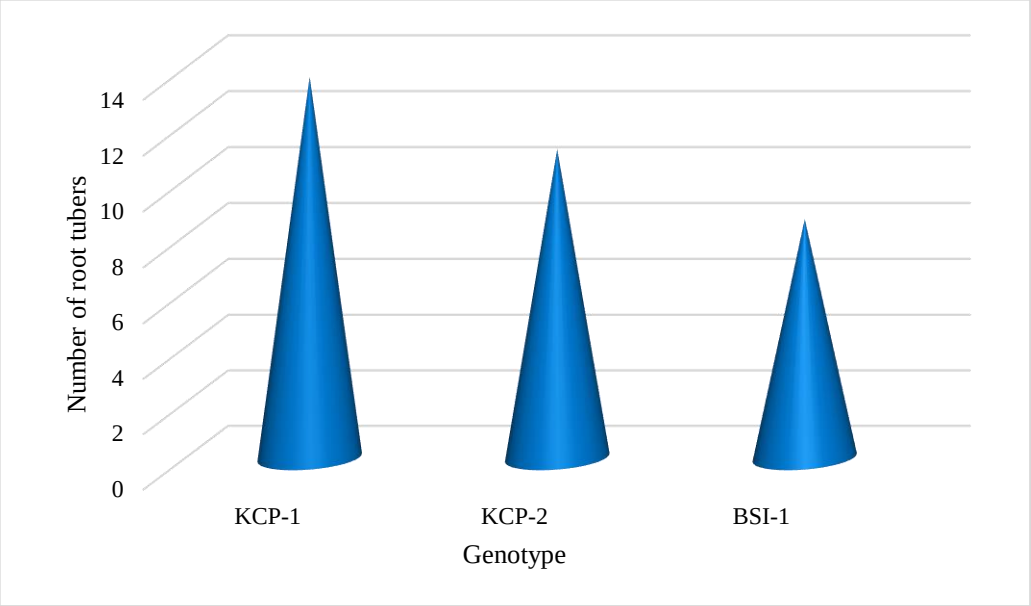


Figure 25: Number of root tubers in *K. parviflora* genotypes

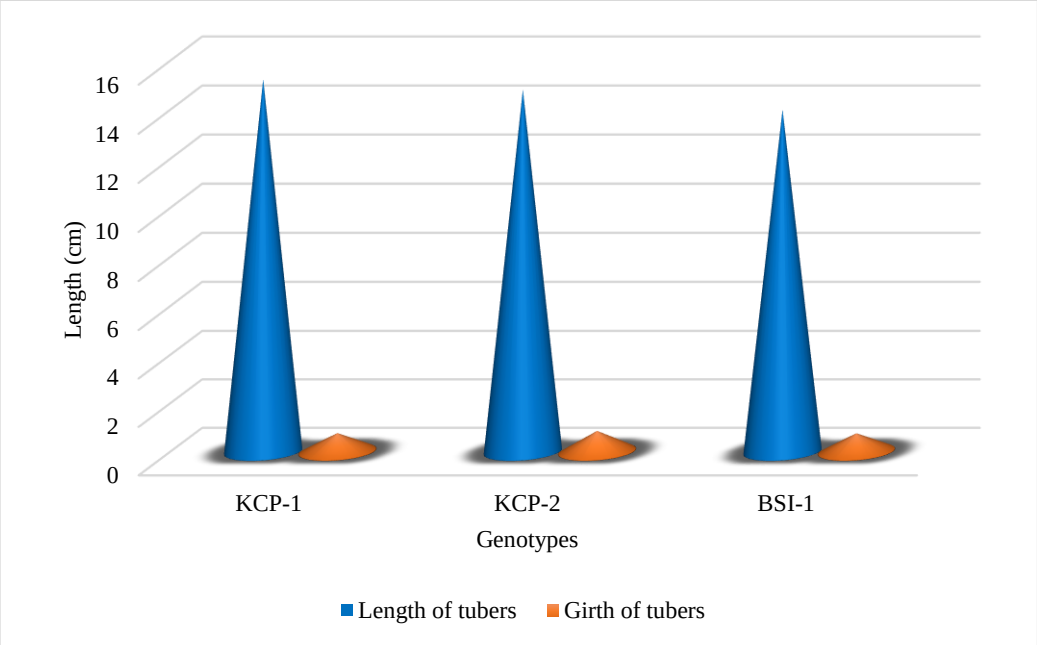


Figure 26: Length and girth of root tubers of *K. parviflora* genotypes

5.1.2.12 Length and girth of root tubers

There was no significant variation among the genotypes with respect to the size of root tubers. However during 2017-18, the root tubers had more girth.

In a similar crop *C. alismatifolia*, Ruamrungsri *et al*. (2004) observed root tuber length of 17.32 cm in plants applied with 200:200 N: K treatment. He also reported that with increase in quantity of nitrogen application, length of root tubers increased.

In *K. parviflora*, root tuber length of 2.9 to 5.5 cm and girth of 0.3 to 1.7 cm was reported by Hongpakdee *et al*. (2010) when grown under different night temperature and day length conditions.

5.1.2.13 Fresh and dry weight of root tuber

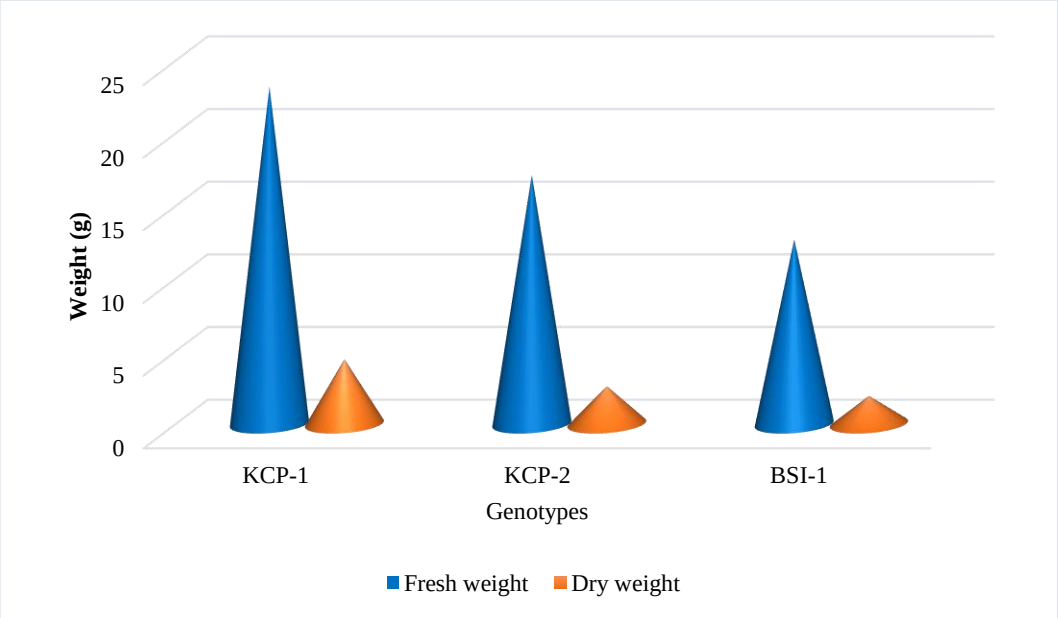


Figure 27: Fresh weight and dry weight of root tubers in *K. parviflora* genotypes

There was variation among the genotypes and years with respect of fresh and dry weight of root tubers also (Figure 27). As in the case of number of root tubers, the fresh and dry weight was also high in the genotype KCP-1 and the year 2017-18.

Here again, in *C. alismatifolia*, Hongpakdee *et al* (2010) have reported fresh weight of root tuber as 48.4 ± 4.9 g/plant and dry weight as 13.4 ± 2.2 g/plant when grown under short day condition. Mean dry weight of root tuber was 3.5 ± 0.56 g at zero week after planting in *C. alismatifolia* according to Anuwong *et al*. (2014).

In *K. parviflora*, Nurul-Azilla and Wan-Zaliha (2017) have reported the fresh and dry weight of roots as 31.17g and 1.88g respectively when grown in cocopeat as the growing media.

5.1.2.14 Biological yield

Genotype KCP-2 showed significantly highest biological yield and season 2018-19 recorded higher biological yield. Variation in biological yield was also noticed in *K. galanga* accessions by Latha (1994.)

5.1.2.15 Harvest index

In harvest index, genotype BSI-1 showed significantly higher value. Higher harvest index can be viewed as increase in economical yield of the plant (Berrocal-Ibarra *et al*., 2002).

Overall performance of *K. parviflora* genotypes.

When we take the average performance of the three genotypes over two years, much variation could not be observed with respect to economic parameters.

5.1.3 Vivipary in *Kaempferia parviflora*

According to Tweddle *et al*. (2003) seeds that do not tolerate desiccation are called recalcitrant seeds. In the recalcitrance test, for proving the viviparous nature of *K. parviflora*, the dried seed showed zero germination per cent while the freshly harvested seeds had 55 per cent germination, thus confirming the vivipary in *K. parviflora*. Recalcitrance of seeds, as in viviparous plant species, had ecological advantages. The absence of a dormancy period during which metabolism is extremely low enables seeds to continue building up reserves while it is attached to the parent plant (Juncosa, 1982; Farnsworth, 2000). When there is no dormancy at all and seeds germinate even before abscission, it is called true vivipary. In viviparous plants the offspring grows continuously while still attached to the mother tree (Goebel, 1905).

Vivipary is an important reproductive mechanism allowing better germination and survival of seeds regardless of climatic and edaphic factors. In spite of being a relatively unusual event in angiosperms, vivipary is a phenomenon of evolutionary and biological significance. This phenomenon can be correlated as an adaptive reproduction strategy in plants growing under environmental stress. In Cactaceae, vivipary is regarded as a strategy allowing species with an otherwise low seed germination rate to reproduce in extreme environmental conditions. The fruit characters of Cactaceae enable it to protect the seeds and young seedlings from desiccation, insulating them from environmental conditions and thus ensuring the survival of the immature seedlings (Cota-Sanchez, 2004). It had also been reported that in species showing high frequency of sterility, which may be either due to polyploidization as in arctic plants or due to hybridization as in some members of Poaceae, viviparous germination offers the scope of survival of the seedlings when normal seeds fail to germinate (Lee and Harmer, 1980). Among seed plants, vivipary is most prominently developed in mangrove species, especially in some genera of Rhizophoraceae (Tomlinson and Cox, 2000), which is another example of a reproductive adaptation strategy under adverse conditions.

Though a rare event among members of Zingiberales, viviparous germination of seeds has been reported in *Costus lucanusianus* and *Alpinia mutica*. It may be assumed that vivipary is not a natural trend among gingers and occurs only under exceptional conditions.

Unusual occurrence of vivipary in certain species is attributed to a number of unfavourable environmental factors such as high humidity, incessant rainfall, frosting etc. Majumdar *et al*. (2010) reported germination in Cupressaceae following frost. Vivipary in *Jatropa curcas* was reportedly brought on by continuous rainfall (Deore and Johnson, 2008). *Trifolium repens* also reportedly undergoes viviparous germination following continuous rainfall (Majumdar *et al*., 2004). In *Hedychium elatum* also, vivipary was observed after continuous rainfall during monsoon followed by dry spell (Bhadra *et al*., 2013). In all cases, the authors listed wetting of inflorescence heads as possible triggers of viviparous germination. In the present study, in *K. parviflora*, viviparous seedlings were observed during July which falls under heavy monsoon coupled with high humidity. Weather data presented in Appendix 1 showed that both the experimental years have been characterised by high rainfall and relative humidity. This might have triggered seed germination while still inside the inflorescence. Thus, it can be presumed that continuous rainfall resulting in high humidity played an important role in inducing vivipary in *K. parviflora*.

5.1.3.1 Growth characters of viviparous seedlings

The viviparous seedlings which were attached to the mother plant as seen in Plate 4 were separated and then transplanted into grow bags in order to evaluate the growth and development as well as for confirming rhizome formation. The seedlings produced three leaves at 30 DAT and they had 9.8 leaves at 120 DAT. No tiller production was noticed until 90 DAT. However, the plants had 2.7 tillers at 120 DAT. Leaves were small with leaf area of 3.09 cm^2 at 30 DAT which reached 37.39 cm^2 at 120 DAT (Figure 28).

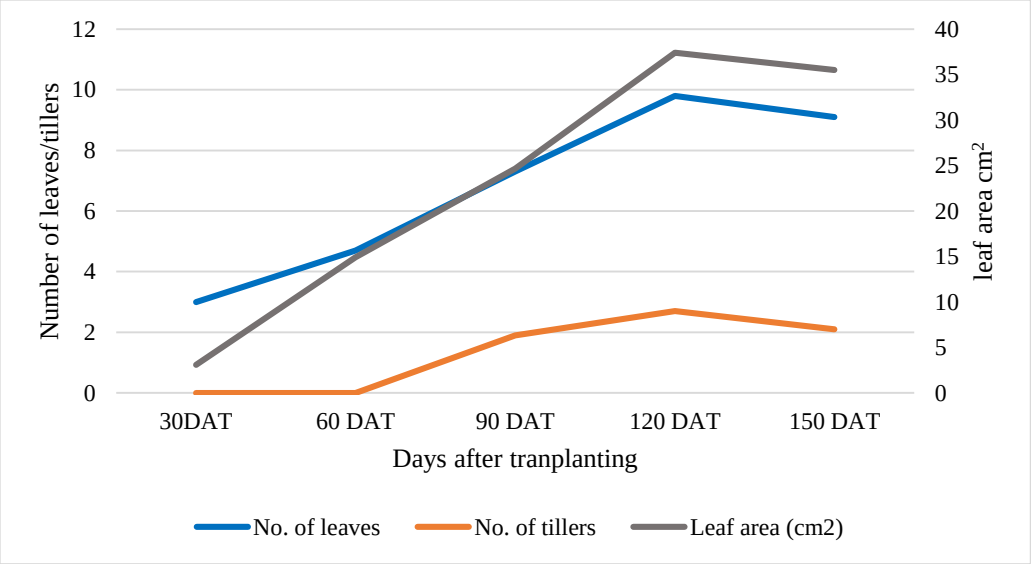


Figure 28: Growth characters of viviparous seedlings

It can be inferred that plant stature of viviparous seedlings was small as we see in micro propagated plants in Zingiberaceae crops like ginger, galangal *etc*. In a study of *in vitro* shoot culture in *K. parviflora*, Alveno (2012) had recorded 3.27 leaves at three month after culture. An average of 3.39 leaves per shoot at 6 weeks after acclimatization was observed in the transplanted *in vitro* propagated plantlet of *K. marginata* (Saensouk *et al*., 2016).

The rhizome produced by the viviparous seedlings was minute and almost round in shape with length of 20.18 mm and girth of 15.80 mm. The fresh weight of rhizome was very less with only 1.41 g at 150 DAT. This rhizome also resembled the *in vitro* produced micro rhizomes in Zingiberaceae crops. A micro rhizome weight of 265 mg/plantlet had been reported *in vitro* study of *K. parviflora* (Zuraida *et al*., 2015). Similarly, in ginger and turmeric, *in vitro* micro rhizome weight of 0.12 to 1.83 g and 0.1 to 2 g, respectively have been reported (Mrudul *et al*., 2001; Archana *et al*., 2013). The normal growth and development observed in viviparous seedlings is advantageous as a means for multiplication. Reports are available on vivipary in few species of Zingiberaceae *viz*. *Hydechium*, *Alpinia* and *Globba*. Vegetative reproduction is the common method in Zingiberaceae which helps in spreading a number of genetically identical propagules over a large area in a short

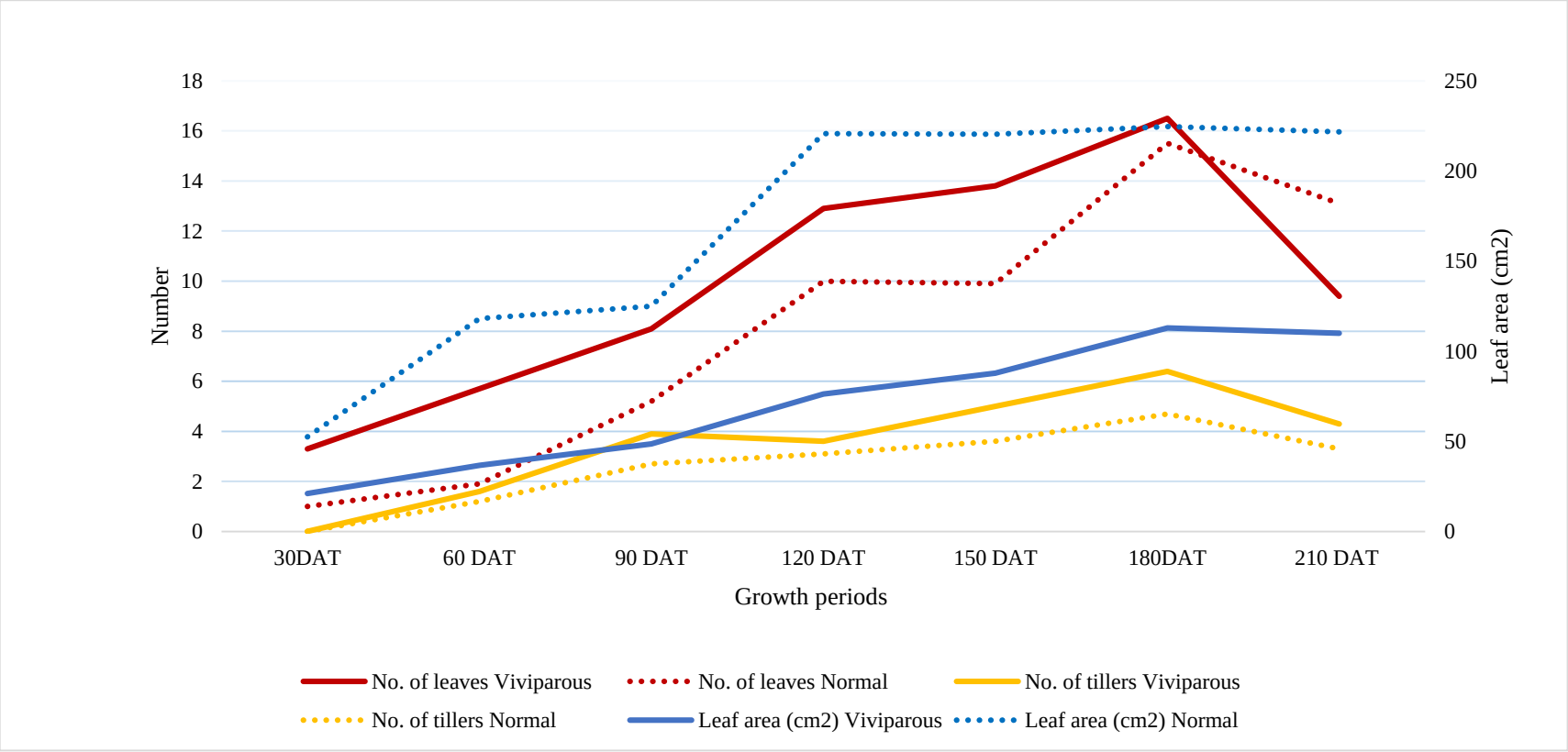


Figure 29: Comparison of growth characters between viviparous and normal plants

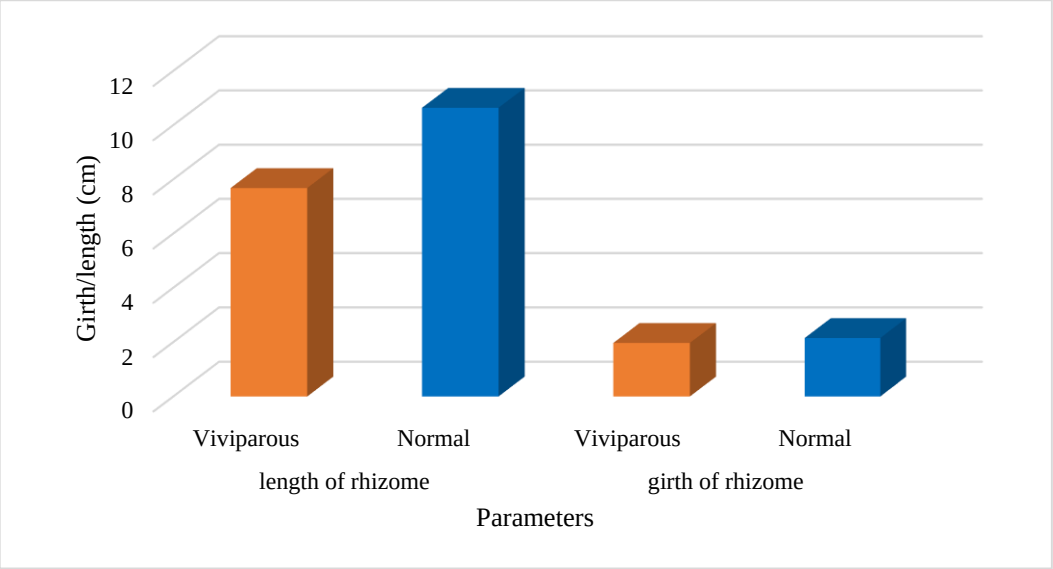


Figure 30: Comparison of length and girth of rhizome between viviparous and normal plants

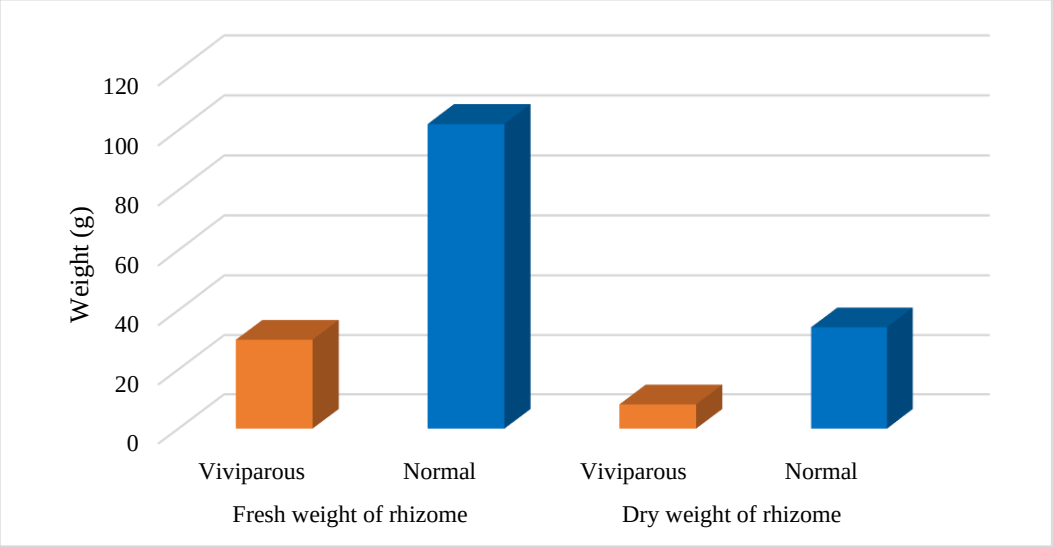


Figure 31: Comparison of fresh and dry rhizome weight between viviparous and normal plants

period of time. However, viviparous plants produced through germination of seeds on mother plant are genetically diverse (Bhadra *et al*., 2013).

5.1.3.2 Comparison of viviparous and normal plants of K. parviflora

In the next season the *in vivo* micro rhizomes collected from viviparous seedling were grown along with macro rhizomes collected from normal plants. The plants were observed for complete growth period.

There was a huge variability between the viviparous and normal plants both in the growth and yield parameters (Figure 29-31). The viviparous plants took 28.5 days for sprouting while normal plants took only 16.7 days which might be due to the bigger size of mother rhizome used for planting. In crops like ginger, bigger rhizome took least days for sprouting (Mahender *et al*., 2015). The higher quantity of reserve food material helps in early sprouting, better crop growth and development (Asafa and Akanbi, 2018).

The normal plants were taller when compared with viviparous plants (Plate 8). The number of tillers as well as number of leaves produced by viviparous plants were consistently higher when compared to normal plants. However with respect to leaf area, the normal plant had significantly higher leaf area at all the growth stages. The size of leaves from normal plant were almost double the size of viviparous plants and this justifies the higher leaf area recorded in normal plants. However, fresh weight of leaves of viviparous plant was significantly higher while dry weight was almost the same in both the cases. The higher fresh weight may be correlated with higher number of leaves and dry weight may be correlated with small size as well as thin nature of leaves.

In case of yield parameters, normal plants produced bigger sized rhizome with higher length, girth as well as fresh and dry weight of rhizome per plant. Fresh yield of rhizome from the normal plant was almost three times the rhizome yield of viviparous plants.

In the comparative evaluation, it was seen that the viviparous plants which were raised from micro rhizome of 1.5 g produced 30.06 g at the end of crop period.

In case of normal plants raised from a rhizome bit of 25-30 g, the final rhizome yield was 102.06 g/plant. This indicated that vivipary in *K. parviflora* reported for the first time in the present study can be exploited in the commercial cultivation of this species after standardising the propagation and agronomic practices.

5.1.4 *Kaempferia galanga*

K. galanga was included in this study as a reference species only since the plant has been researched in-depth and it has already reached the status of a commercial crop. However, as there were two genotypes one each from Kerala and Arunachal Pradesh, a general evaluation with respect to growth and yield was made between the two and an overall comparison was made with *K. rotunda* and *K. parviflora*.

5.1.4.1 Qualitative parameters

K. galanga rhizome were dark reddish brown in colour and inner core was pearl white. Rhizome had tuberous roots. Dark green leaves were round ovate with acute tip and entire margin.

Different rhizome colours *viz*. light brown colour in variety Kasthuri and creamish brown colour in variety Rajani had been reported by Indrayan *et al*. (2007) in *K. galanga*. Aiyer and Kolammal (1964) had reported deep colour leaf with varying shapes as orbicular or sub-orbicular, orbiculate-ovate or ovate cordate having margin are wavy and conical shaped root tubers of greyish or light brown colour.

5.1.4.2 Evaluation of genotypes of K. galanga

There was not much difference noticed between the two genotypes with respect to most of the morphological parameters except leaf characters. ArPCG-1 was characterised by smaller leaves compared to the Kerala counterpart. Both the number of leaves as well as number of tillers were significantly higher in genotype ArPCG-1 during both years.

In the yield parameters, except rhizome girth all other parameters did not show any noticeable variation.

Divya (2008) found that *K. galanga* produced maximum number of leaves after six months of planting which is in accordance with the findings of the present study. She also observed variation in leaf area among the genotypes. But Latha (1994) observed no significant variation among the *K. galanga* genotypes for rhizome characters.

5.1.5 Comparison of three *Kaempferia* species

Among the three *Kaempferia* species evaluated in the study, only *Kaempferia galanga* had been exploited as a commercial species. Even though *K. rotunda* is widely utilized in several Ayurvedic formulations, it is not cultivated on a large scale. *Kaempferia parviflora* is a newly introduced species which has immense potential as a drug. In the following tables comparative evaluation of the three species with respect qualitative parameters is presented.

Table 114: Leaf characters of *Kaempferia* species

Characters	*K. rotunda*	*K. parviflora*	*K. galanga*
Growth habit	Sprawling	Sprawling	Sprawling
Shape of leaf	Erect lanceolate	lanceolate	Ovate
Colour of leaf	Light green	green	Dark green

Table 115: Rhizome characters of *Kaempferia* species

Characters	*K. rotunda*	*K. parviflora*	*K. galanga*
Presence of root tuber	Present	Present	Present
Outer rhizome colour	Light brown	Muddy brown	Dark brown
Inner rhizome colour	Off-white	Violet	Pearl white
Number of root tubers	Abundant	Few	Few

5.2. FLORAL BIOLOGY OF *Kaempferia* SPECIES

5.2.1 Study of floral biology and morphology

Floral morphology and pollen study of *Kaempferia* species is discussed here.

The *Kaempferia* species in the present study exhibited different times of flowering. *K. rotunda* flowered by first week of March and flowering continued upto April end. The inflorescent stalk emerged first after dormancy period and in most of the plants the new leaves emerged after all the flowers withered. In the natural habitat also the same type of flowering was observed by Saensouk *et al*. (2016) in *K. rotunda*. In the terrestrial orchid, *Nervillia aragoana*, Shankar (2011) reported that the flowering occurred immediately after the summer showers in March and the leafy shoot emerged after the drying up of inflorescence.

In *Kaempferia parviflora*, flowering was observed from last week of May to second week of November. The inflorescence was visible only after the unfurling of first leaf. Devi *et al*. (2016) found flowering and fruiting of *K. parviflora* during April- September in Manipur forest.

Kaempferia galanga flowered for one month only *i.e*. from first week of June to first week of July. The same period was reported by Latha (1994) in *K. galanga*. Flowering was visible after around 30 per cent of leaves unfurled.

The number of flowers produced was more in *K. parviflora*, medium in *K. rotunda* and low in *K. galanga* (Latha, 1994).

The inflorescence of all the species was a scape covered by a lathery sheath, bearing flowers in bracts, which opened in succession. The inflorescences arised directly from the rhizome (Plate 32). The flower had only one fertile stamen. Flowers were larger in *K. rotunda*, medium in *K. galanga* and small in *K. parviflora* (Plate 33). Similar floral features had been reported in *K. galanga* (Bhurke, 2002; Rekha, 1993). Devi *et al*. (2016) had reported pedunculate inflorescence of 5.1-5.4 cm bearing white flowers with purple tinge in *K. parviflora*.

The pistil of the flower was characterized by long style which passed through the groove present between the two anther lobes and ends in a spiny stigma reaching just above the anther lobes (Plate 34-35). Ovule of *K. rotunda* was larger in size with length of 4.15 mm, while *K. parviflora* and *K. galanga* were almost similar (Plate 35). Number of ovules varied in all the species with 15.0 in *K. rotunda*, 12.4 in *K. parviflora* and 8.0 in *K. galanga*. In contrast to present observation, Bhurke, (2002) found varying number of ovules ranging from 18.60 to 22 in *K. galanga*.

The anthesis occurred between 5.00 to 7.15 am in *K. parviflora*, 3 to 5 am in *K. rotunda* and 4-5 am in *K. galanga* and dehiscence between 5.15 to 6.30 am in *K. parviflora*, 4:30 to 5:30 am in *K. rotunda* and 4:30 to 5.00 am in *K. galanga* (Plate 31). The flower buds were not visible on the previous day of anthesis in *K. parviflora* and *K. galanga*, while a whitish bud was visible on the previous day in *K. rotunda*. Bhurke, (2002) also noticed no visibility of flower bud in *K. galanga*.

5.2.2 Pollen studies

Pollen size of *K. parviflora* was larger when compared with the other two species (Plate 36). Pollen size in the range of 106-128 µm was noticed in *K. galanga* by Bhurke, (2002). Pollen fertility was high for all the three species with 94.24 per cent in *K. rotunda*, 90.71 per cent in *K. galanga* and 85.18 per cent in *K. parviflora*. Stigma remained receptive for 24 hours in *K. rotunda*, while it was only 8 and 9 hours, respectively for *K. parviflora* and *K. galanga*.

Studies on floral morphology revealed that the floral characters are well adapted for entomophily. White showy tepals with purple coloured labellum (Plate 33), mild fragrance of the flower, presence of nectar and nocturnal anthesis are all adaptations for insect pollination. Adaptation favouring self-pollination are also prevelant in the flowers *viz*. proximity of anther and stigma as well as homogamy *i.e*. simultaneous maturity of anther and stigma (Plate 34 and Fig. 32-34). Even though the flowers are well adapted for pollination by any means no seed set has so far been reported in *K. galanga* and *K. rotunda*. In the present study, seed set was

noticed in *K. parviflora*. Hand pollination using pollen grains from the same flower or flower of different plants belonging to various morphotypes were also done so as to confirm whether lack of pollination was the factor responsible for seedlessness in *K. galanga* and *K. rotunda.* This attempt was also unsuccessful as no seed set could be obtained from artificial pollination. All these factors show the possibility for some floral adaptations especially self-incompatibility which prevent seed set in *K. rotunda* and *K. galanga*.

Germination of the pollen grains was studied both under *in vitro* and *in vivo* conditions. However due to the bursting of pollen, *in vitro* studies was not success and *in vivo* stigmatic pollination was followed.

In the detailed studies on the morphology of stigma it was noticed that the stigmatic surface was spiny for all the species with and area of 0.07 mm in *K. parviflora*, 0.13 mm in *K. rotunda* and 0.06 mm *K. galanga*. *In vivo* growth of the pollen tube examined using fluorescent microscopy showed that under natural pollination the percentage of germination was found to be low and the penetration of the pollen tube through the style also was lacking. In *K. galanga*, many of the pollen grains seemed suspended on the spines without getting adherence to the stigmatic surface for germination (Plate 38). While half of the pollen were germinated in *K. parviflora*, no germination at all was noticed in *K. rotunda* on the stigmatic surface (Plate 39 and 37). Presence of spines hindering pollen germination was observed in *K. galanga* (Bhurke, 2002). Length of style was 6.19 cm in *K. parviflora*, 7.72 cm in *K. rotunda* and 4.6 cm in *K. galanga* (Plate 33). Fluorescent microscopic studies in *K. galanga*, by Bhurke, (2002) revealed that penetration of pollen tube beyond the stigma occurs only under chemically aided pollination. Even after 24 hours the pollen tube length did not reach ovary in *K. galanga* and did not germinate at all in *K. rotunda*, while pollen tube of *K. parviflora* reached ovule at 12 hours after pollination (Plate 37-39). The retention of the flower in *K. galanga* was only 12 to 16 hours and 24 hours in *K. rotunda* while it was 11 to 12 hours in *K. parviflora*. Length of style as the major hindrance of fertilization was reported in *K. galanga* (Bhurke, 2002). Rekha (1993) also observed the not sufficient pollen

tube growth so as to surpass the lengthy style and to reach the ovary below in *K. galanga* and suggested that the seedlessness might be mainly due to incompatibility reaction prevalent in the style and stigma. The non-germination of pollen in *K. rotunda* might be due to sporophytic self-incompatibility and non-reaching of pollen tube of *K. galanga* in the ovary might be due to gametophytic self-incompatibility, which has to be confirmed by further studies.

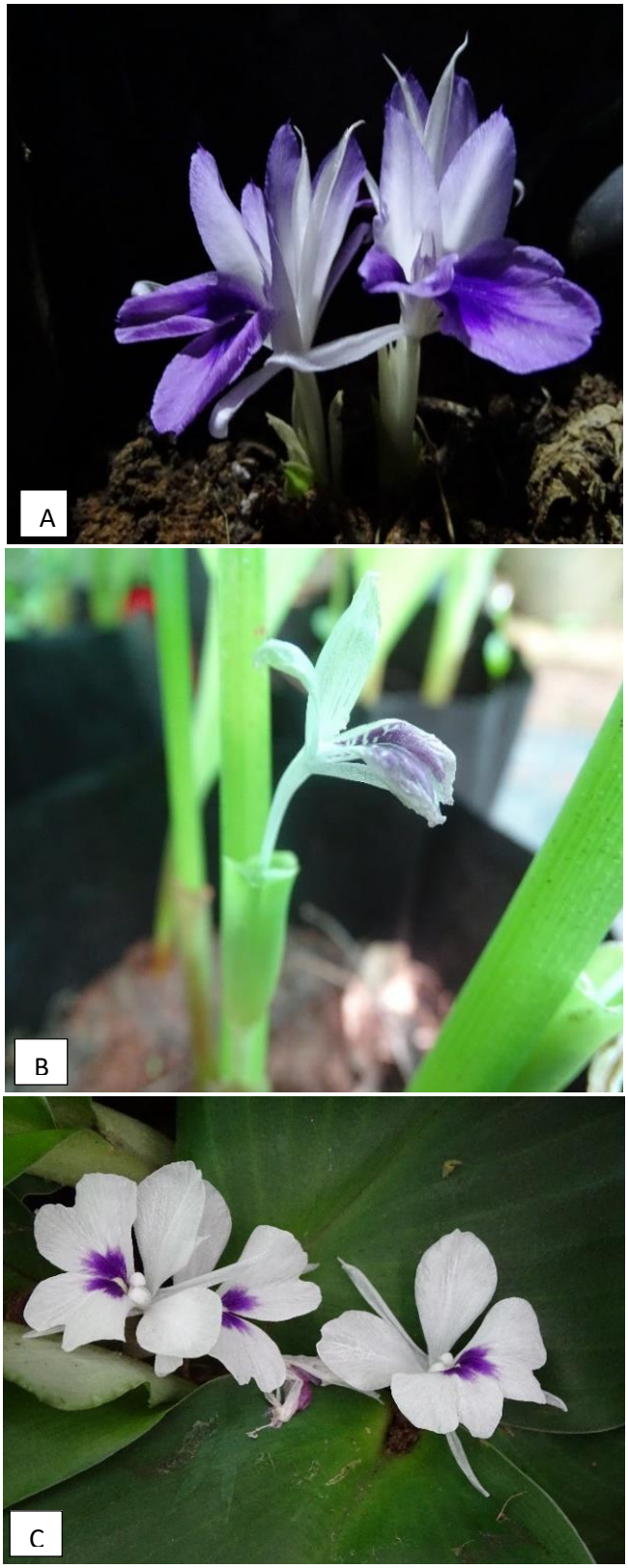


Plate 31: Flowers *Kaempferia* species at the time of anthesis. (A)- Opened flower of *K. rotunda* at 4 am, (B): Opened Flower of *K. parviflora* at 6 am, (C): Opened flower of *K. galanga* at 6 am.

Plate 32: Inflorescence arising directly from rhizome. (A): *K. rotunda*, (B): *K. parviflora*, (C): *K. galanga*

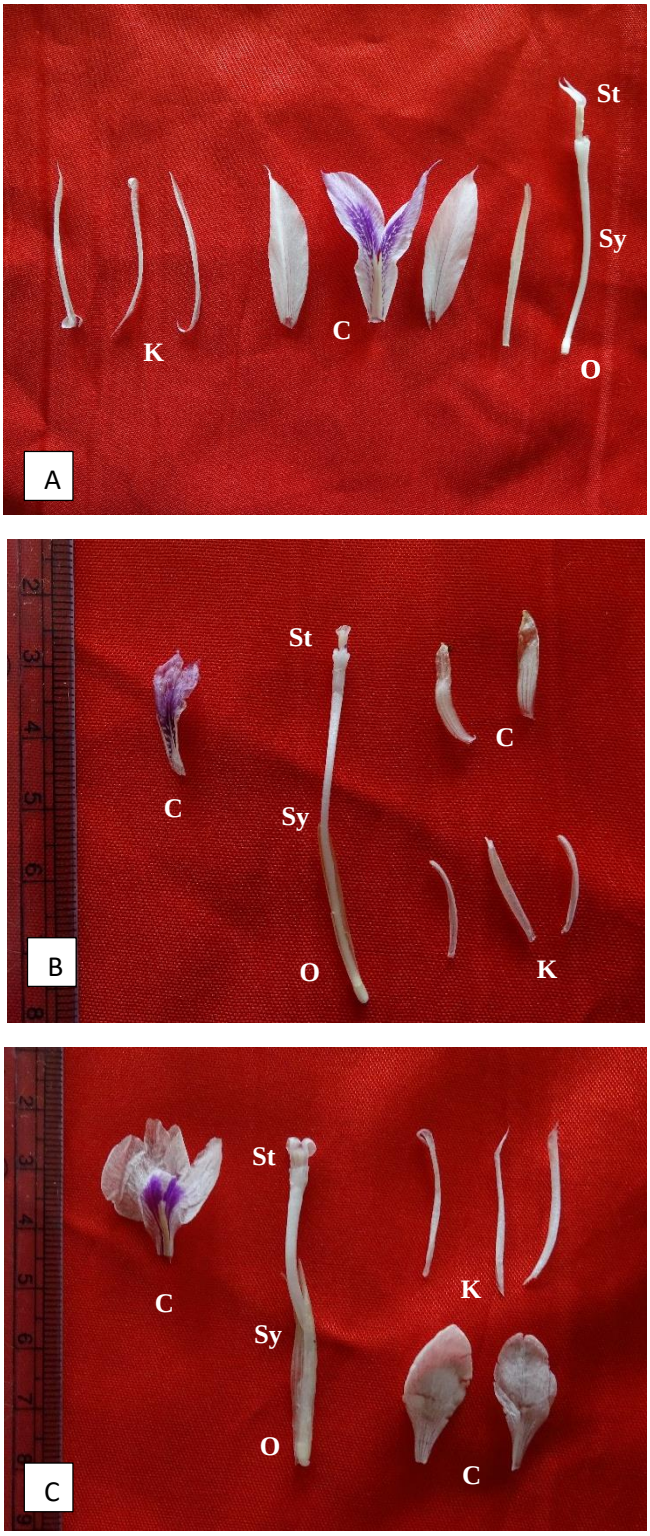


Plate 33: Floral parts of *Kaempferia* species. (A) *K. rotunda*, (B) *K. parviflora*, (C) *K. galanga.* (K-Calyx, C-Corolla, St-Stigma, Sy-Style, O-Ovary)

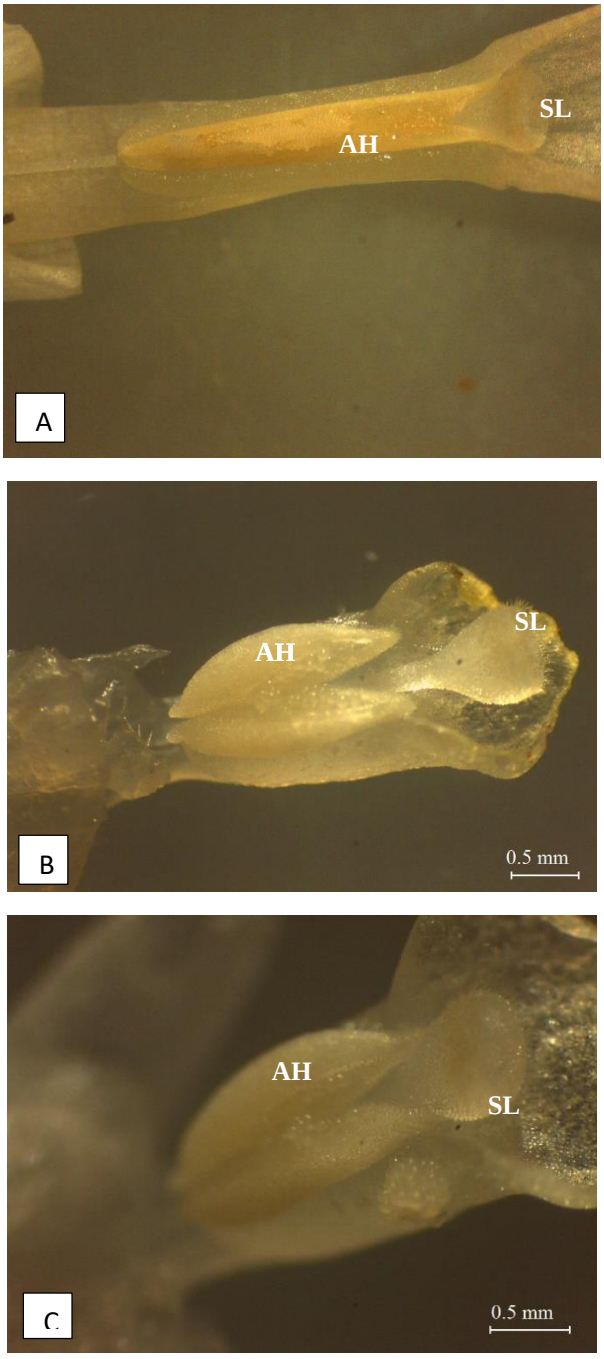


Plate 34: Stamen and stigmatic lobe of *Kaempferia* species. (A) *K. rotunda*, (B) *K. parviflora*, (C) *K. galanga*. (AH-Antherhood, SL-Stigmatic lobe)

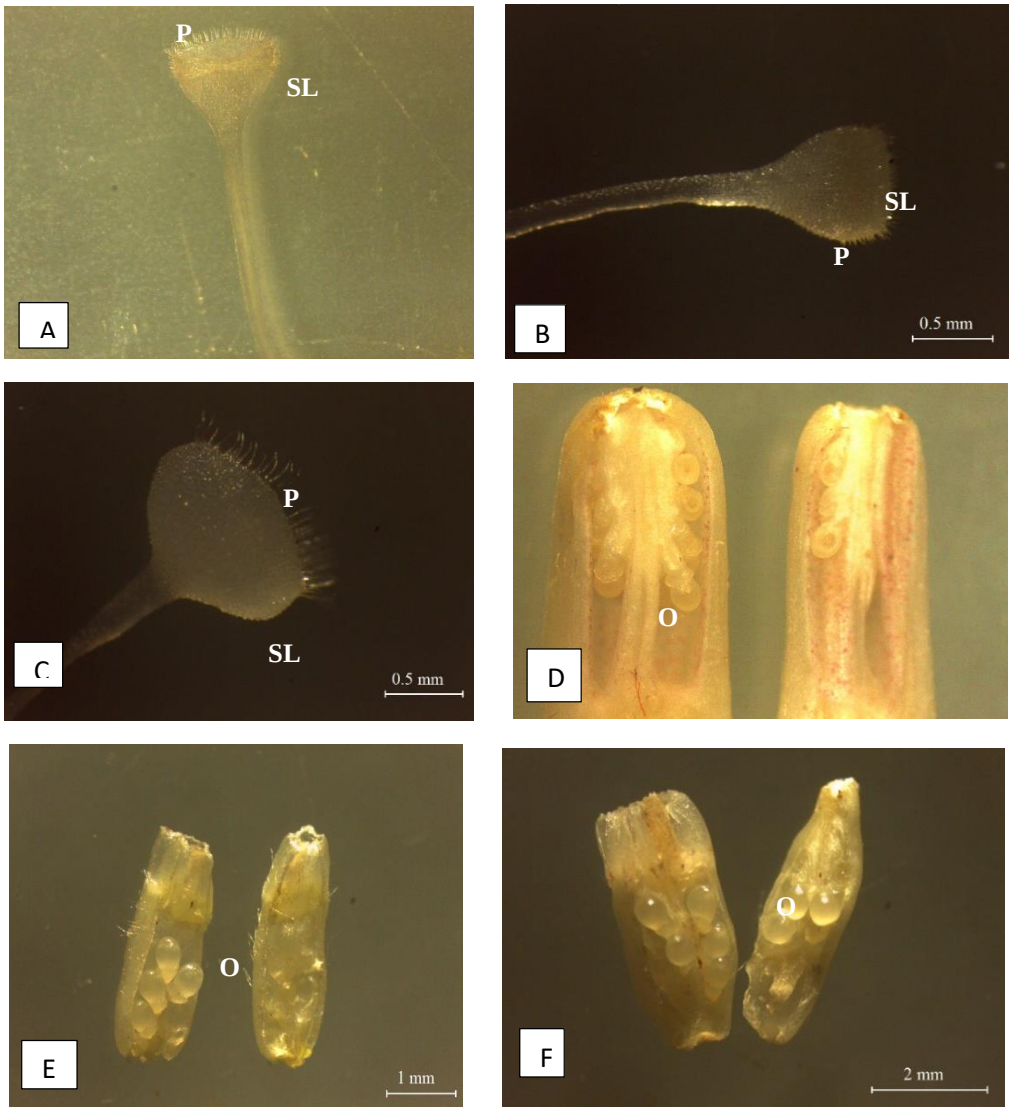


Plate 35: Stigmatic lobe with papillae and ovary in *Kaempferia* species (A) Stigmatic lobe with papillae in *K. rotunda*, (B) Stigmatic lobe with papillae in *K. parviflora*, (C) Stigmatic lobe with papillae in *K. galanga*, (D) Ovary with ovules of *K. rotunda*, (E) Ovary with ovules of *K. parviflora*, (F) Ovary with ovules of *K. galanga*. (SL-Stigmatic lobe, P-Pappilae, O- Ovules)

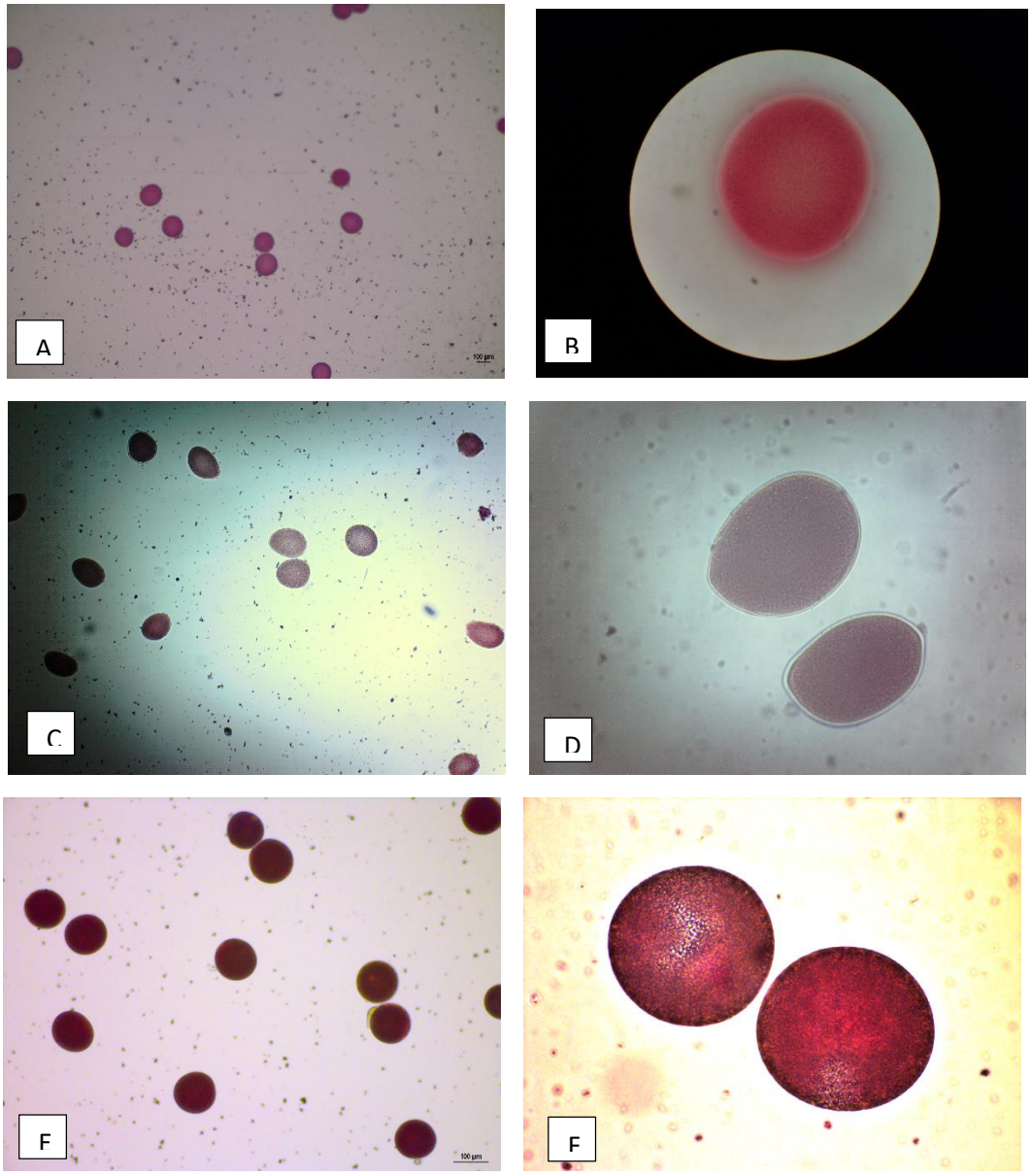


Plate 36: Pollen grain of *Kaempferia* species. (A) Pollen grains of *K. rotunda* 10X, (B) Pollen grain of *K. rotunda* 40X, (C) Pollen grain of *K. parviflora* 10X, (D) Pollen grain of *K. parviflora* 40X, (E) Pollen grain of *K. galanga* 10X, (F) Pollen grain of *K. galanga* 40X.

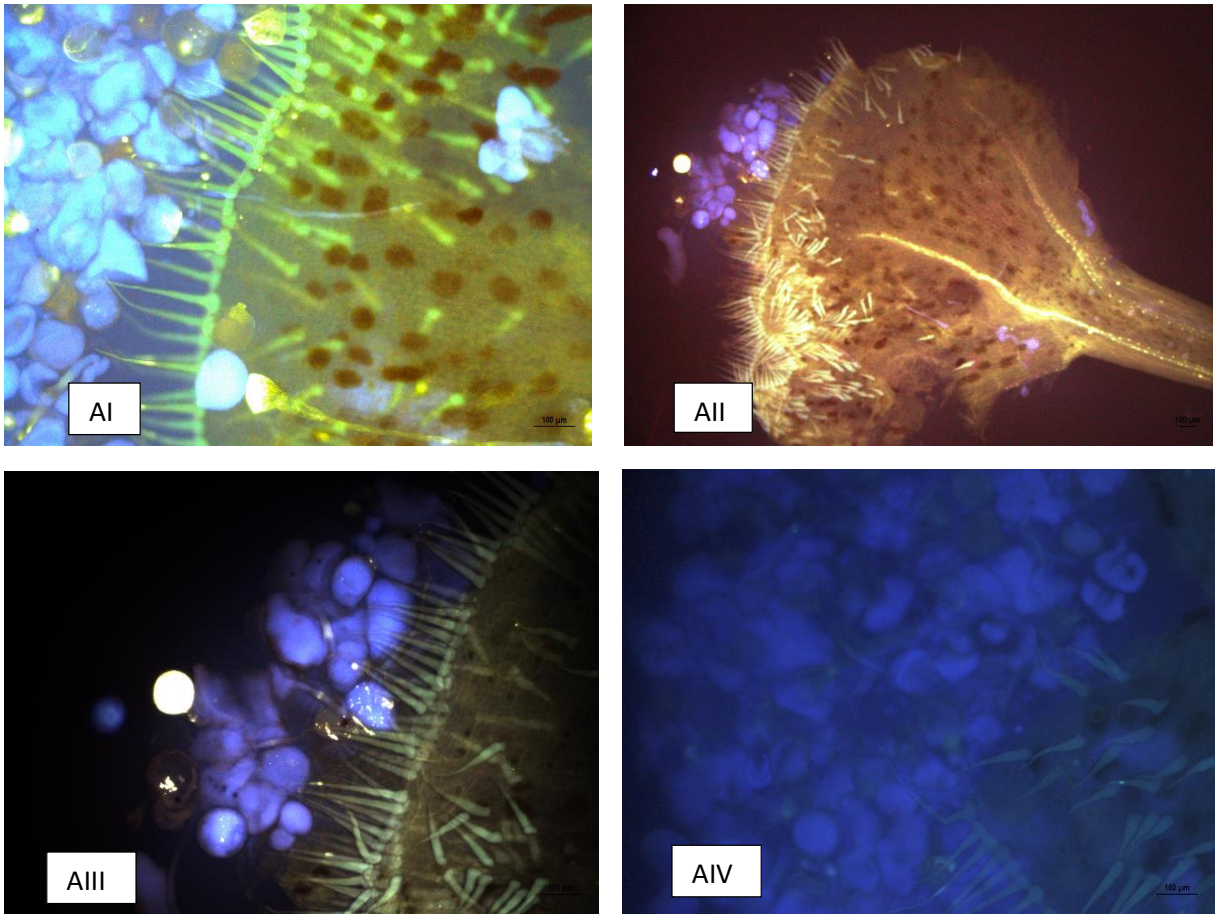


Plate 37: *In vivo* pollen germination studies in *K. rotunda*. (A) Non-germinating pollens of *K. rotunda* in the stigmatic lobe, AI- at 6h after pollination (10X), AII- at 9h after pollination (4X), AIII-at 12h after pollination (10X), AIV2- at 4h after pollination (10X)

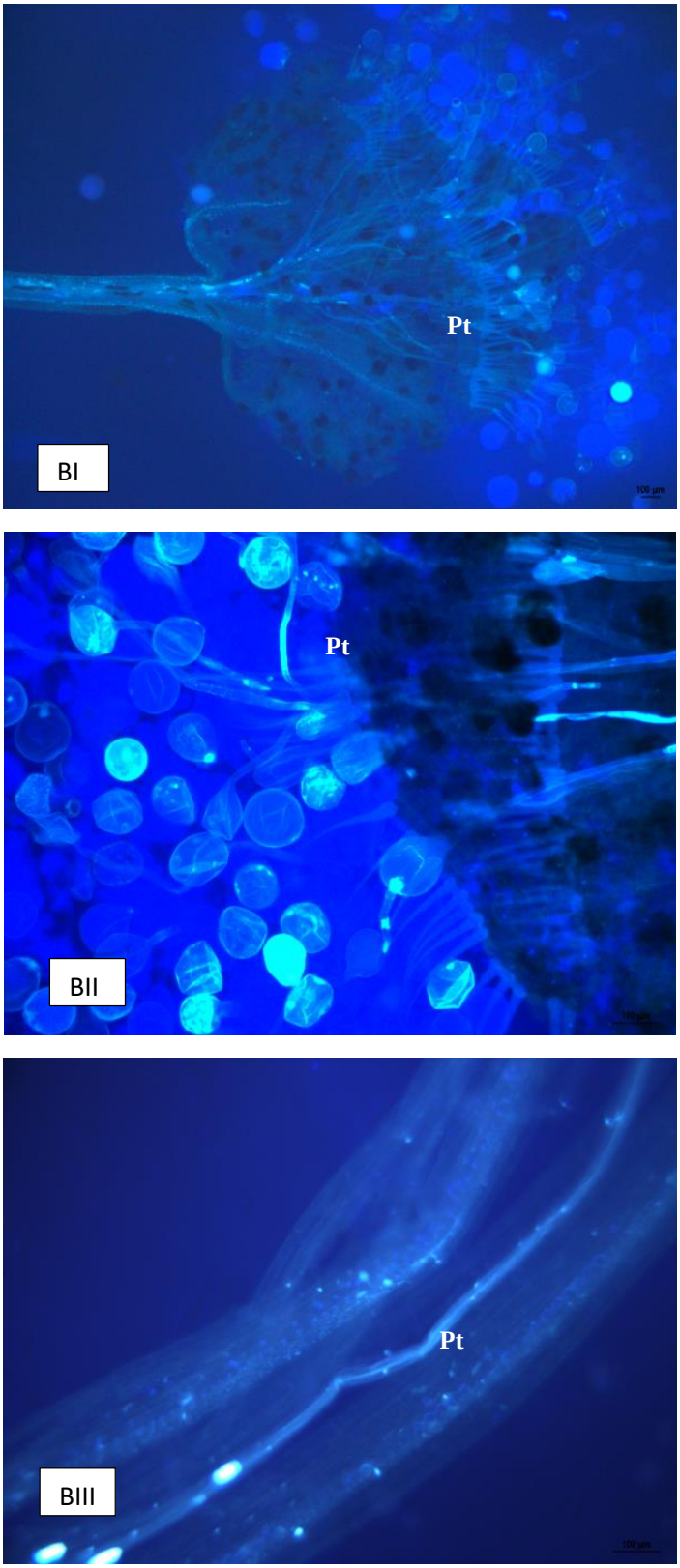


Plate 38: *In vivo* pollen germination in *K. galanga*. (B) Pollens with tube growth in *K. galanga*, BI- 6h after pollination (10X), BII-12h after pollination (10X), BIII- pollen tube growth at 12h after pollination (20X), (Pt-pollen tube)

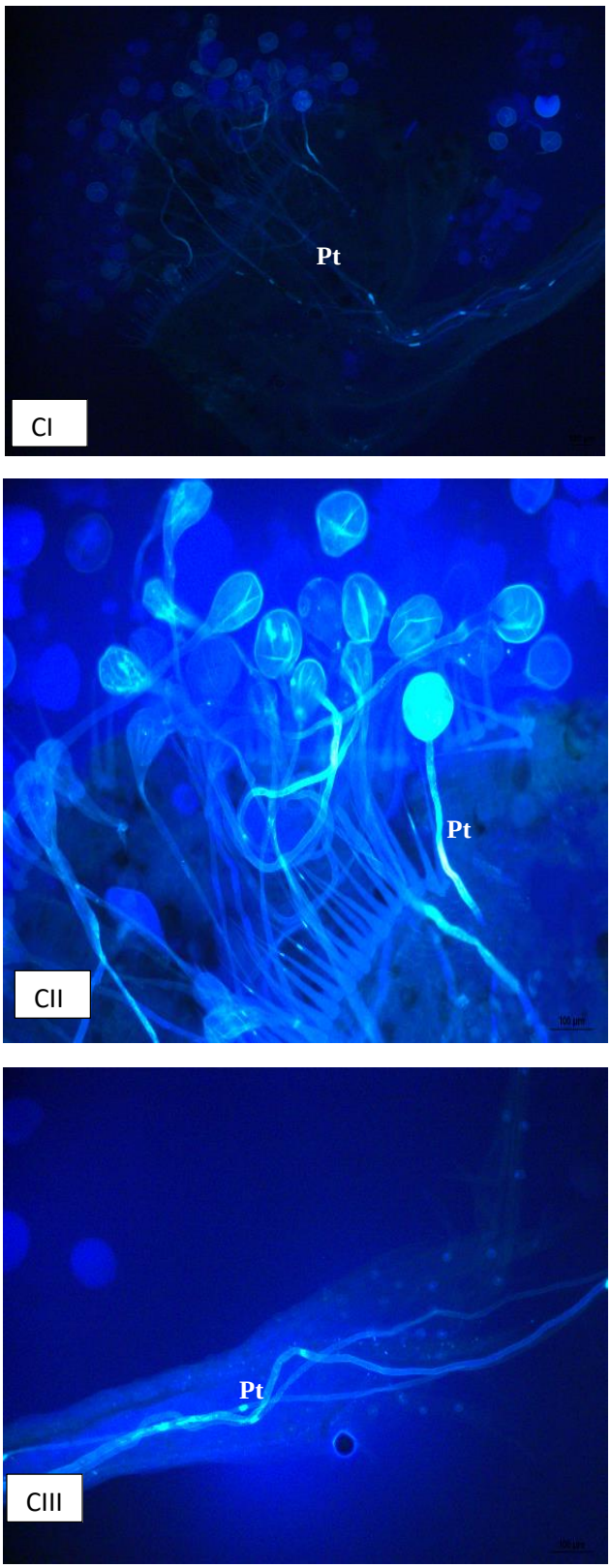


Plate 39: *In vivo* pollen germination in *K. parviflora*. (CI)- Pollen tube growth along the style of *K. parviflora* (4X), (CII)- at 6h after pollination (10X), (CIII)- pollen tube (10X). (Pt-Pollen tube)

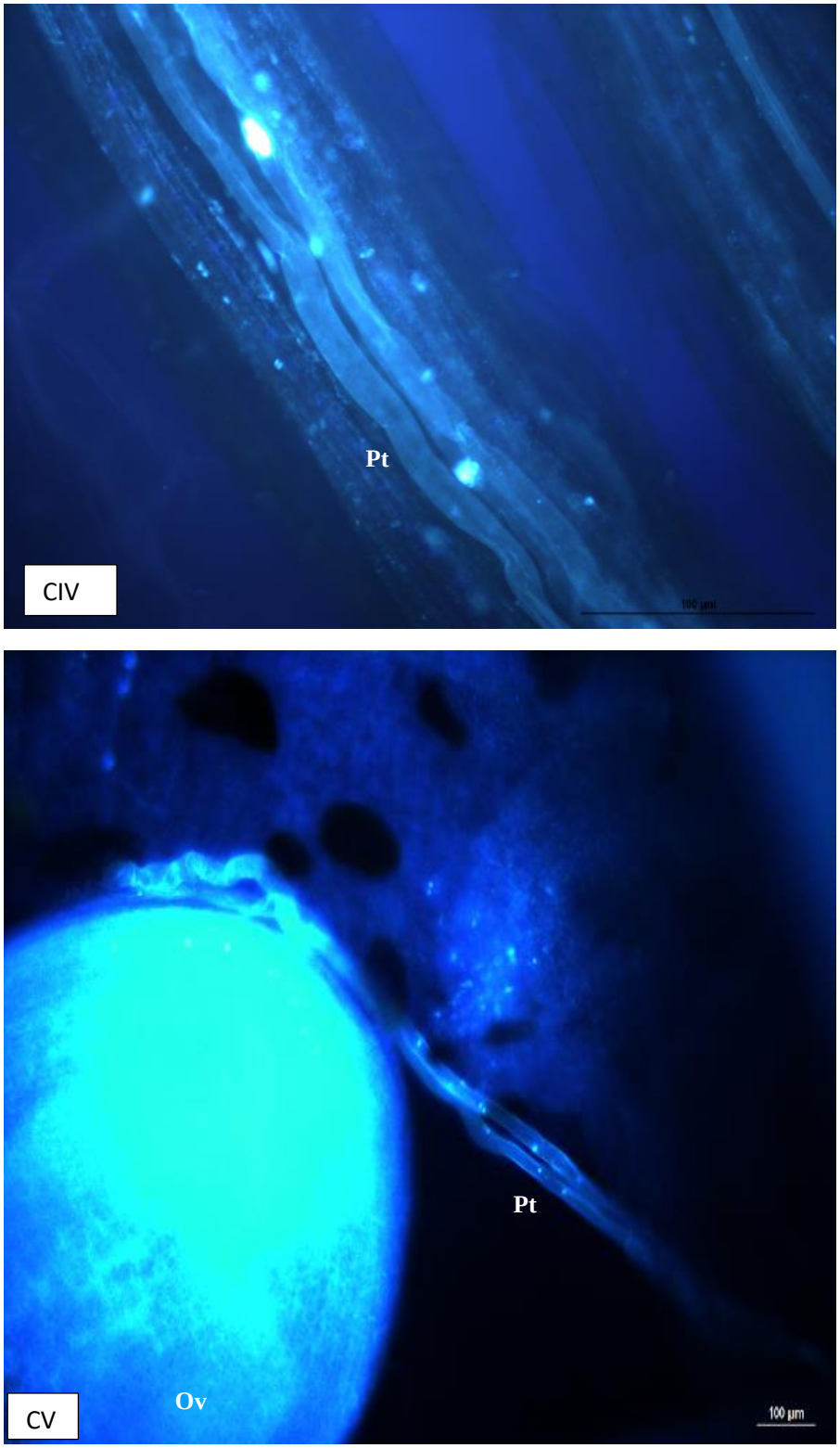
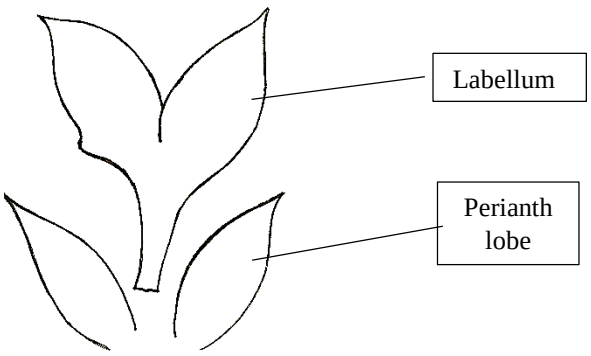
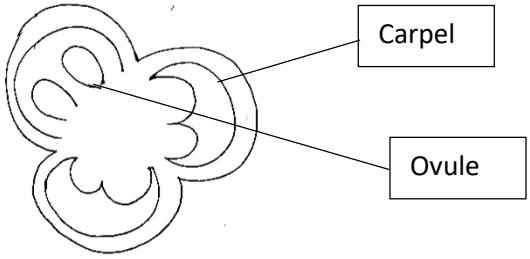
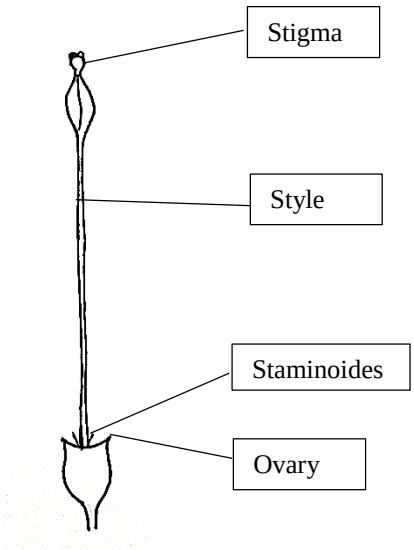


Plate 39: *In vivo* pollen germination in *K. parviflora*. (CIV)- pollen tube (40X), (CV)- Pollen tube reaching ovule 12 h after pollination (40X). (Pt-Pollen tube, Ov-Ovule)





C.S of ovary

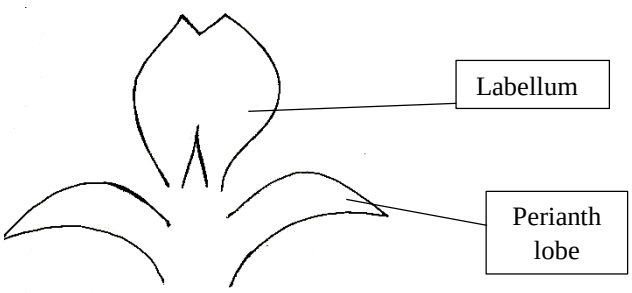


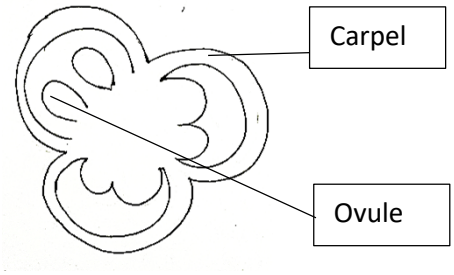
Pistil

Floral diagram

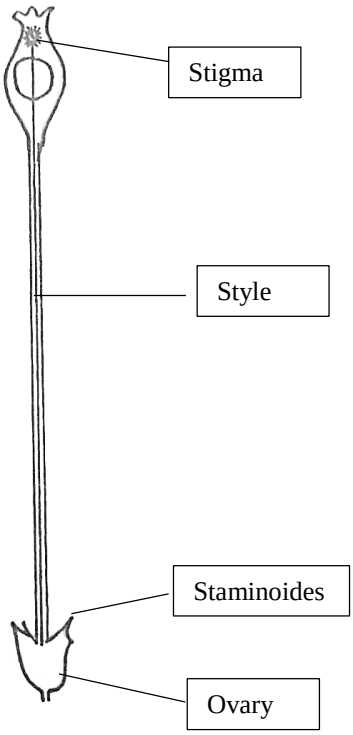
$$\overset{\circ}{\varphi}\ K_{(3)}\ \overset{\frown}{C_{(3)}}\ A_1\ \underline{G}_{(3)}$$

Figure 32: Floral formula of *K. rotunda*

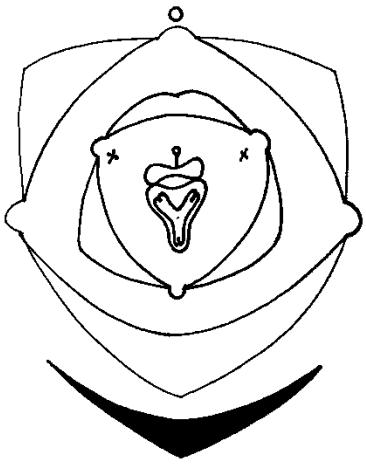




C.S of ovary



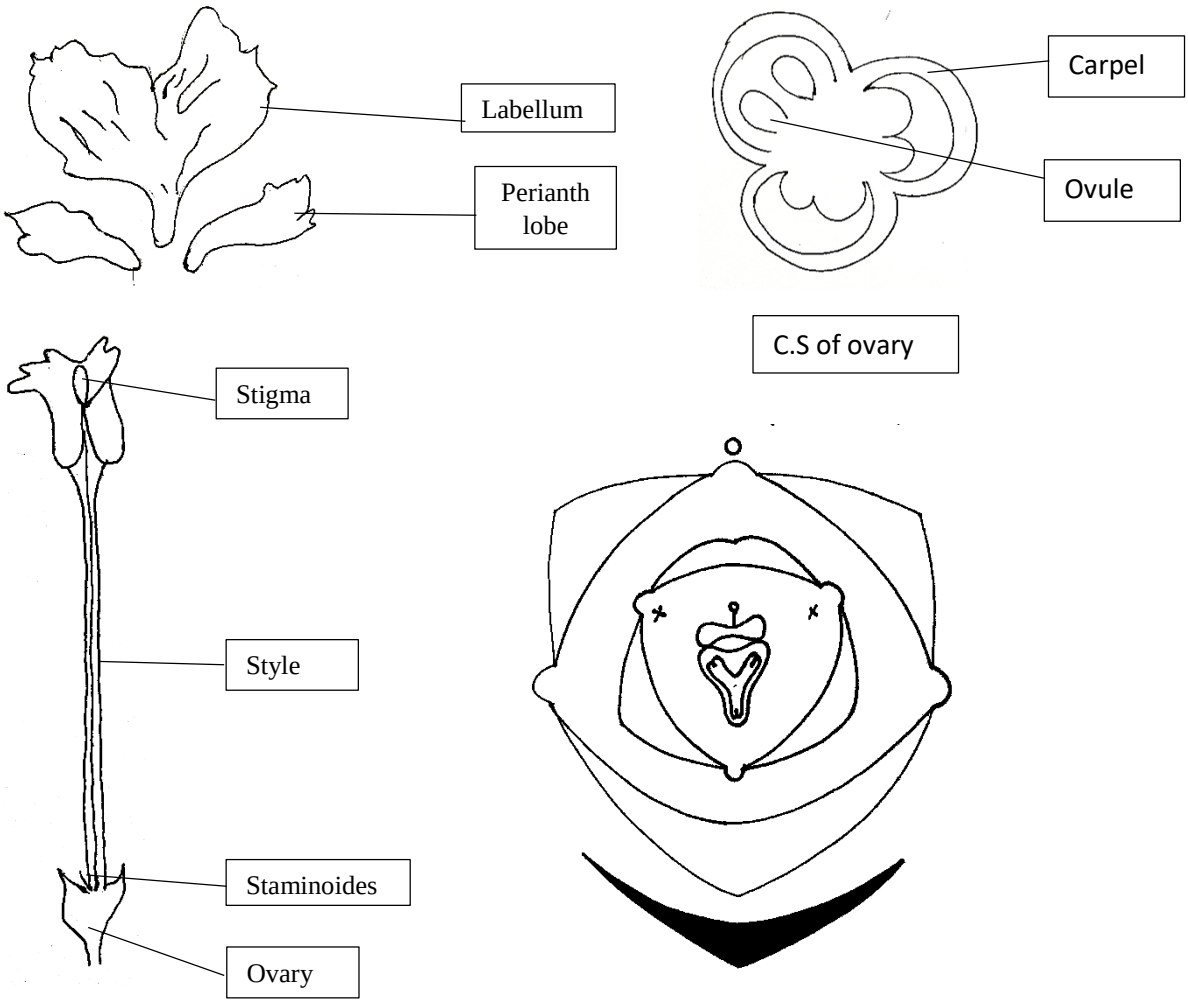
Pistil



Floral diagram

$$\female\ K_{(3)}\ \overset{\frown}{C_{(3)}}\ A_1\ \underline{G}_{(3)}$$

Figure 33: Floral formula of *K. parviflora*



$$\female\ K_{(3)}\ \overset{\frown}{C_{(3)}}\ A_1\ \underline{\overline{G}}_{(3)}$$

Figure 34: Floral formula of *K. galanga*

5.3 ANATOMY OF *Kaempferia* SPECIES

The anatomy of *Kaempferia* species studied in the present investigation revealed many common characters typical of the genus as those mentioned by Tomlinson (1960). Many are also common to the family Zingiberaceae.

In all the species, epidermal cells were irregular or polygonal and usually run perpendicular to veins (Plate 12, 17 and 22). Trichomes present on the leaf lamina were simple and unicellular and the stomata were hexacytic.

In *K. rotunda*, the stomatal index was higher in the adaxial surface, while the length of stomata was higher on the abaxial side. Calcium oxalate crystals were observed in epidermal cells of *K. rotunda* (Plate 23). In *K. parviflora*, length and breadth of stomata were almost similar in both the surfaces, while the stomatal index was higher in upper surface. In *K. galanga*, stomatal length was more in upper surface than the lower and the stomatal index was higher on the lower surface.

Stomata appeared on both the surfaces in ten *Curcuma* species and they were more prominently distributed on the abaxial surface in comparison with the adaxial surface revealing an adaptation to water loss (Seema, 2015). In *Globba marantina* belonging to the family Zingiberaceae, Roy *et al*. (2016) observed larger stomatal size in the upper surface of the leaf and higher stomatal index in the lower surface. They also observed prismatic calcium oxalate crystals in the leaf epidermis.

Transverse section of leaves of all the species possessed uniserriate upper and lower epidermis (Plate 13, 18 and 23). *Kaempferia parviflora* and *K. rotunda* had larger epidermal cells when compared with *K. galanga*. All the three species had collateral and closed type of vascular bundles. Oil globules were present in *K. galanga* leaves, while it was absent in the other two species. Prismatic calcium oxalate crystals were noticed in *K. parviflora* in the mesophyll cells (Plate 18). Anuwong *et al*. (2014) have also reported collateral type of vascular bundle in TS of leaf in *Curcuma alimatifolia*. Variations were noticed in the transverse sections of the leaf lamina, midrib and petiole in 20 species of *Alpinia* by Hussin *et al*. (2000).

Transverse section of stem had single layer epidermis in all the species with smaller size in *K. galanga* when compared with other two species. Outline of hypodermis was irregular in all the species (Plate 14, 19 and 24). Air chambers and bigger vascular bundles were seen alternately arranged towards the lower epidermis. This arrangement was observed in other species of zingiberales by Roy *et al*. (2016) also.

The TS of rhizome showed some variation among the species especially with regard to the number of oil globules as well the distribution of starch granules in the cortex region. All the species had brown colour cork cells in 6-8 layers in *K. rotunda*, 8-9 layers in *K. parviflora* and 6-7 layers in *K. galanga*. Small vascular bundles were seen embedded in the endodermal layer. In *K. galanga*, starch granules were seen near the endodermal layer only while it was scattered throughout the rhizome section and found to be more concentrated near the endodermal layer in both *K. rotunda* and *K. parviflora* (Plate 15, 20 and 25). Starch granules were oval to spherical shaped in *K. galanga* and *K. parviflora* but it was oval shaped in *K. rotunda*. Dark orange coloured oil globules and light orange coloured oleoresin mass were present in all *Kaempferia* species. Oil globules were abundant in *K. parviflora*. Apart from oil and oleoresin mass, *K. parviflora* also contained flavonoid vacoules in the rhizome section.

Roy *et al*. (2013) described the rhizome anatomy of *Kaempferia galanga*, *Curcuma aromatica*, *C. caesia* and *C. longa* with 1- cell-layered epidermis followed by 1-2 celled-layered hypodermis or the absence of hypodermis, then the cork cambium layer of few cell-layered thick; then the parenchymatous cortical zone of massive cell layers, then endodermis encircling the ground tissue with the numerous vascular bundles.

In ginger, Eltahir *et al*. (2018) observed collateral and closed type of vascular bundles scattered across the rhizome. Pitakpawasutthi *et al*. (2018) observed a visible endodermis, oil droplets and starch grain in the parenchyma cells of rhizome section in *K. parviflora*. Sereena *et al*. (2011) observed a distinct endodermis like layer in the rhizome in *K. rotunda* and oval shaped starch granules.

Zulfa (2012) found numerous starch grains, varying in shape, size and number present in both sides of intermediate zone in *K. parviflora*. Many plant cells accumulated water-soluble flavonoid pigments which range in colour from orange-red to purple in vacuoles (Taiz, 1992).

The outline of the transverse section of root tuber was circular in all the species (Plate 16, 21 and 26). Exodermis was single layered and thin walled. Vascular tissues in endodermal layer were polyarch in arrangement. Starch granules were present abundantly in the root tubers in all the species. The occurrence of polyarch (8–18) vascular bundles in *Kaempferia* is similar to the observations made by Uma and Muthukumar (2014) in gingers. They also reported that tuberous roots of *Globba*, *Hedychium* and *Kaempferia* had a wide, starch-filled cortex with stele diameter similar to non-tuberous roots.

The anatomy of three *Kaempferia* species is tabulated in the following table.

Table 116: Comparative anatomy of *Kaempferia* species

Character	*K. rotunda*	*K. parviflora*	*K. galanga*
Type of stomata	Hexacytic	Hexacytic	Hexacytic
Stomatal index	16.15	11.55	10
Leaf epidermis	Uniserriate and large	Uniserriate and large	Uniserriate and small
Presence of oil globule in leaf epidermis	Absent	Absent	Present
Presence of calcium oxalate crystal in epidermal cell of leaf	Present	Absent	Absent
Presence of calcium oxalate crystal in TS of leaf lamina	Absent	Present	Absent

Table 116 contd.

Character	*K. rotunda*	*K. parviflora*	*K. galanga*
Presence and intensity of oil globules in rhizome	Present abundantly	Present abundantly	Present sparsely
Presence and intensity of starch in rhizome	Scattered throughout and concentrated near endodermis and present abundantly	Scattered throughout and concentrated near endodermis and present abundantly	Concentrated near endodermis only and present sparsely

5.4. BIOCHEMICAL STUDIES

Discussion on the biochemical constituents of the three Kaempferia spices is furnished here.

5.4.1 Volatile oil and oleoresin content of *Kaempferia* species

Volatile oil per cent ranged from 0.057 to 0.317 per cent in *K. rotunda*, 0.20 per cent to 0.21 per cent in *K. parviflora* and 0.64 to 0.71 per cent in *K. galanga*. Kumar (2014) has earlier reported that *K. galanga* rhizomes gave yellow coloured oil yielding 3.01 per cent oil. Wide variation of essential oil content has been reported in *K. galanga* ranging from 0.3 to 1.9 per cent by Raina and Abraham (2016). Nimisha (2018) reported volatile oil ranging from 2.26 to 4.45 per cent while Dev (2013) reported oil recovery of 1.20 to 2.32 per cent in ginger genotypes. In the present study even though the anatomy of rhizome revealed abundant oil globules in *K. parviflora* followed by *K. rotunda*, the oil recovery was lowest in *K. parviflora*.

The oleoresin content ranged from 0.60 to 4.95 per cent in *K. rotunda*, 2.03 to 4.17 per cent in *K. parviflora* and 2.12 to 2.67 per cent in *K. galanga*. In all the species the oleoresin content varied greatly among the genotypes. The rhizome

anatomy of the three species also located oleoresin mass, *K. parviflora* possessing more number. In ginger genotypes, oleoresin content ranged from 4.12 to 6.01 per cent (Nimisha, 2018). Kavitha and Menon (2013) found oleoresin content ranging from 2.07 to 3.07 per cent in *K. galanga*.

5.4.2 Biochemical parameters

Starch the main component of rhizome. Starch content was three fold high in *K. parviflora* (13.2 mg/100 mg) compared to *K. galanga* (4.4 mg/100 mg) and *K. rotunda* (3.8 mg/100mg). Total sugar content was high in *K. rotunda* (1.74 mg/100 mg) followed by *K. galanga* (1.23 mg/100 mg) and *K. parviflora* (9.00 mg/100 mg). Total free amino acid was highest in *K. rotunda* (1.15 mg/100 mg). *K. parviflora* rhizome contained huge quantity of flavonoids (26.1 mg eq. quercetin/100 mg) when compared to the other two species.

In *K. galanga*, Kalaiselvi and Ponmozhi, (2011) reported total carbohydrate content of 1.9 mg/g, starch content of 1.7 mg/g, amino acid content of 3.1 mg/g and flavone and flavanol content of 30.8 mg/g. Total carbohydrate content of *Curcuma* species varied from 767.90 ± 6.3 to 560.64 ± 13.9 µg/mg eq to glucose (Jain and Parihar, 2017) and total free amino acid ranged from 73.44 µg/mg to 859 µg/mg (Jain and Parihar, 2017).

Higher contents of sugars and amino acids are advantages to medicinal plants especially in plants used as rejuvenating drugs, tonics, adaptogens *etc*. Shankar (2011) has reported high sugars and amino acids in *Nervilia aragoana* which is used as a tonic, Jacob (2017) reported higher amino acids in medicinal ashgourd which is used as a rejuvenating drug, Miniraj (2011) reported high sugars and amino acids in *Seidenfia rheedii*, a medicinal orchid.

Total flavonoid content of 24.41 mg/100ml wine was reported in *K. parviflora* wine (Vichitphan *et al*., 2007). Methanolic extract of *K. parviflora* contained 17.20 mg of flavonoids per gram of extract (Nanasombat *et al*., 2014). Diversity of flavonoid content in *K. parviflora* from 12 different origins of Thailand investigated by Sutthanut *et al*. (2007) found that total flavonoid content ranged

from 23.86 to 60.98 mg/g. Flavonoids are associated with health-promoting effect because of their antioxidant, anticarcinogenic and anti-inflammatory properties (Panche *et al*., 2016; Asif and Khodadadi, 2013).

Studies focussing on flavonoids and the other phenolic compounds from medicinal plant species have increased considerably because of their versatile benefits for human health (Tungmunnithum *et al*., 2018). Mishra *et al*. (2013) found that leaf extracts of *Bauhinia variegata* contained flavonoid compounds, and possessed antioxidant properties against oxidative damage. Phenolic compounds especially flavonoids have long been reported as chemopreventive agents in cancer therapy (Ahmed *et al*., 2016; Mishra *et al*. 2013; Brusselmans *et al*., 2003; Block *et al*., 1992). The present study also proved the *in vitro* antioxidant, anticancer, antimicrobial and *in vivo* immunomodulatory properties of *K. rotunda* and *K. parviflora* which might have been contributed by the abundant presence of flavonoids, sugars and total free amino acids in the rhizome.

5.4.3 GCMSMS profile for volatile oil of *K. parviflora*

A total of 34 compounds were identified in the volatile oil of *K. parviflora* (Table 88 and Fig. 35). Ethanol, 2-(trimethylsilyl) occupied the largest area. Compounds belonging to flavone group identified were 3,5-Dihydroxy-4',7-dimethoxyflavone; 3',4',5,7-Tetramethoxyflavone; Flavanone, 3,5,7-trihydroxy-4'-methoxy; 4',5,7-Trihydroxyflavanone, tris(trimethylsilyl) ether; Flavanone, 3-hydroxy-2',4',5,7-tetramethoxy-, acetate, trans; 7-methoxy-4'-hydroxyisoflavone and Acacetin. Acacetin which is a flavonoid was reported in essential oil of leaves of *Myrtus communis* (Dellaoui and Berroukche, 2019). The present study also exhibited the abundant presence of flavonoids in the anatomy of rhizome, phytochemical screening, biochemical analysis of rhizome and GCMS profiling of the ethanolic extract of *K. parviflora*.

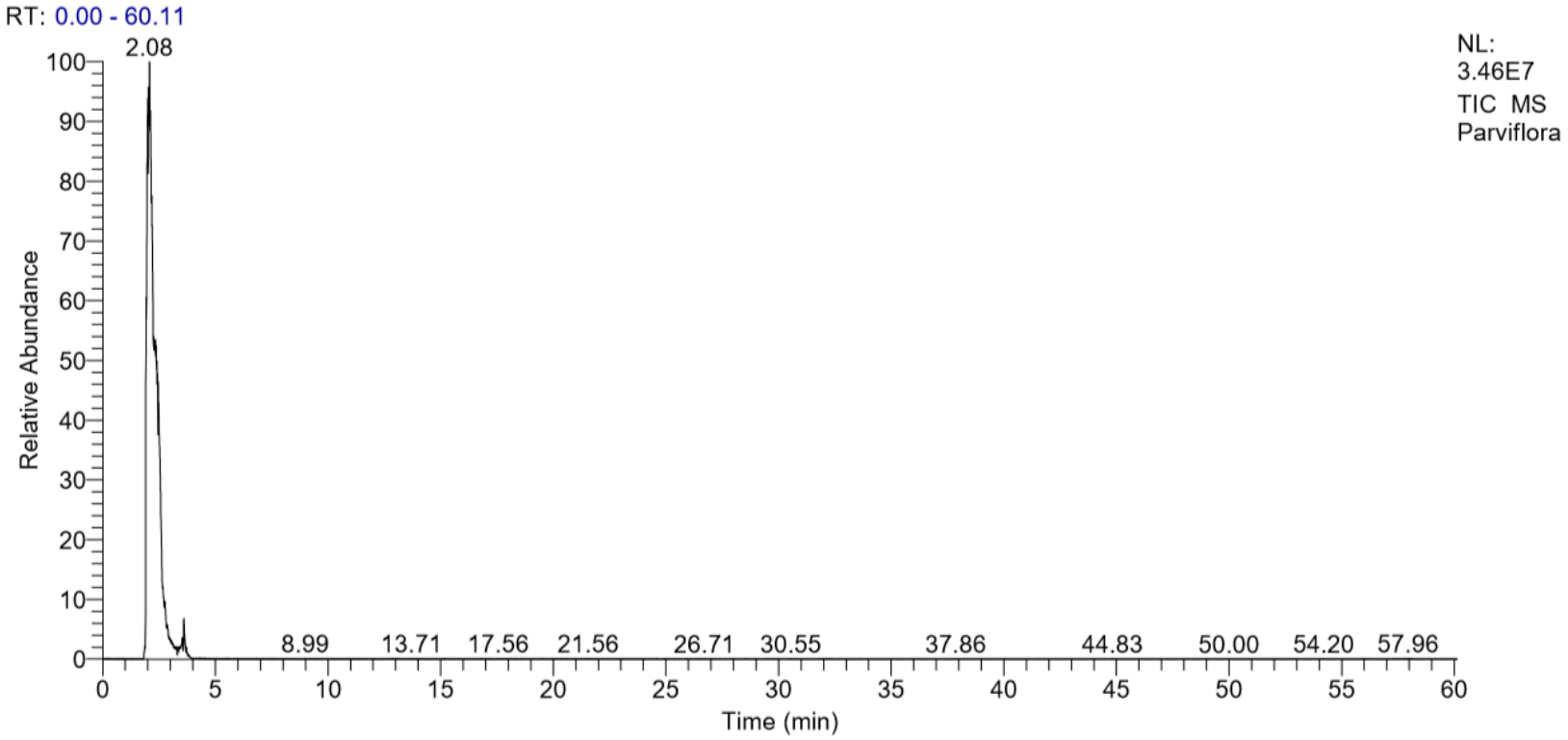


Figure 35: Chromatogram of GCMSMS profile for volatile oil of *K. parviflora*

5.4.4 GCMSMS profile for volatile oil of *K. rotunda*

Twenty two compounds were identified from volatile oil of *K. rotunda* (Table 89 and Fig 36). Camphene (32.88 %) was the major compound identified followed by Bornyl acetate (18.52 %), Benzyl Benzoate (15.39 %), Camphor (11.01 %), Pentadecane (6.73 %), Podocarp-7-en-3á-ol, 13á-methyl-13-vinyl- (6.67 %), endo-Borneol (2.03 %) and à-ylangene (1.05 %).

Sirat *et al*. (2005) reported pentadecane (25.4 per cent), bornyl acetate (24.9 %), benzyl benzoate (15.3 %) and camphor (12.1 %) as the major constituents of the essential oil of *K. rotunda*.

Jamalluddin (2014) reported 33 components in the essential oil of *K. rotunda* with high concentration of benzyl benzoate (31.48 %), bornyl acetate (5.56 %), camphor (5.45 %) and camphene (5.04 %).

Feng (2009) identified main constituents of rhizome oil from Malaysia in genotypes of *K. rotunda* as bornyl acetate (9.6 %), benzyl benzoate (8.4 %) and camphor (5.6 %), while the rhizome oil from Indonesia as rich in benzyl benzoate (87.7 %) and n-pentadecane (4.2 %).

5.4.5 GCMSMS profile for volatile oil of *K. galanga*

In the present study, 27 compounds were identified (Table 90 and Fig. 37). Eucalyptol (20.94 %) was the the major compound identified followed by Ethyl p-methoxycinnamate (16.44 %), Pentadecane (15.63 %), à-Pinene (12.76 %), Camphene (10.82 %), 2-Propenoic acid, 3-phenyl-, ethyl ester (8.15 %), endo-Borneol (5.54 %), 2,6-Dimethyl-1,3,5,7-octatetraene, E,E- (4.75 %) and ç-Muurolene (1.24 %).

Twenty chemical compounds were identified by Tunsaringkarn *et al*. (2007) from *K. galanga* oil using GC finger printing. Major compounds were ethyl cinnamate, pentadecane and ethyl p-methoxy cinnamate.

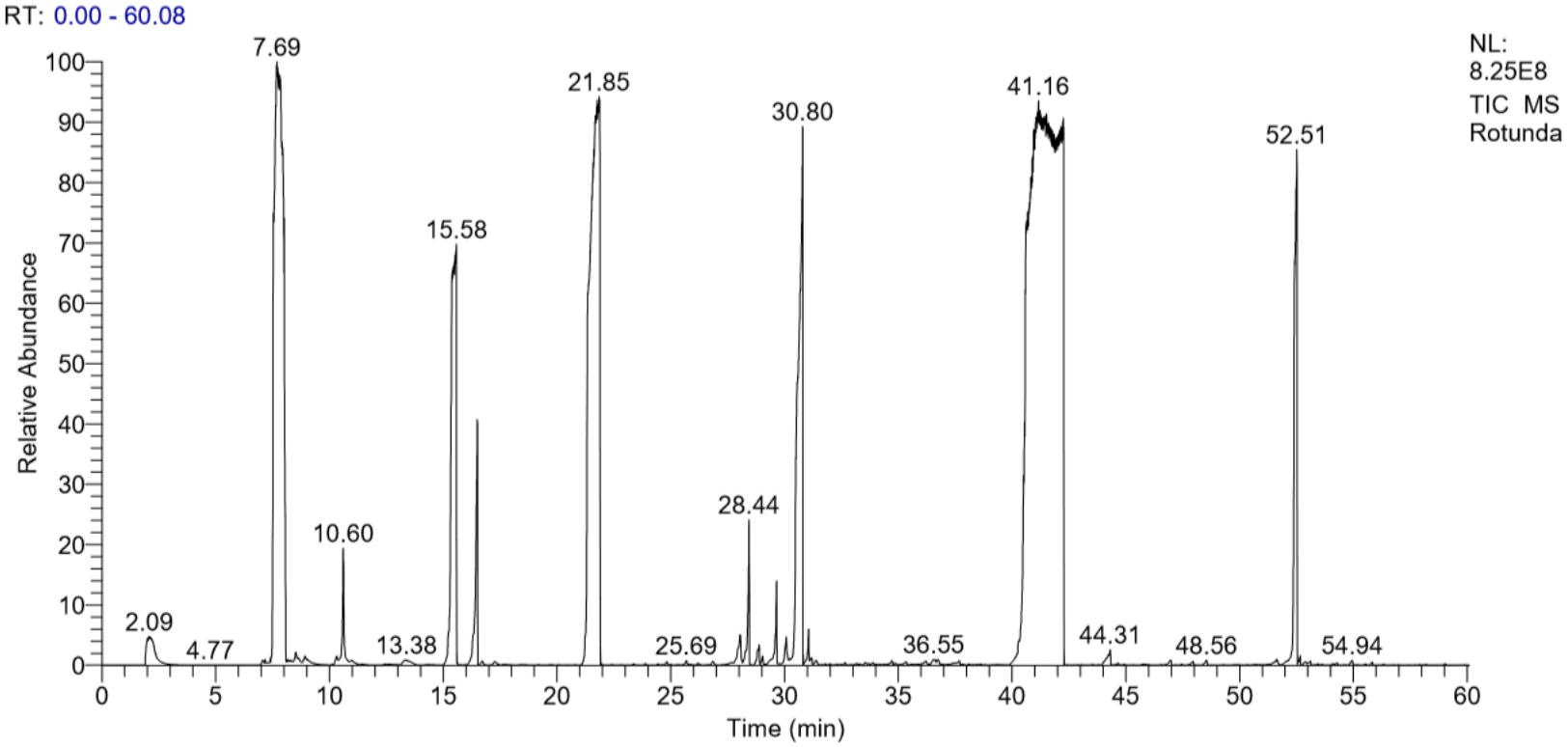


Figure 36: Chromatogram of GCMSMS profile of *K. rotunda* volatile oil

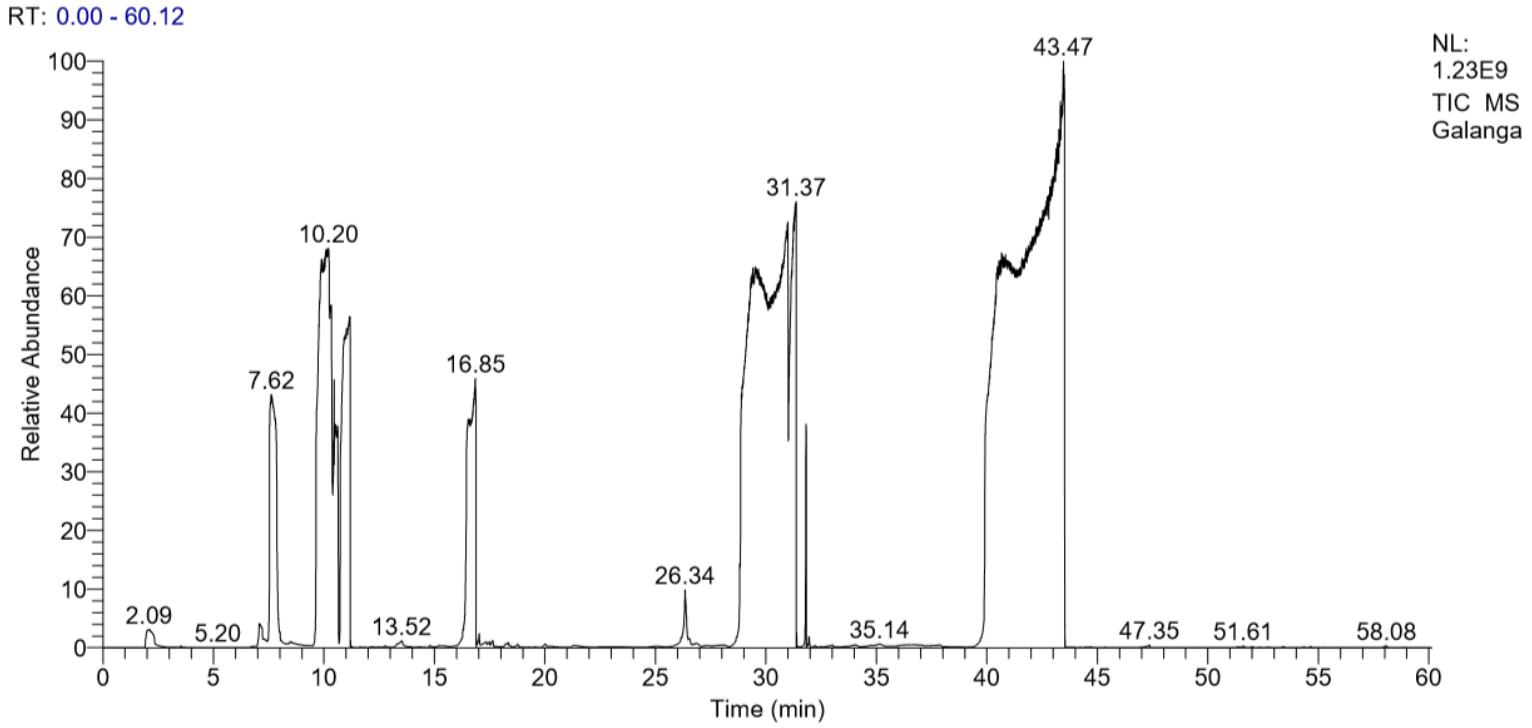


Figure 37: Chromatogram of GCMSMS profile of *K. galanga* volatile oil

Tewtrakul *et al*. (2005) found major components of *K. galanga* oil to be α-pinene (1.28 %), camphene (2.47 %), carvone (11.13 %), benzene (1.33 %), eucalyptol (9.59 %), borneol (2.87 %), methyl cinnamate (23.23 %), pentadecane (6.41 %) and ethyl-p-methoxycinnamate (31.77 %).

Li *et al*. (2017) identified forty-one components from essential oil of *K. galanga* using GCMS analysis and reported the strong nematicidal activity of major component *i.e*. ethyl cinnamate, ethyl ρmethoxy cinnamate and trans-cinnamaldehyde.

Many authors have reported the chemical constituents of *K. galanga* oil by GCMS analysis, thirty-eight aroma compounds (Raina and Abraham, 2016), 25 compounds (Yang *et al*., 2018), eighty one components (Bhuiyan *et al*., 2008).

5.4.6 GCMS profile of ethanolic rhizome extract of *K. parviflora*

A total of 8 compounds were identified from ethanolic extract of *K. parviflora* (Table 91 and Fig. 38). The major compounds present in the extract were dimethylchrysin, techtochrysin, benzaldehyde, (diphenylmethylidene) hydrazine and tri-o-methylapigenin. The same compounds were previously reported by various authors in *K. parviflora* (Yenjai *et al*., 2004 and Sutthanut, 2007). Tri-o-methylapigenin (synonym: 4',5,7-trimethoxyflavone) was reported to have antiplasmodial, antifungal and antimycobacterial activity (Yenjai *et al*., 2004). Techtochrysin, a methoxyflavone, was found to induce skeleton muscle hypertrophy (Sultana *et al*., 2011). Flavonoids isolated from different plant sources namely flavonoids from *Phyllanthus niruri* (Jose *et al*., 2014) and flavonoids from *Vitis vinifera* (Liu *et al*., 2012) showed immunomodulatory effect.

5.4.7 GCMS profile of ethanolic rhizome extract of *K. rotunda*

Eighteen compounds were identified from ethanolic extract of *K. rotunda* (Table 92 and Fig. 39). Major compounds identified were Benzoic Acid, Phenylmethyl Ester (34.49 %), Pentadecane (16.35%), Andrographolide (8.09 %), L-.Alpha.-Bornyl Acetate (7.90 %), 4,8,13-Cyclotetradecatriene-1,3-Diol, 1,5,9-Trimethyl-12-(1-Methylethyl)- (7.27 %), Heptadecane (4.32 %), 2,5-Furandione,

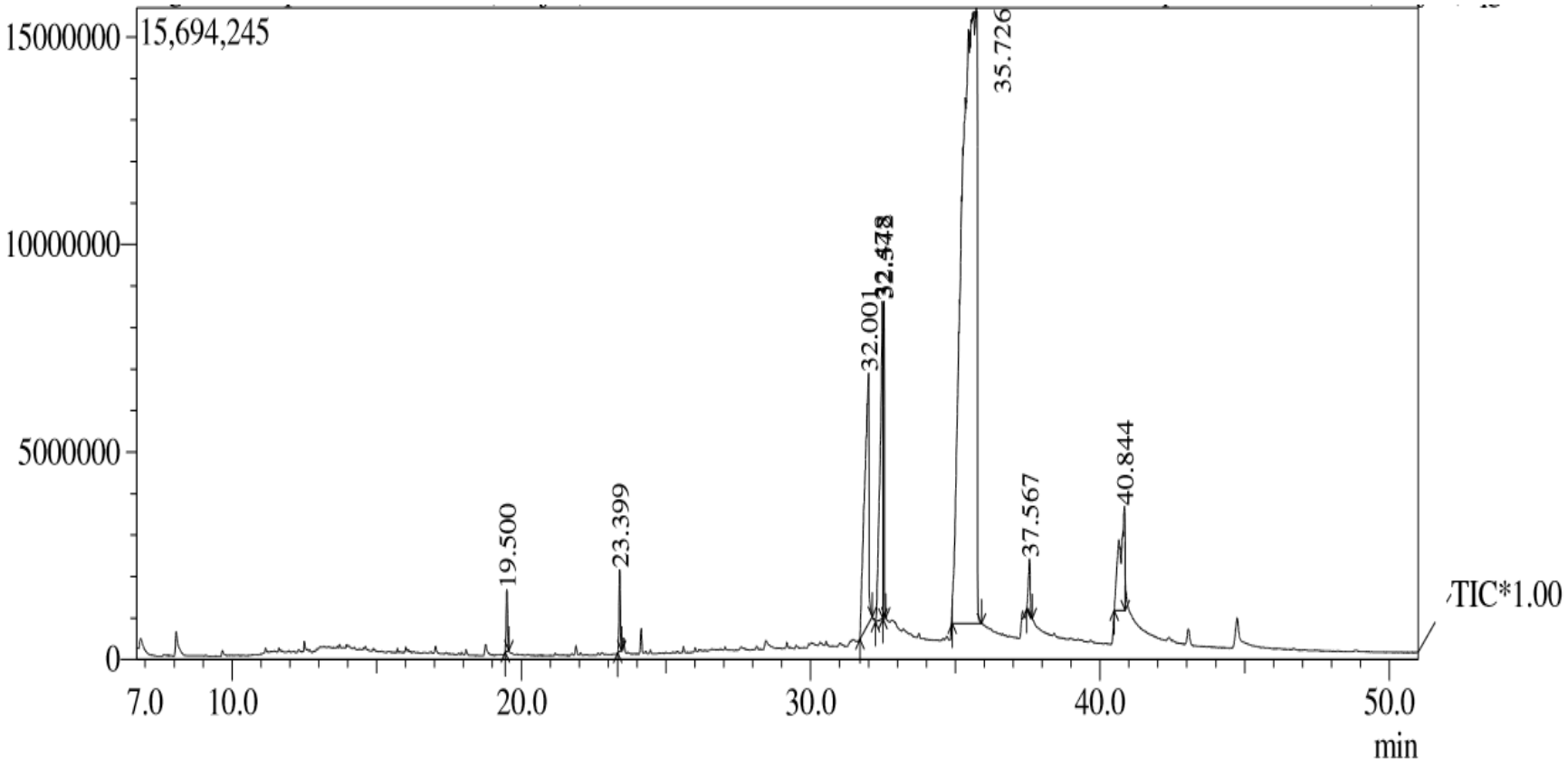


Figure 38: Chromatogram of GCMS profile of ethanolic rhizome extract of *K. parviflora*

3-(Dodecenyl)Dihydro- (4.34 %), trans-Z-.alpha.-Bisabolene epoxide (3.31 %), Sandaracopimar-15-en-8.beta.-yl acetate (3.24 %), Glycerol 2-Hexadecanoate (1.77 %).

A total of nine compounds were identified by Suphrom *et al*. (2017) from the ethanolic rhizome extract of *K. rotunda* and the major constituents were benzyl benzoate (5.46 %) and crotepoxide (42.92 %), pentadecane (2.39 %) and heptadecane (0.29 %). Pentadecane was detected in essential oil of *K. rotunda* by Sirat *et al*. (2005) and heptadecane was detected from the essential oil of *K. galanga* (Indrayan *et al*., 2007).

Andrographolide is bioactive chemical constituent of *Andrographis paniculata* is also detected in the present study which has got potent immunomodulatory and anti-angiogenic activities in tumorous tissues as reported by Varma *et al*. (2011).

5.4.8 Phytochemical screening of ethanolic rhizome extract of *K. parviflora* and *K. rotunda*

Results of phytochemical screening of ethanolic extract of *K. rotunda* using different tests revealed the presence of steroids, flavonoids, glycosides and saponins and ethanolic extract of *K. parviflora* indicated the presence of flavonoids and glycosides (Table 93). This finding is in agreement with Kumar *et al*. (2015) in *K. rotunda*. Steroids derived from plants could be used for prevention of cancer and in reducing cholesterol (Sultan and Raza, 2015). In the present study also anticancer property was noticed in both *K. parviflora* and *K. rotunda* extracts. Flavonoids are associated with health-promoting effect on account of their antioxidant, anticarcinogenic and anti-inflammatory activities (Panche *et al*., 2016; Asif and Khodadadi, 2013). In the present study also antioxidant property was observed in both species. Gycosides extracted from plant sources are shown to possess antimicrobial activities (Zearah *et al*., 2013). Antimicrobial property against *E. coli*, *S. aureus*, *S. enterica* and *P. aeruginosa* was also noticed from the ethanolic extract of both *K. parviflora* and *K. rotunda* in the present study.

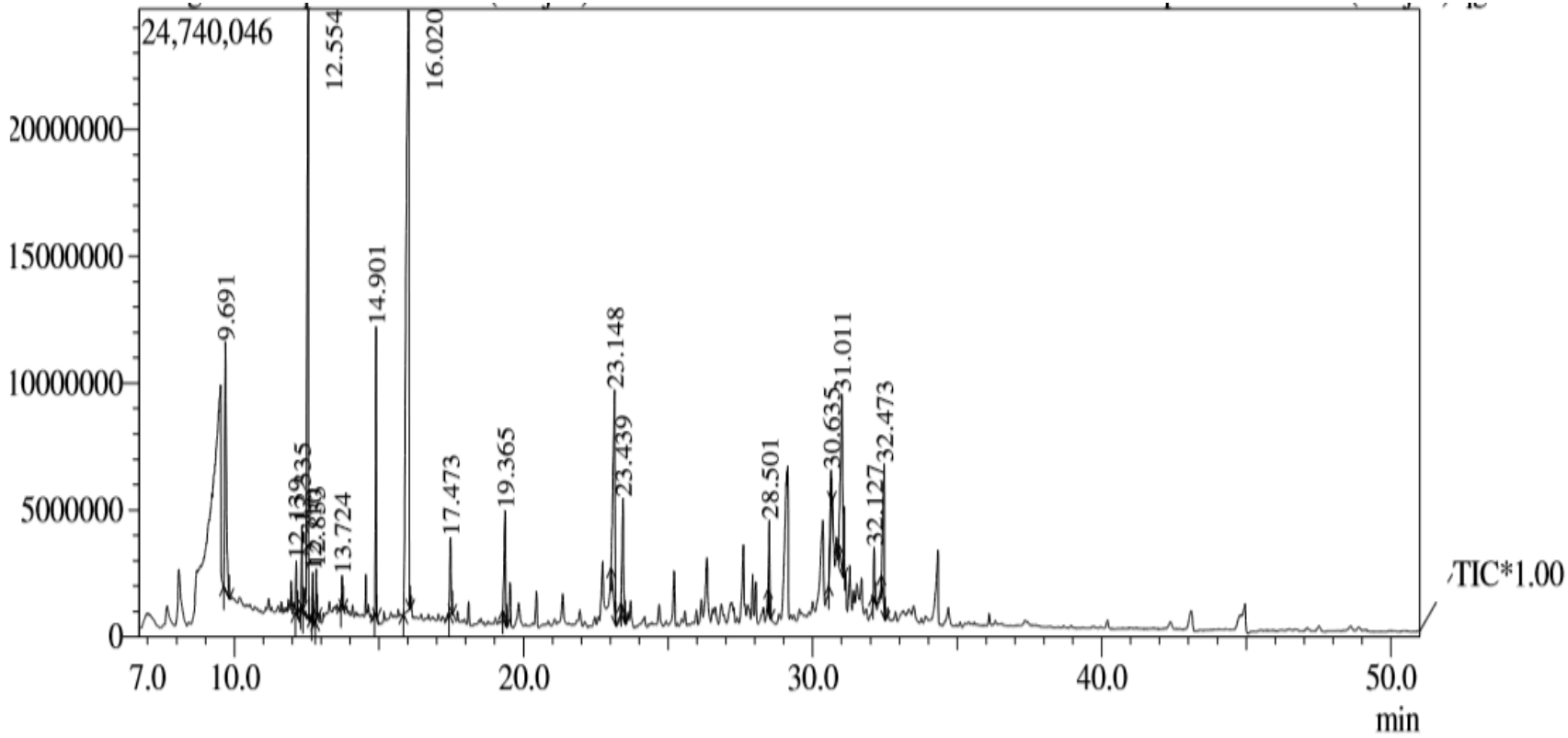


Figure 39: Chromatogram of GCMS profile of ethanolic rhizome extract of *K. rotunda*

5.5 PHARMACOLOGY STUDY

5.5.1 *In vivo* studies

5.5.1.1 Acute toxicity test of ethanolic rhizome extract of K. parviflora and K. rotunda

No mortality or adverse symptoms were observed with the ethanolic extract of *K. parviflora* and *K. rotunda* rhizome treated animals in the 14 day study period. Further, there was increased in body weight at 7^{th} and 14^{th} day of the experiment in both *K. parviflora* and *K. rotunda*. Post mortem observation also showed no gross and lesion in all the organs. Thus the LD_{50} value of the extract was found to be greater than 2000 mg/kg per oral treatment. Sini *et al*. (2014) also reported no toxic sign in *K. rotunda* with dose of 2000 mg/kg in Wistar albino rats. Similarly, aqueous and methanolic extract of *K. rotunda* with dose of 5 to 2000 mg/kg did not produce any toxic effect in albino rats (Imam *et al*., 2013). In the acute toxicity test in *Kaempferia parviflora* extract, up to 2 g/kg, orally as a single dose did not produce any clinical sign of toxicity (Sae-wong *et al*., 2009).

5.5.1.2 In vivo immunomodulatory study

5.5.1.2.1 Physiological parameters

5.5.1.2.1.1 Body weight and organ weight

In the present study, there was significant increase in body weight on 12^{th} day for all the groups when compared with A_{II} (Table 98). The increase in body weight was noticed in herbal extract treated cyclophosphamide induced immunosuppressed mice (Kuttan, 2009). The increase in body weight may be due to better feed utilization (Kumar, 2005). Reduction in body weight was also observed in cyclophosphamide treated group in Swiss albino mice by Smalatha *et al*. (2012).

There was no significant difference in liver weight among the treatments on both 12^{th} and 19^{th} days. However, groups A_I, A_{III}, A_{IV}, A_V and A_{VI} showed higher relative liver weight when compared with cyclophosphamide control group. Liver

is the important organ that responds immediately to any antigen. Relatively higher liver weight when compared with cyclophosphamide control and similar weight with normal control showed the restoration of normal activity of the liver (Kumar, 2005). Immunocompetency can be viewed as a slight increase in spleen weight (Lydyard *et al*., 2003; Gnanasekaran *et al*., 2015) and this increase in spleen weight was recorded in plant alone treated (A_{III} and A_V) and plant administered cyclophosphamide treated immunosuppressed group (A_{IV} and A_{VI}). Similar fingdings have been reported by Kumar (2005) and Naseema (2014).

5.5.1.2.2 Haematological parameters

In the study, a significant increase in leukocyte count was observed in plant alone treated (A_{III} and A_V) and plant administered cyclophosphamide treated immunosuppressed group (A_{IV} and A_{VI}). On both 12^{th} and 19^{th} days, *K. parviflora* treated cyclophosphamide immunosuppressed mice showed significantly higher total leukocyte count than *K. rotunda* treated cyclophosphamide immunosuppressed mice. Similar studies have also shown that there is significant increase in leukocyte count of plant treated cyclophosphamide induced immunosuppressed mice (Kajaria *et al*., 2013; Sultana *et al*., 2011). Increase in WBC count could be viewed as an important contributing factor in reducing the risk of various diseases (Lawrence and Amado, 1984).

An increased lymphocyte count was observed in plant alone treated (A_{III} and A_V) and plant administered cyclophosphamide treated immunosuppressed group (A_{IV} and A_{VI}) on 19^{th} day. The major innate of immune cells are phagocytes and lymphocytes, increase in lymphocytes show immunostimulatory effect (Shruthi *et al*., 2018; Nfambi *et al*., 2015).

No significant difference was observed in monocyte count. However, monocyte count was higher in groups A_{III}, A_V and A_{VI} on 12^{th} day when compared with cyclophosphamide treated group. On 19^{th} day, A_{III}, A_{IV}, A_V and A_{VI} showed higher count when compared with A_{II}. The innate immune response was strengthened by increase in monocytes as observed by Umair *et al*. (2016).

In the present experiment, neutrophil count was found highest in cyclophosphamide control group. The neutrophilia in the cyclophosphamide control group might be a compensatory mechanism to the drop in lymphocyte count observed. Concomitant administration of cyclophosphamide along with plant extract showed a significant decrease in neutrophil count comparable with that of normal group indicating the enhanced efficacy of plant extract in preventing cyclophosphamide induced neutrophilia. Increase in neutrophil count is attributed to marginalization of phagocytic cells *i.e*. improved defensive response under normal circumstances as explained by Sultana *et al*. (2011). It can be concluded that the plant extract stimulated haematopoietic system by increasing the lymphocytes and decreasing the neutrophil counts as observed by Kumar (2005).

5.5.1.2.3 Immunological parameters

Haemagglutination test

The groups $A_{IIII,}$ A_{IV}, A_V and A_{IV} showed significant increase in titre value, while the lowest was recorded in cyclophosphamide treated group (A_{II}). Increase in proliferation and transformation of B lymphocytes in plasmocytes might increase the antibody titre as explained by Mungantiwar *et al*. (1999). The result of the present study suggests the increase in immune response when treated with *K. rotunda* and *K. parviflora*. *K. parviflora* had higher immune response on 12^{th} day.

Bone marrow cellularity

The increase in number of bone marrow cells shows the effect of *Kaempferias* on enhancing immunological response (Table 106). Bone marrow is a foremost hematopoietic organ. Bone marrow hematopoietic stem cells (HSC) can self-duplicate, proliferate and differentiate to the subordinate cells of positive lineage. Drugs and radiation could damage all the systems, organs and tissues of the whole body and the hematopoietic tissue is the most sensitive to ray. Thus there was depletion in hematopoietic stem cell leading to depletion of mature hematopoietic cells due to administration of cyclophosphamide. The marked increase in bone marrow cell count on administration of *K. parviflora* and *K.*

rotunda is an indication of its immunomodulatory property. Gnanasekaran *et al*. (2015) observed that administration of *chyawanprash* and *brahma rasayana* showed the enhanced level of bone marrow cellularity indicating that hematopoietic cells was stimulated and differentiated. Cyclophosphamide potentially affect the bone marrow cellular production. However, *K. parviflora* as well as *K. rotunda* extract was found to be counteractive as it show an active protective effect in Swiss albino mice used in the present study as reported by Morris *et al*. (2011).

5.5.1.2.4 Biochemical parameters

Serum protein have role in maintaining homeostasis and resistance to infections (Tothova *et al*., 2016). The result on the significantly higher serum protein in plant *Kaempferia* alone treated (A_{III} and A_V) and *Kaempferia* treated immunosuppressed mice showed that higher immune response of the plant might have contributed to higher values in terms of immunoglobulin and other humoral factors as explained by Shwetha *et al*. (2012). Serum protein and serum albumin globulin ratio is one of the earliest indicators of normal serum chemistry of an individual. A change in serum protein concentration and albumin ratio would hint about the altered immune response status of the individual. In the present study, the serum protein levels were higher in plant extract treated groups. The higher immune response of the plant extract treated groups might have contributed to the higher serum protein in terms of different molecules such as immunoglobulin and other humoral factors.

Globulins are principally responsible for both the natural and acquired immunity that an individual has against invading organism (Lawrence and Amado, 1984).The increase in the level of globulin on *K. parviflora* alone treated mice indicate the antimicrobial action of this plant.

5.5.1.2.5 Histopathology of Spleen

Depletion of lymphoid cells was observed in white pulp region in cyclophosphamide group (Plate 28B and 29B). Similar effects were observed in experiments conducted by other researchers showing immunosuppression (Yoon *et al*., 2012). The proliferation of lymphocytes in the white and red pulp in *K. parviflora* alone treated group and attenuation of cyclophosphamide induced depletion in *K. parviflora* treated cyclophosphamide immunosuppressed group confirm the immunomodulatory effect of *K. parviflora* (Kim *et al*., 2018). Administration of *K. rotunda* induced hyperplasia of lymphocyte in white and red pulps while there was proliferation of lymphocytes in white pulp and mild depletion of lymphocytes observed in the marginal zone which showed moderate immunomodulatory activity of *K. rotunda*.

5.5.1.2.6 Delayed type hypersensitivity

Kaempferia alone treated (A_{III} and A_{V}) and *Kaempferia* treated immunosuppressed mice (A_{IV} and A_{VI}) showed significant increase in foot pad thickness when compared with cyclophosphamide control group (Plate 27 and table 108). One of the parameter to measure cell mediated immune response is to study the delayed type hypersensitivity in animal model. Various experiments conducted in animal models showed increase in foot pad thickness indicating the cell mediated immunity (Shabbir *et al*., 2016; Manure and Naikwade, 2018). The increased in response indicates that both *K. parviflora* and *K. rotunda* possesses stimulatory effect on cells required for the influence on biological mediators as reported by Shukla *et al*. (2011).

Plants hold abundant phytochemical constituents which are well-known to be biologically active compounds and shows various pharmacological activities. The results of preliminary chemical testing confirmed the presence of various classes of bioactive chemical constituents in ethanol extract of *K. rotunda* including steroids, flavonoids, glycosides and saponins and in *K. parviflora* flavonoids and glycosides were identified. The phytochemical constituents like flavonoids

(Hosseinzade *et al*., 2019), steroids (Khare, 2008), saponins (Bafna and Mishra, 2006) are considered to exhibit immunomodulatory property. Andrographolide showed immunomodulatory effect on both innate and adaptive immune responses (Wang *et al*., 2010). Bornyl acetate exhibited induced anti-inflammatory effects which was mediated via up-regulation of IL-11 in human primary chondrocytes (Andrade and Sousa, 2013). *Trichopus zeylanicus* ssp. *travancoricus* Burkill ex K. Narayanan which possesses immunomodulatory activity also exhibited the presence of pentadecane and heptadacane as chemical constituents (Nambi and Raju, 2017).

Tri-o-methylapigenin (synonym: 4', 5, 7-trimethoxyflavone) detected from *K. parviflora* extract was reported to have antiplasmodial, antifungal and antimycobacterial activity (Yenjai *et al*., 2004). Techtochrysin, a methoxyflavone, was found to induce skeleton muscle hypertrophy (Sultana *et al*., 2011).

5.5.2 *In vitro* studies

5.5.2.1 Antioxidant activity

The per cent inhibition was 78.95±1.37 at 500 µg/ml, and 35.20±1.39 at 62.5 µg/ml and calculated IC_{50} was 131.15±4.83 µg/ml for *K. rotunda*. For *K. parviflora*, it was 68.427±0.73 at 500 µg/ml and 31.613±0.36 at 62.5 µg/ml Inhibitory concentrations 50 (IC_{50}) calculated for *K. parviflora* was 198.68±7.62 µg/ml. Inhibitory concentrations 50 (IC_{50}) calculated for Vitamin C was 116.1±5.05 µg/ml

1, 1-Diphenyl-2- picryl- hydrazyl (DPPH) radical scavenging activity is relatively a stable organic radical largely used for the determination of antioxidant property of various plant extracts (Köse and Ocak, 2018). The antioxidant property was observed in all the concentrations of the extract. Significantly highest inhibition was observed in the highest concentration *i.e*, at 500 µg/ml. Fidrianny *et al*. (2015) observed that IC_{50} < 50 µg/ml range in very strong antioxidant, 50-100 µg/mL was a strong antioxidant and 101-150 µg/ml was a medium antioxidant, while a weak antioxidant was with IC_{50} >150 µg/ml. Thus, the IC_{50} of this plant ranged under

strong antioxidant activity. Similar scavenging effect was observed using n-hexane, chloroform, ethyl acetate, n-butanol and water soluble extracts of *K. rotunda* (Puspa *et al*., 2008). The phytochemical screening showed the presence of flavonoids which might have helped in the antioxidant property of this plant. The result suggested the medicinal suitability of ethanolic extract of *K. rotunda* in various antioxidant applications.

5.5.2.2 In vitro anticancer property

The per cent inhibition for MCF-7 cells was 76.70±1.18 at 500 µg/ml, and 27.82±1.69 at 80 µg/ml while for MDA-MB-231 cells, per cent inhibition were 62.82±1.457 at 500 µg/ml and 12.77±0.73 at 80 µg/ml. The half maximal inhibitory concentration (IC_{50}) for *K. rotunda* was found to be 194.8±8.97 µg/ml for MDA-MB-231 cells and 167.1±5.60 µg/ml for MCF-7 cells. In *K. parviflora*, the per cent inhibition for MCF-7 cells was 92.519±0.25 at 500 µg/ml, and 15.991±0.18 at 5 µg/ml, while for MDA-MB-231 cells, per cent inhibition were 69.853±0.39at 500 µg/ml and 17.976±0.41at 5 µg/ml. The half maximal inhibitory concentration (IC_{50}) for *K. parviflora* was found to be 126.35±2.53 µg/ml for MDA-MB-231 cells and 143.03±2.70 µg/ml for MCF-7 cells.

Results of the present study indicated the low cytotoxicity for MCF-7 cells and no cytotoxicity for MDA-MB-231 cells in the ethanolic extract of *K. rotunda* and *K. parviflora* in human breast cancer cell lines (Kuete and Efferth, 2011). However, earlier reported studies highlighted the cytotoxic effect of *K. rotunda* against MDA-MB-231 cells (Lallo *et al*., 2014). *In vitro* and *in vivo* cytotoxic investigations on the extracts or isolated compounds of *K. rotunda* have revealed anticancer activities (Atun and Arianingrum, 2015). Purified lectin from the extract of *K. rotunda* showed anti proliferative activity against Ehrlich ascites carcinoma (EAC) cell line tested for antitumor activity (Kabir *et al*., 2011; Ahmed *et al*., 2017). The species is known to contain a wide range of secondary metabolites such as flavonoids, alkaloids, steroids, terpenoids and saponins (Kumar *et al*, 2015; Desmiaty *et al*, 2018). This low cytotoxicity might be due to type of solvent used for extraction. Saponins derived from plant have shown to possess cytotoxicity (Yu

et al, 2015). Presence of flavonoids and saponins was detected in the present phytochemical screening (Table 4). These compounds might have contributed to the anticancer property.

5.5.2.3 Antimicrobial studies

Zone of inhibition for *K. rotunda* extract against *E. coli* was 11.13±0.16 mm, *S. enterica* was 11.47±0.29 mm, *S. aureus* was 14.18±0.32 and *P. aeruginosa* was 11.52±0.38 mm at concentration of 250 mg/ml (Plate 40). In *K. parviflora*, zone of inhibition was 12.32±0.12 mm, 13.8±0.16 mm, 15.48±0.23 mm and 11.17±0.31 mm, respectively for *E. coli*, *S. enterica*, *S. aureus* and *P. aeruginosa* at extract concentration of 250 mg/ml (Plate 41).

The family Zingiberaceae is a good source of plants possessing antimicrobial properties. Ethanolic extract of *K. rotunda* and *K. parviflora* exhibited appreciable antimicrobial activity on *E. coli*, *S. enterica* and *P. aeruginosa* and showed a potent activity on *S. aureus* (14.18±0.32 mm and 15.48±0.23 mm respectively). Similar results have been reported against *S. aureus* by Malahayati *et al* (2018). Kumar *et al*. (2015) demonstrated the antimicrobial activity of *K. rotunda* on *S. aureus* and *P. aeruginosa*. Study on the purified lectin extracted from *K. rotunda* showed partial inhibition of the growth of *E. coli* and *S. aureus* (Kabir and Reza, 2014). Glycosides from plants are reported to have antimicrobial properties (Zearah *et al*, 2013; Soulef *et al*. 2014). Phytochemical screening of *K. rotunda* also showed the presence of glycosides (Table 93). As suggested by Mostafa *et al*. (2018), the interaction of this compound with the cell membrane of microorganism might have induced cell death and inhibited enzymes necessary for amino acids biosynthesis which have attributed to the antimicrobial property.

GCMS chromatogram of the ethanolic extract of *K. rotunda* has demonstrated the occurrence benzoic Acid, Phenylmethyl Ester with an area of 34.49 per cent. This compound was also found with highest area in GCMS analysis of the essential oil of *Salvia urmiensis* and it was proven to have antimicrobial properties against *Staphylococcus epidermidis* and *S. cerevisiae* (Farjam, 2012).

Pentadecane exhibited antimicrobial activity against Amastigotes parasites and *Leishmania infantum Promastigotes*, antifungal activity against *Fusarium oxysporum* and antioxidant activity (Bruno *et al*., 2015; Yuan *et al*., 2012; Begum *et al*., 2016). Ethanolic extract of the nutmeg seed which contained 30,40,7-trihydroxyflavone showed effective potential against MDR gram-negative bacteria *e.g.*, *Providencia stuartii*, *Escherichia coli* (Dzotam *et al*., 2018).

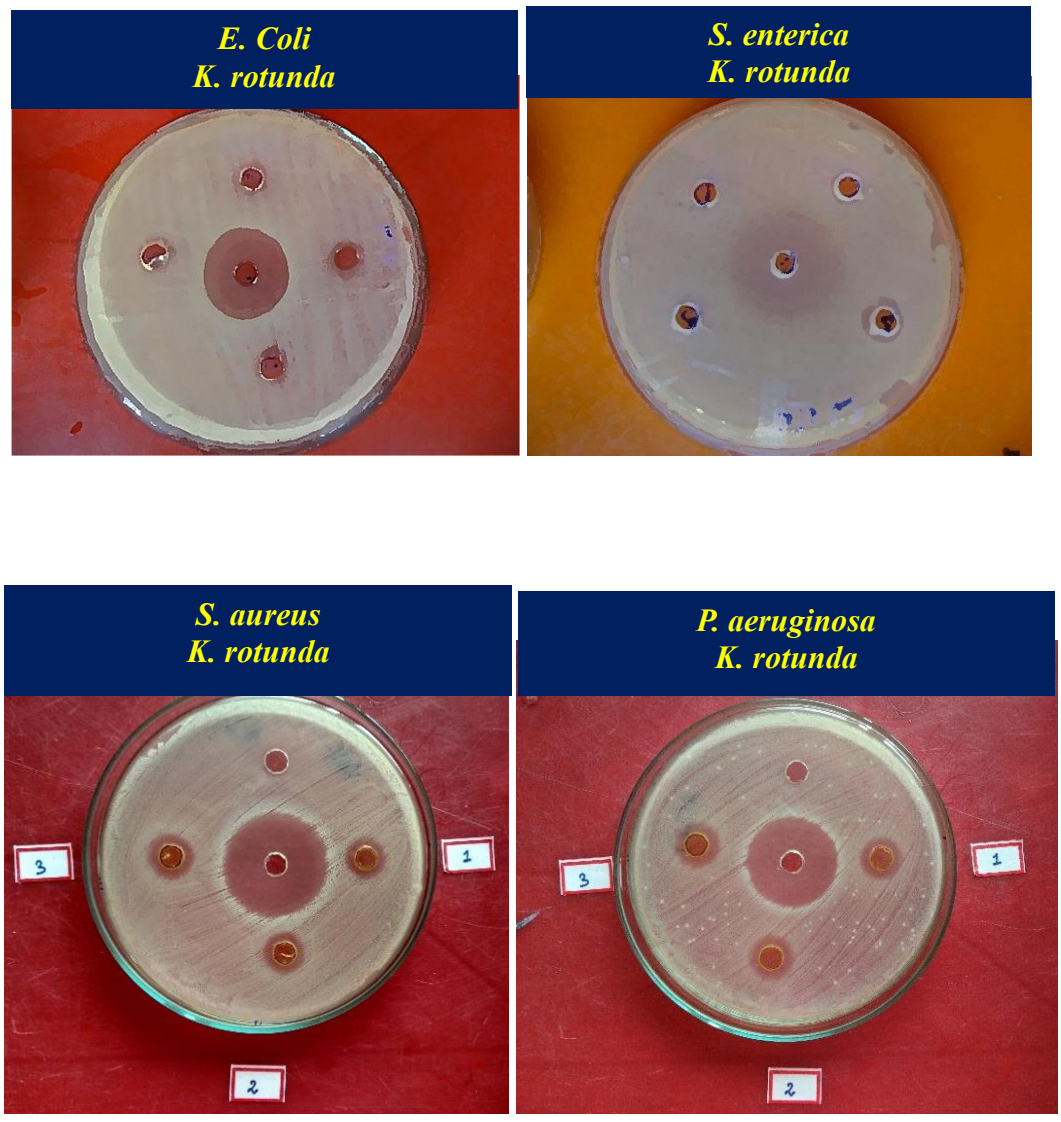


Plate 40: Zone of inhibition for *K. rotunda* extract against *E. coli*, *S. enterica*, *S. aureus* and *P. aeruginosa*

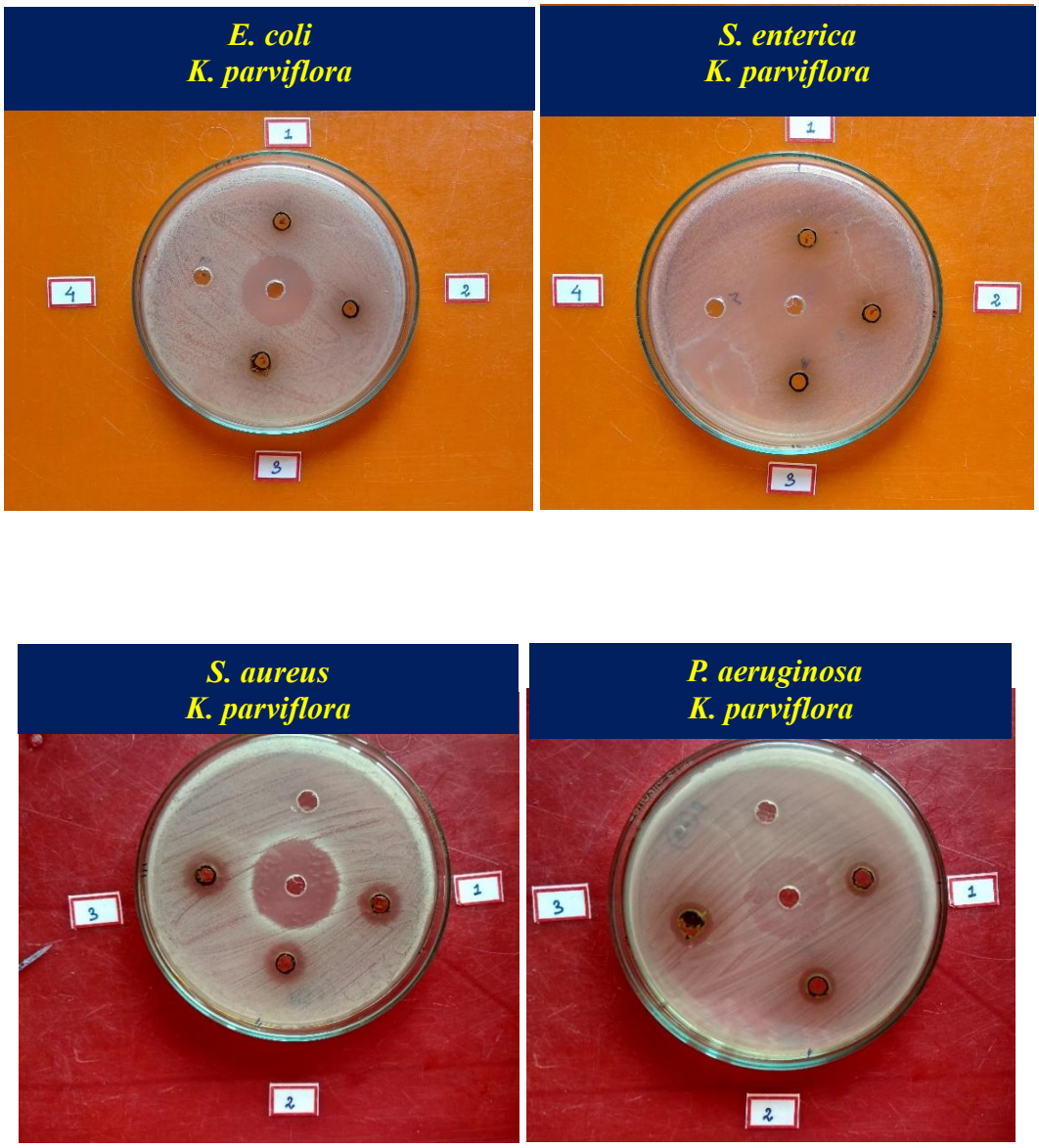


Plate 41: Zone of inhibition for *K. parviflora* extract against *E. coli*, *S. enterica*, *S. aureus* and *P. aeruginosa*.

Summary

6. SUMMARY

The present investigation entitled “Performance analysis of medicinal *Kaempferia* species” was carried out at the Department of Plantation crops and Spices, College of Horticulture, Kerala Agricultural University, Thrissur during 2016-18. The pharmacological studies were carried out at the Department of Pharmacology and Toxicology, College of Veterinary and animal Sciences, Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur with the objectives to evaluate the medicinal *Kaempferia* species in terms of growth and development, morphology, anatomy, floral biology, yield, medicinal properties and pharmacological aspects.

Salient findings of the study are summarised here.

Morphological evaluation of *K. rotunda*

Thirteen genotypes of K. rotunda collected from Kerala and Manipur were evaluated in the study.

In *K. rotunda*, there was no variation exhibited in the qualitative parameters among the genotypes. The globose rhizome had brown colour outer scale and off-white inner core, abundant root tubers were present and the mature green leaves were erect lanceolate.

The rhizomes of *K. rotunda* took 5.67 to 17.92 days for sprouting. The significantly lowest number of days (5.67) for sprouting was recorded by KCR-2 which was on par with KCR-1 and the highest days to sprout (17.92) was recorded by MCR-1.

The tiller production was highest at 180 DAP in all the genotypes. Genotype KCR-3 produced significantly highest number of tillers (3.83) which was on par with KCR-2.

Significant variation was noticed in the number of leaves among the genotypes at all the growth periods. The leaf production increased steadily and reached peak at 180 DAP. The significantly highest value (17.17) was recorded by the genotypes KCR-1 and KCR-2 and it was on par with MCR-1.

Significantly highest leaf length was recorded in the genotype MCR-6 which was on par with MCR-4 and the significantly highest leaf breadth was noticed in the genotype MCR-2 which was on par with MCR-1, MCR-3, MCR-4, MCR-4, MCR-5, MCR-6 and KCR-5 at 180 DAP.

The genotype MCR-5 showed significantly highest leaf area at 60 DAP, while at 180 DAP, genotype MCR-6 recorded significantly highest (372.95 cm^2) leaf area and the lowest leaf area (248.12 cm^2) was recorded in MCR-1.

The plant spread in both North-South and East-West showed significant variation among the genotypes. North-South plant spread was significantly highest in genotype MCR-3 and it was on par with MCR-4 and MCR-6. In the E-W spread, KCR-1 was noticed to have significantly highest value.

The plant height at 180 DAP ranged from 68.17 to 118.17 cm. Significantly highest plant height was recorded in the genotype MCR-6 which was on par with KCR-1.

The fresh and dry weight of leaves per plant recorded at 180 DAP showed significant variation among the genotypes. Significantly highest (187.95 g) fresh weight of leaves was recorded in the genotype MCR-4 which was on par with MCR-6. Dry weight of leaves per plant was recorded significantly highest value in the genotype MCR-6.

A significant variation was noticed among the genotypes in both length and girth of rhizome. Genotype KCR-1 recorded significantly highest rhizome length (8.33 cm) and girth (2.56 cm).

The fresh rhizome yield ranged from 15.21 to 52.44 g/plant and dry yield of rhizome ranged from 5.00 to 17.72 g/plant. Both fresh and dry yield of rhizome recorded significantly highest value (52.44 g, 17.73 g) in the genotype MCR-6.

The drying percent varied significantly among the genotypes and it ranged from 52.41 to 63.98 per cent. Genotype MCR-1 registered the significantly highest (63.98) value and it was on par with MCR-2, KCR-2 and KCR-6

The root tubers arising from rhizome showed significant variation and it was highest in the genotype KCR-1. Length of tuber ranged from 4.88 to 10.57 cm, significantly highest tuber length was recorded in the genotype MCR-4. In the girth of root tubers, genotype KCR-1 recorded the highest (1.67) value.

The fresh weight of root tubers per plant was significantly highest in the genotype KCR-6 and significantly highest dry weight of root tubers was recorded in genotype MCR-6.

The biological yield registered significantly highest value in the genotype MCR-6 (122.16 g). In harvest index, KCR-6 showed significantly highest index which was on par with KCR-4.

Based on both growth and yield parameters, Manipur collection MCR-6 performed the best followed by Kerala collection KCR-5.

Morphological evaluation of *K. parviflora*

Three collection of *K. parviflora*, on each from Thailand, IISR, Kozhikode and BSI, Shillong were evaluated in the study.

The irregular shape, branch or palmate rhizome had brown colour outer scale and purple inner core. Off white root tubers were present and the light green mature leaf had semi erect/lanceolate shape. Not much variation was observed among the genotypes regarding qualitative parameters.

The number of days required for sprouting recorded significantly lowest value in the genotype KCP-2 (15.42) and genotype KCP-1 took highest (18.25) days to sprout which was on par with BSI-1.

At 180 DAP tiller production reached the peak and the significantly highest value was registered by the genotype, BSI-1 (6.67).

In plant spread, both N-S and E-W spread showed no significant difference among the genotypes.

All genotypes had only single leaf at 30 DAP and highest leaf production was recorded in all the genotypes at 180 DAP. No significant difference was observed among the genotypes AT 180 DAP.

The leaf length and breadth at 60 DAP recorded significantly highest values in the genotype KCP-2 (20.61 cm and10.83 cm respectively). At 180 DAP, both parameters reached peak. However, no significant difference was observed in leaf length but leaf breadth exhibited significant variation which ranged from 13.97 to 14.87 cm.

The leaf area recorded at different growth periods reached peak at 180 DAP. Significantly highest leaf area (249.42 cm^2) was recorded by the genotype KCP-2 followed by BSI-1 (233.11 cm^2).

Fresh weight of leaves recorded significantly highest value (122.58 g) in the genotype KCP-2 which was on par with KCP-1. Significantly highest dry weight of leaves was recorded in the genotype KCP-1.

Not much variation was observed in the plant height, length and girth of rhizome in *K. parviflora* genotypes.

Significantly highest (114.60 g) fresh weight of rhizome was recorded by the genotype KCP-1. Dry yield of rhizome recorded significantly highest value (35.90 g) in genotype KCP-1 which was on par with BSI-1. Drying per cent ranged from 31.51 to 42.15 and it showed significant variation among the genotypes.

No significant variation was observed in the number and dimension of root tubers. However, fresh weight and dry weight of root tubers showed significant difference. Genotype KCP-1 recorded significantly highest (23.23 g and 4.47 g respectively) fresh and dry weight of root tubers.

The genotype BSI-1 recorded significantly highest (76.77) harvest index which was on par with KCP-1.

Based on the growth and yield parameters, the best performing genotype was Thailand collection KCP-1 followed by Shillong collection BSI-1.

Vivipary in *K. parviflora*

The phenomenon of vivipary was noticed in *K. parviflora* in all the accessions. Recalcitrance test proved the viviparous nature of *K. parviflora*.

In the transplanted viviparous seedlings, highest leaf length (15.3 cm), leaf breadth (6.2 cm) and leaf area (37.39 cm^2) was observed at 120 DAT. The fresh yield of rhizome was 1.41 g at 150 DAT.

The number of leaves and tillers were more in viviparous plants when compared with normal plants. However, leaf dimensions and plant height was significantly higher in normal plants.

The rhizome length and girth was significantly higher in normal plants when compared with viviparous plants. Fresh and dry weight of rhizome from viviparous plants were 30.05 g and 8.20 g respectively. Rhizome yield of viviparous plant was only $1/3^{rd}$ of that in normal plants. Viviparous plants took two years for appreciable yield.

Morphological observations of *K. galanga*

Kaempferia galanga was used as a reference species and there were two genotypes one each from Kerala and Arunachal Pradesh.

The rhizome of *K. galanga* are dark reddish brown in colour with pearl white inner core. Rhizome bear creamy white tuberous roots. Dark green leaves were round ovate.

The genotype ArPCG-1 showed significantly higher (29.16, 28.50) E-W spread. Fresh and dry weight of leaves were significantly higher in the genotype KCG when compared with ArPCG-1 in both the years.

In tiller and leaf production, genotype ArPCG-1 showed better performance at all the growth stages upto harvest. However leaf dimension were significantly higher in genotype KCG-1.

There was not much variation in rhizome characters between the two genotypes.

Floral biology of *Kaempferia* species

The flowering was observed from last week of May to second week of November in *K. parviflora*. *K. rotunda* started flowering by first week of March which continued upto last week of April. *K. galanga* flowered for one month only (first week of June to first week of July).

All the three *Kaempferia* species possess scape type of inflorescence directly arising from the rhizome. Floral parts are large in *K. rotunda*, medium in *K. galanga* and small in *K. parviflora*. Flowers are bisexual, complete, trimerous and zygomorphic in all the species. Gynoecium consists of tricarpellary syncarpous inferior ovary with ovules arranged in axile placentation.

Kaempferia parviflora produced 14.2 number of flowers per inflorescence while *K. rotunda* produced 8.9 and 5.6 in *K. galanga*. Time of anthesis in *K. parviflora* was 5.00 am to 7.15 am, *K. rotunda* was at 4.00 to 5.00 am and in case of *K. galanga*, peak anthesis time was 4.00 to 5.00 am.

The pollen grain of *K. parviflora* was spherical to spherical elongated shape, slightly heterogeneous. In *K. rotunda* and *K. galanga*, pollen grains were spherical, homogenous.

The pollen fertility was 85.18 per cent for *K. parviflora*, 94.24 per cent for *K. rotunda* and 90.71 per cent for *K. galanga*.

In the i*n vivo* stigmatic pollination, germinated pollen tube reached ovule 12 hours after pollination in *K. parviflora*. Pollen germination occurred on the spiny stigma/papillae of *K. galanga* and growth was observed upto 3/4th of the style length. Pollen germination did not occur at all in *K. rotunda*.

Biochemical parameters

The volatile oil content of *K. parviflora* genotypes ranged from 0.203 to 0.213 per cent. In *K. rotunda*, genotypes KCR-3 recorded significantly highest (0.32 %) oil content which was on par with the genotypes MCR-6 and MCR-5.

The oleoresin content of *K. rotunda* recorded significantly highest (4.947 %) in the genotype KCR-3. Oleoresin content of *K. galanga* genotypes was significantly higher in the genotype KCG (2.670 %)**.**

The content of starch, total sugar and flavonoids were high in *K. parviflora* whereas total free amino acids was high in the rhizome of *K. rotunda*.

There were 34 chemical compounds in GCMSMS profile of *K. parviflora* volatile oil. Ethanol, 2-(trimethylsilyl) was present in high quantity (89.6 %). Twenty two compounds are identified from volatile oil of *K. rotunda* and 27 compounds were identified from *K. galanga*.

In the GCMS profiling, a total of eight compounds were identified from ethanolic extract of *K. parviflora* and eighteen compounds were identified from *K. rotunda*.

The phytochemical screening of ethanolic extract of *K. parviflora* showed the presence of flavonoids and glycosides and that for *K. rotunda* showed the presence of steroids, flavonoids, glycosides and saponins.

Anatomical studies

In all the three species, epidermal cells were irregular or polygonal and usually run perpendicular to veins. Trichomes present on the leaf lamina were simple and unicellular and the stomata were hexacytic.

In *K. rotunda*, the stomatal index was higher in the adaxial surface while in *K. galanga*, the stomatal index was higher in lower surface and in *K. parviflora*, it was higher in upper surface.

All the three species had collateral and closed type of vascular bundles in transverse section of leaves. Oil globules were present in *K. galanga* leaves while it was absent in the other two species. Prismatic calcium oxalate crystals were noticed in *K. parviflora* in the mesophyll cells.

The transverse section of rhizome showed some variation among the species especially with regard to the number of oil globules as well the distribution of starch granules in the cortex region. Apart from oil and oleoresin mass, *K. parviflora* also contained flavonoid vacuoles in the rhizome section.

Pharmacology study

***In vivo* studies**

No acute toxicity was noticed in ethanolic extract of both *K. rotunda* and *K. parviflora* as there was no mortality of animals as well as no gross and lesions observed in the organs of sacrificed animals.

The immunosuppression in cyclophosphamide control animals was confirmed from decreased body weight, total leukocyte count, haemaglutination titre, total protein and bone marrow cellularity.

Both *Kaempferia* species significantly increased in body weight in plant alone treated animals when compared with cyclophosphamide treated group.

A significant increase in total leukocyte count, decrease in neutrophil count were observed in normal as well as immunosuppressed mice.

The total serum protein was significantly higher in *Kaempferia* alone treated as well as immunosuppressed mice.

A significant stimulation of humoral immune response was indicated by increase in antibody titre.

The bone marrow cellularity and DTH reaction showed significant stimulation in *Kaempferia* alone treated as well as *Kaempferia* treated immunosuppressed animals on both 12^{th} and 19^{th} day of the experiment.

A significant increase in foot pad thickness was recorded in *K. parviflora* and *K. rotunda* treated normal as well as immunosuppressed mice.

In histopathology of spleen, proliferation of lymphocytes in the white and red pulp in *K. parviflora* alone treated group and attenuation of cyclophosphamide induced depletion in *K. parviflora* treated cyclophosphamide immunosuppressed group confirmed the immunomodulatory effect of *K. parviflora.*

K. rotunda induced hyperplasia of lymphocyte in white and red pulps while there was proliferation of lymphocytes in white pulp and mild depletion was also observed in the marginal zone which showed moderate immunomodulatory activity of *K. rotunda.*

***In vitro* studies**

K. rotunda exhibited similar antioxidant property as that of Vitamin C and *K. parviflora* had lower antioxidant property when compared with Vitamin C. IC_{50} for *K. rotunda* was 131.15 µg/ml, *K. parviflora* was 198.68±7.62 µg/ml and that of Vitamin C was 116.1±5.05 µg/ml.

Both *K. rotunda* and *K. parviflora* showed cytotoxicity against MDA MB231 and MCF-7 cell lines. IC50 for *K. rotunda* were 167.1±5.60 and 194.8±8.97 respectively for MCF-7 and MDA MB231 cell lines while for *K. parviflora* they were 143.03±2.70 and 126.35±2.53 respectively. This result indicated the anticancer property of *K. parviflora* and *K. rotunda*.

The ethanolic extract of *K. rotunda* and *K. parviflora* exhibited appreciable antimicrobial activity on *E. coli* (11.13±0.16 mm, 12.32±0.12 mm), *S. enterica* (11.47±0.29 mm, 13.8±0.16 mm) and *P. aeruginosa* (11.52±0.38 mm, 11.17±0.31 mm) and showed potent activity on *S. aureus* (14.18±0.32 mm, 15.48±0.23 mm).

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PERFORMANCE ANALYSIS OF MEDICINAL *Kaempferia* SPECIES

By

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ABSTRACT OF THE THESIS

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ABSTRACT

The medicinal *Kaempferia* species are a good source of valuable bioactive compounds. *Kaempferia rotunda* is widely used in the ancient systems of medicine in India and Indonesia. *Kaempferia parviflora*, popularly known as black ginger or Thai ginseng, has potential for great exploitation on commercial basis. Rhizomes of *K. parviflora* are used as aphrodisiac in traditional medicine in Thailand. The present study was undertaken at Department of Plantation crops and Spices, Kerala Agricultural University, Thrissur to evaluate the medicinal *Kaempferia* species in terms of morphology, anatomy, floral biology, yield, medicinal as well as pharmacological properties.

A total of 18 genotypes belonging to three different species of *Kaempferia* formed the material for the study. *Kaempferia galanga* was taken as a reference species. The morphological evaluation was done consecutively for two years (2017-18 and 2018-19) based on qualitative and quantitative parameters. In *K. rotunda*, there was not much variation in qualitative parameters while the quantitative parameters exhibited tremendous variation among the 13 genotypes evaluated. The fresh rhizome yield ranged from 15.21 to 52.44 g/plant and dry yield of rhizome ranged from 5.00 to 17.73 g/plant. The Manipur collection MCR-6 performed best with the highest fresh as well as dry rhizome yield of 52.44 g and 17.73 g respectively followed by KCR-5 from Kerala. In *K. parviflora* also, the qualitative parameters did not show much variation whereas the quantitative characters exhibited significant variation among the genotypes. Significantly highest (114.60 g) fresh weight of rhizome was recorded by the genotype KCP-1. The Thailand collection KCP-1 was the best performer followed by BSI-1 from Shillong. The two genotypes of *K. galanga* evaluated in the study exhibited morphological variation in certain characters, however no significant variation was noticed for rhizome yield.

The flowering was observed from May to November in *K. parviflora*, March to April in *K. rotunda* and June to July in *K. galanga*. In the floral biology studies, variation was noticed in floral parts including the pollen grains of the three species

with respect to size and shape. The flowers were bisexual, complete, trimerous and zygomorphic in all the species. There were on an average 14.2 number of flowers per inflorescence in *K. parviflora*, 8.9 flowers in *K. rotunda* and 5.6 flowers per inflorescence in *K. galanga*. The time of anthesis was 5.00 am to 7.15 am in *K. parviflora*, 4.00 to 5.00 am in *K. rotunda* and in case of *K. galanga*, peak anthesis time was 4.00 to 5.00 am. The stigma was receptive upto eight hours after the anthesis in *K. parviflora* whereas *K. rotunda* and *K. galanga* remained receptive for 24 hours and nine hours respectively after anthesis. In the *in vivo* pollen germination studies, pollen germination was noticed only in *K. parviflora* and *K. galanga*. The seed set was observed only in *K. parviflora*.

The vivipary was observed in *K. parviflora* and recalcitrance of the seed was confirmed, thus proving its viviparous nature. The viviparous plants were compared with rhizome borne plants. The number of leaves and tillers were more in viviparous plants when compared with normal plants but they took two years for appreciable yield. However, rhizome yield of viviparous plant was only 1/3rd of that in normal plants.

In the anatomical studies, all the three species had collateral and closed type of vascular bundles in transverse section of leaves. The oil globules were present in the leaf lamina of *K. galanga*. Calcium oxalate crystals were present in the leaf epidermis of *K. rotunda* and leaf lamina in *K. parviflora*. Oil globules were abundant in the rhizome of *K. rotunda* and *K. parviflora*. Starch granules in rhizome and root tuber were concentrated near endodermal layer in all the species. The flavonoid vacuoles were abundantly present in the rhizome section of *K. parviflora*.

In the biochemical studies, the volatile oil content in *K. rotunda* rhizome ranged from 0.057 to 3.17 per cent and oleoresin content was to the tune of 0.60 to 3.17 per cent. In *K. parviflora*, volatile oil content was negligible; oleoresin content ranged 2.03 to 4.17 per cent. The content of starch, total sugars and flavonoids were high in *K. parviflora* whereas total free amino acid content was high in *K. rotunda* rhizome. The profiling of volatile oil of *K. rotunda* by GCMSMS detected 22 compounds, that of *K. parviflora* indicated 34 compounds and in *K. galanga* there were 27 compounds. The profiling of ethanolic extract of *K. rotunda* rhizome by

GCMS showed the presence of 18 compounds and that of *K. parviflora*, eight compounds.

The ethanolic extract of rhizome of *K. rotunda* and *K. parviflora* were subjected to detailed *in vitro* as well as *in vivo* pharmacological studies at the Dept. of Pharmacology and Toxicology, KVASU, Mannuthy. The *in vivo* acute toxicity and immunomodulatory study was carried out in Swiss albino mice using cyclophosphamide as immunosuppressive agent. No acute toxicity was noticed in the ethanolic extract of both *K. rotunda* and *K. parviflora*. Both the species significantly increased the body weight, total leukocyte count, serum protein and decreased neutrophil count in normal as well as immunosuppressed animals. A significant stimulation of humoral and cellular immune response was indicated by increase in antibody titre, bone marrow cellularity and DTH reaction. Histopathology of spleen confirmed the high immunomodulatory effect of *K. parviflora* and moderate immunomodulatory activity of *K. rotunda*. In the DPPH assay, *K. rotunda* exhibited high antioxidant activity (IC_{50} 131.15 μg/ml) while *K. parviflora* showed lower activity (IC_{50} 198.68±7.62 μg/ml). Ethanolic rhizome extract of both the species exhibited anticancer property in breast cancer cell lines. Both *K. rotunda* and *K. parviflora* showed cytotoxicity against MDA MB231 and MCF-7 cell lines. IC_{50} for *K. rotunda* was 167.1±5.60 and 194.8±8.97 respectively for MCF-7 and MDA MB231 cell lines while for *K. parviflora* it was 143.03±2.70 and 126.35±2.53 respectively. The ethanolic extract of *K. rotunda* and *K. parviflora* exhibited appreciable antimicrobial activity on *E. coli* (11.13±0.16 mm, 12.32±0.12 mm), *S. enterica* (11.47±0.29 mm, 13.8±0.16 mm) and *P. aeruginosa* (11.52±0.38 mm, 11.17±0.31 mm) and showed potent activity on *S. aureus* (14.18±0.32 mm, 15.48±0.23 mm).

APPENDIX

Weather parameters recorded at Vellanikkara

Month	Maximum temperature (° C)	Minimum temperature (° C)	Relative humidity (%)	Rainfall (mm)
2017				
June	30.4	23.5	87	630.2
July	30.8	22.8	85	385.5
August	30.1	23.3	87	478
September	31.5	22.9	84	413.91
October	31.7	22.3	81	183.4
November	33	21.8	73	58.3
December	32.4	21.1	63	11.5
2018				
January	33.5	20.9	53	0
February	35.7	22.5	47	5.2
March	36.7	24	59	33.2
April	36.1	24.8	69	28.9
May	33.2	22.6	79	483.6
June	29.8	23.8	89	730
July	29.6	22.5	88	793.2
August	29.2	22.2	87	928
September	32.2	22.5	75	290
October	32.8	22.2	76	393
November	32.7	22.9	68	66
December	33	23.3	63	0
2019				
January	32.9	20.4	55	0
February	35.8	23.4	59	0
March	36.7	24.8	65	0