VARIABILITY STUDIES IN LESSER GALANGAL

(*Alpinia calcarata* Roscoe)

By

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(2020-12-010)

DEPARTMENT OF PLANTATION, SPICES, MEDCICAL AND

AROMATIC CROPS

COLLEGE OF AGRICULTURE

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THESIS

Submitted in partial fulfillment of the requirement for the degree of

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DEPARTMENT OF PLANTATION, SPICES, MEDCICAL AND AROMATIC CROPS
COLLEGE OF AGRICULTURE
VELLANIKKARA, THRISSUR – 680656
KERALA, INDIA

2023

DECLARATION

I, hereby declare that the thesis entitled **"Variability studies in lesser galangal (*Alpinia calcarata* Roscoe)"** is a bonafide record of research work done by me during the course of research and the thesis has not been previously formed the basis for the award to me any degree, diploma, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

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Introduction

1. INTRODUCTION

Nature has offered a complete storehouse of remedies for all human maladies. Plant based therapies are as old as human civilization. Today, there is a vast knowledge about therapeutic benefits of various plants. In both the traditional and modern systems of medicine, plants continue to be a major source of drugs (Raj and Radhamany, 2012). Phytochemicals are non–nutritive chemicals that are produced by plants to protect themselves. Scientific investigations show that many phytochemicals can also be used for the prevention and cure of human diseases. These compounds have different health benefits including antioxidant, anti-inflammatory, anti-microbial, and cancer preventive effects (Savithramma *et al.*, 2012). But most herbal medicines have not undergone thorough exploration or scientific validation. Moreover, just a small portion of the world's biodiversity has been studied so far.

Lesser galangal (*Alpinia calcarata* Rosc.) belongs to the family Zingibercaeae is a widespread rhizomatous perennial herb, commonly used in Indian and Sri Lankan traditional medicinal systems. It is one of the under-exploited herbs and has great potential for exploitation in view of its pharmaceutical properties. *Alpinia calcarata* is cultivated throughout tropical and subtropical Asian countries including India, Sri Lanka, Bangladesh, Thailand, and Malaysia. It is commonly known as lesser galangal, cardamom ginger, snap ginger or Indian ginger. In ancient Ayurvedic texts, it is mentioned as *Rasna* in Sanskrit.

The economic part of *Alpinia calcarata* is rhizomes and are widely used in the indigenous systems of medicine for relieving throat inflammation, purifying blood, stimulating digestion and improving the voice. Lesser galangal is an important constituent of many ayurvedic formulations like *Rasnadi kashayam, Rasnadi churnam, Rasnasavam, Maharasnadikashayam, Rasna saptakam, Rasnai randadi kashayam* and *Rasna dasamoola kwatham*. Regular application of *Rasnadi*

churnam on the head after bath helps to prevent cold. In traditional medicine, the decoction of the rhizome is widely used to treat cough, respiratory ailments, bronchitis, asthma, arthritis and diabetics (Jayaweera, 1982). Drugs prepared from this plant are used in the treatment of rheumatism, bronchial catarrh and for reducing pain. It also yields an essential oil of commercial importance used for preparation of perfumes, lotion, pharmaceuticals and cosmetic products (Raina and Abraham, 2015). The plant also possesses several pharmacological properties namely anti-oxidant, anti-ulceric, anti-spasmodic, antiemetic, anti-bacterial, anti-fungal, anti-helminthic, gastro-protective and anti-diabetic.

Various pharmaceutical properties of *Alpinia calcarata* are due to the presence of a wide range of volatile compounds in the essential oil of different parts from the plant. Compounds like 1,8-cineole, α-fenchyl acetate, α-terpineol, camphor, terpinen-4-ol and borneol were the major constituent present in the roots, rhizome and leaves of lesser galangal (Bhuiyan *et al.*, 2011). The preliminary phytochemical studies on rhizomes revealed the presence of proteins, carbohydrates, phenol, tannin, alkaloids, terpenoids, steroids, and saponins. Few research works have been made on essential oil and the biochemical composition of the rhizomes, roots and aerial shoots. However, there are no comprehensive investigation on variability including morphological, yield and quality parameters in this plant.

Therefore, an experiment entitled **Variability studies in lesser galangal (*Alpinia calcarata* Roscoe)** was taken up with the objective to assess variability in morphological, yield and quality parameters of lesser galangal germplasm collected from southern parts of India and also to identify elite genotypes with desirable traits for further crop improvement programme.

Review of literature

2. REVIEW OFLITERATURE

This chapter provides insight into previous studies on *Alpinia calcarata*, other species under the genus *Alpinia* and Zingiberales wherever information specific to lesser galangal is insufficient. The available literature has been gathered and reviewed under the following parameters:

2.1 Morphological characteristics

2.2 Biochemical characteristics

2.3 Anti-microbial properties

Alpinia calcarata

Lesser galangal (*Alpinia calcarata* Rosc.) is a widely distributed aromatic medicinal plant native to India, belongs to the Zingiberaceae family. It is cultivated in tropical countries, including India, Sri Lanka, Bangladesh and Malaysia. The diploid chromosome number of lesser galangal is found to be 48 (Raghavan and Venkatasubban, 1943).

Botanical Classification of *Alpinia calcarata* is given below:

Kingdom : Plantae

Phylum : Magnoliophyta

Class : Liliopsida

Order : Zingeriberales

Family : Zingiberaceae

Subfamily : Alpinioideae

Tribe : Alpinieae

Genus : *Alpinia*

Species : *calcarata*

Lesser galangal is a perennial herb with a non-tuberous pungent rhizome and full leafy stem. Its leaves are simple, alternative 35 to 45 cm long and 2.5 to 5 cm broad (Rahman and Islam, 2015). The inflorescence is a terminal panicle and 12 to 15 cm long. The fragrant flowers are waxy, light pink flower buds open to tubular flowers with yellow inside lips and red throats. There is no fruit and seed set. Rhizomes are less branched, creamy yellow coloured, 2 to 6 cm long and 2 cm wide (Mathew *et al.*, 2014).

2.1 Morphological variation

2.1.1 Tiller characters

Dissanayake *et al.* (2013) compared the morphological features of six different species of the Zingiberaceae family: *Alpinia galanga, A. calcarata, A. malaccensis, Hedychium flavescens, H. coronarium* and *H. coccineum.* They observed the mean tiller height and mean internodal length of *A. calcarata* as 170 cm and 12 cm respectively.

KAU (2016) reported that *Alpinia calcarata* plant produces 24 tillers per clump per year.

Pooja *et al.* (2020) conducted a variability study in fourteen accessions of *Alpinia galanga* and recorded the mean plant height as 187.36 cm. In another study, Trimanto *et al.* (2021) reported that the leafy stem of *A. galanga* was erect with 1.5 to 2.0 cm diameter and reached 2.1 to 3.0 m tall; also, the base of leafy shoot was found reddish green. According to Trimanto and Hapsari (2021), *A. warburgii* was a herbaceous, perennial plant wherein the plant height reached upto 2.8m.

2.1.2 Leaf characters

Hussin *et al.* (2000) investigated the leaf anatomy of twenty *Alpinia* species from China and noted that there was interspecific variation in the structure of the midrib and petiole of leaves which could be utilized for species identification.

Leaf anatomy of twenty two *Alpinia* species from the Malay Peninsular was

examined by Talip *et al.* (2003). The results indicated that a combination of characteristics, including the presence of hypodermal fibres in the lamina, midribs, and petioles, as well as the shapes of midribs, petioles, and leaf margins and their relative sizes in transverse sections, could be exploited to identify the species.

Sabu (2006) described that leafy stem of *Alpinia calcarata* was 1 to 1.5 m in height. The leaves were sessile, lamina glabrous, and of 40 to 45 cm in length and 2 to 2.5 cm in breadth. He also pointed that the shape of leaf was linear lanceolate, narrowed towards the base and tip acuminate.

Kasarkar and Kulkarni (2011) compared the phenology of three *Zingiber* species and two *Alpinia* species. They recorded leaf measurements of 26.9 cm mean length and 3.12 cm mean breadth in *A. calcarata.*

Dissanayake *et al*. (2013) studied the morphological features of six different Zingiberaceae species and observed that number of leaves per tiller of 12 in *Alpinia calcarata* and mean leaf length of 48 cm and mean width of 8 cm.

Anatomical variations in the leaf of *Alpinia calcarata* and *A. galanga* was evaluated by Mathew *et al.* (2014) and found that the leaf anatomy in both species showed numerous vascular bundles and bundle sheath was extended up to lower epidermis.

Pooja *et al*. (2020) studied genotypic variability, heritability and genetic advance for yield and yield contributing characters in fourteen accessions *of Alpinia galanga* and they recorded the mean leaf length, width and mean leaf area of 39.7 cm, 10.46 cm and 331.50 cm^2 respectively.

Trimanto *et al.* (2021) described the plant morphological characters of *Alpinia galanga* and recorded that there were 10 to 11 leaves per tiller with lanceolate oblong shape leaves with dimension 26 to 53 x 8 to 10 cm with short petiole of 3 mm.

2.1.3 Rhizome characters

Wijayasiriwardena and Premakumar (2012) conducted a microscopic study of the rhizome powder of *Alpinia calcarata* and *A. galanga* and concluded that microscopical detection is a reliable and cost-effective tool for detection of adulteration.

Dissanayake *et al.* (2013) compared the morphological features of six different species of the Zingiberaceae family: - *Alpinia galanga*, *A. calcarata* Roscoe, *A. malaccensis*, *Hedychium flavescens*, *Hedychium coronarium* and *Hedychium coccineum* and reported that the mean perimeter of *A. calcarata* rhizome was 5 cm.

Mathew *et al.* (2014) conducted a study on anatomical variations in the rhizomes of *Alpinia calcarata* and *A. galanga* and found that the rhizomes of *A. galanga* is highly branched with yellowish colour while *A. calcarata* rhizome is less branched with creamy yellow colour.

Namdeo and Kale (2015) determined the macroscopic characters of rhizomes of *Alpinia galanga* and *A. officinarum*. They observed that the rhizomes of *A. galanga* were cylindrical and branched, externally reddish- brown, internally orange yellow in color, pleasant and aromatic odour, spicy and sweet in taste, whereas *A. officinarum* rhizomes were a slightly curved and cylindrical, sometimes branched, externally red-brown to dark brown with fine striped lines, odour was pleasant and taste is extremely pungent in taste.

2.1.4 Yield parameters

Ponmozhi and Kalaiselvi (2011) compared morphological characters among *Alpinia* species and found that the mean rhizome weight of *A. calcarata* and *A. officinarum* was 24.54 and 28.87 g per tiller respectively.

KAU (2016) stated that average rhizome yield of *Alpinia calcarata* as 23 $t.ha^{-1}$ and the dry recovery of rhizome was 25 per cent.

Pooja *et al.* (2020) conducted study on genotypic variability, heritability and

genetic advance for yield and yield contributing characters in fourteen wild accessions of *Alpinia galanga*. They found that genotypic coefficient of variation was less than the phenotypic coefficient of variation in all the characters studied and also reported the mean value of rhizome yield of 373.43g per tiller.

2.1.5 Floral characters

Raghavan and Venkatasubban (1943) studied the floral morphology and cytology of *Alpinia calcarata* and they reported the reason for the non-seed setting nature of this species was due to failure of the embryo-sac development and pollen degenerations.

In a study by Sabu (2006), the inflorescence of *Alpinia calcarata* was noted as terminal panicle, 10 to 15 cm long and densely paniculate. Flowers were shortly pedicellate, 3 cm long, 1.5 to 1.8 cm wide in the lower half, and variegated with dark purple and yellow. He also explained that flowers were pollinated by insects of the order Diptera.

The phenology of three *Zingiber* species and two *Alpinia* species was studied by Kasarkar and Kulkarni (2011). They observed the variations in the life cycle including the inflorescence development period, flowering period, longevity of flowers and opening of flower bud to anthesis of flower. Two flowering seasons were recorded for *A. calcarata* during October and April and it took 70 to 80 days for the development of inflorescence. The longevity of flowers was one day but they failed to develop fruits and seeds.

Trimanto and Hapsari (2021) conducted a detailed morphological study of *Alpinia warburgi* and observed the flowering of *A. warburgi* from May to June. The mean inflorescence length was recorded as 15 cm with 20 to 40 flowers per inflorescence and mean flower length and width were 3.5 cm and 1.6 cm respectively.

2.1.6 Floral phenology and pollen studies

Phenological studies are important for a better understanding of interactions between different species, ecological adaptations and germplasm conservation (Stern and

Roche, 1974; Waser, 1979).

Kasarkar and Kulkarni, (2011) studied the phenological stages of three *Zingiber* species and two *Alpinia* species. These data were useful for knowing the phenological diversity within the two genera which are the key factors of germplasm conservation. The flower of genus *Alpinia* and *Zingiber* opened in early morning but its longevity was one day.

Choon *et al.* (2016) determined the phenological stages of torch ginger *(Etlingera elatior)* inflorescence from emergence to full bloom stage using the extended BBCH scale.

Adaniya and Shoda (1998) conducted the pollen fertility and germination test in ginger (*Zingiber officinale*) and reported pollen fertility of 20.9 per cent and a very low germination rate ranging from 0.00 to 0.22 per cent.

Pollen fertility studies in ginger revealed that very few pollens were fertile and pollen grains failed to germinate in 2 to 15 per cent sucrose solution due to the absence of germinal apertures (Das *et al*., 1999).

2.2 Quality characters of rhizome

Qualitative phytochemical screening of ethanolic extracts of *Alpinia calcarata* and *A. speciosa* rhizomes was carried out by Mohanasundari and Suja (2015) and reported the presence of alkaloids, steroids, tannins, phenols, flavonoids, proteins and carbohydrates by positive reaction with the respective test reagent.

The phytochemical and physicochemical parameters of *Alpinia officinarum* were studied by Naing *et al.* (2020) and indicated the presence of tannins, glycosides, saponins, flavonoids, phenolic compounds and alkaloids, α-amino acid, reducing sugars, terpenoids, starch and carbohydrates in the rhizome powder.

2.2.1 Essential oil

Tewari *et al.* (1999) determined the chemical composition of the rhizome and leaf oil of *Alpinia calcarata* of Indian origin. They noted that there were thirty one constituents in rhizome oil and twenty eight constituents in leaf oil. The main component of the leaf and rhizome oil, was l,8-cineole at 41.4 per cent and 42.2 per cent respectively.

The essential oil of the roots of *Kaempferia galanga* was studied by Jirovetz *et al.* (2001) who reported more than sixty five constituents. The major compounds were ethyl-trans-p-methoxycinnamate (52.5%), ethyl -trans-cinnamate (26.3%), pentadecane (4.9%) and 1,8-cineole (2.4%).

The volatile oil composition of the rhizomes and the leaves of *Alpinia galanga* from Bangalore and Hyderabad in India were evaluated by Mallavarapu *et al.* (2002) and they observed similar components in the oils of the rhizomes and leaves irrespective of the locations under study.

Raina *et al.* (2002) studied the chemical constituents of essential oil of the rhizomes and leaves of *Alpinia galanga*, from the lower Himalayan region of India, which were analysed by GC and GC–MS. They found that 1,8-cineole (39.4%) and β-pinene (11.9%) were the major components in the rhizome oil and 1,8-cineole (32.5%), β-pinene (22.7%), and camphor (12.8%) were the main components of the leaf oil.

Akhtar *et al.* (2004) studied the volatile oil isolated from the rhizome of *Alpinia galanga* by GC and GC-MS analysis. They identified eleven total volatile constituents. Among these, 1,8-cineole (57%) was the most prominent component, followed by geranyl acetate (10.2%), citronellyl acetate (3.4%), and linalool (3.1%).

Jantan *et al.* (2004) evaluated the chemical profile of rhizome and seed oil of *Alpinia galanga* and they reported that 1,8-cineole (40.5%) in rhizome oil and β-bisabolene (8.4%) in seed oil as predominant chemical components oil.

The essential oil of *Alpinia calcarata* extracted from rhizomes, roots and leaves were investigated by Arambewela *et al.* (2005) for their chemical components. They identified around eighteen compounds and identified 1,8-cineole as the major component in the leaf and rhizome oil at 24.7 per cent and 33.3 per cent, respectively, whereas fenchyl acetate (39.8%) was the main component in the root oil.

The essential oils extracted from several parts of *Alpinia calcarata*, growing in Hyderabad, South India, were examined by Kaul *et al.* (2005) and they reported that oil yields from leaf sheath 0.03 per cent, stem 0.05 per cent, flower 0.06 per cent, and root 0.18 per cent. One of the main components of all the oils was 1,8-cineole (12.8 to 33.2%).

The volatile oils obtained from the leaves, rhizomes and roots of *Alpinia calcarata* collected from Berhampur (Orissa) and Bangalore (Karnataka) were examined by Rout *et al*. (2005). The leaf oils of the two accessions were found to be quite similar; whereas, geraniol was the main component of the rhizome oil from the Bangalore accession, but it was not found in the oils from the Berhampur accession.

According to Abeywickrama *et al.* (2006), the main component in the essential oil of *Alpinia calcarata*, 1,8-cineole, exhibited both fumigant and contact toxicity toward *Callosobruchus maculatus*, a major pest of cowpea.

Menon (2006) evaluated the chemical constituents of essential oils of the rhizomes, leaves, stems, and roots of the *Alpinia galanga*, collected from South Kerala. They identified fifty three compounds in the rhizome oil, thirty seven compounds in the leaf oil and thirty nine compounds obtained in the root oil.

The rhizome oil of *Alpinia calcarata* was examined by Pandey *et al.* (2007) using GC and GC-MS and they revealed the presence of twenty three components. 1,8-cineole (42.2%), endo-fenchyl acetate (14.7%), camphene (7.6%), β-pinene (6.9%), α-terpineol (5.3%) and camphor (5.0%) were the main constituents.

Raina *et al.* (2009) conducted an experiment to study the essential oil constituents

of rhizome oil of three *Alpinia* species from South India. Results showed that maximum oil was obtained in *A. calcarata* (0.73–1.26%), followed by *A. galanga* (0.27–0.56%) and *A. officinarum* (0.21%). 1,8-cineole was the major component in *A. calcarata* and that was present in all the three species. In *A. calcarata,* accession IC210421 contained the highest amount of 1,8-cineole and α-fenchyl acetate.

The chemical profile of rhizome oil of the *Alpinia speciosa* collected from Dehradun, India was studied by Indrayan *et al.* (2010) and they identified sixty six compounds. The main constituents were terpinen-4-ol (15.4%), 1,8-cineole (11.1%) and T-cadinol (8.8%).

Padalia *et al.* (2010) compared the essential-oil composition of leaves, flowers, and rhizomes of *Alpinia calcarata, A. galanga, A. speciosa,* and *A. allughas*. They found that the main components in leaf and flower oils were 1,8-cineole, α-terpineol, (E)-methyl cinnamate, camphor, and terpinen-4-ol. The distinct feature of the rhizome oils of *A. galanga, A. calcarata,* and *A. speciosa* was the presence of endo-fenchyl acetate, exo-fenchyl acetate, and endo-fenchol but β-pinene dominated in the rhizome oil of *A. allughas.*

Bhuiyan *et al.* (2011) studied the essential oils extracted from different parts of *Alpinia calcarata* grown in Chittagong, Bangladesh. They reported that 1,8-cineole (28.48%) and camphor (21.40%) were the main components of the leaf oil whereas fenchyl acetate (19.16%) and carotol (16.77%) were components in the stem sheath oil. The major constituents in root oil were fenchyl acetate (51.34%) and borneol (11.44%).

Twenty three components were found in the essential oil extracted from *Alpinia calcarata* rhizomes. The main constituents included camphene (3.86%), beta myrcene (4.39%), eucalyptol (14.05%), linalol (2.48%), pyrazine (1.72%), L-camphor (7.90%) and borneol (5.67%) (Rahman *et al.,* 2012).

Chemical components of volatile oils obtained from four *Alpinia* species, (*A. calcarata, A. galanga, A. malaccensis* and *A. smithiae*) were evaluated by Raj *et al.* (2013) and they reported that thirty seven to forty eight components were identified in the rhizome

oils of these four *Alpinia* spp. and the major component of *A. calcarata, A. malaccensis A. smithiae* and *A. galanga were* 1,8-cineole (35.9%), α-phellandrene (36.4%), α-terpineol (15.1%) and 1,8-cineole (52.9%) respectively.

The chemical composition of essential oils obtained from the rhizomes of *Alpinia galanga* and *A. officinarum* from North East India were compared by Raina *et al.* (2014). They observed that 1,8-cineole (63.4% and 44.2%), α-terpineol (2.8 and 6.3%), α-pinene (1.9 and 2.0%), β-pinene (0.8 and 5.7%), and terpinen-4-ol (2.8 and 4.5%), respectively, were the major constituents found in the oils of *A. galanga* and *A. officinarum.*

Raina and Abraham (2015) investigated the chemical constituents of volatile oils obtained from the rhizomes, roots, and aerial shoots of *Alpinia calcarata* germplasm from south India and they identified that 1,8-cineole, α-fenchyl acetate, α-terpineol, camphor, terpinen-4-ol and borneol were the major oil constituents in all parts of the plant.

Nampoothiri *et al.* (2016) studied the rhizome oil composition of *Alpinia calcarata* and they reported that volatile oil content in dry rhizome was 0.93 per cent and fifty constituents were obtained from *A. calcarata*. Cubenol (15.0%), 1,8-cineole (12.1%), and α- and β-fenchyl acetates (12.9 and 9.7%, respectively) were the main components.

The essential oil content and its chemical composition of rhizomes of *Alpinia calcarata* and *A. galanga* were evaluated by Raina and Abraham (2017). The rhizome oil content was higher in *A. calcarata* (0.29 to 0.96%) as compared to *A. galanga* (0.21 to 0.41%) germplasm. In *A. calcarata,* the highest volatile oil content was obtained in accession IC373610 (0.96%) followed by IC468880 (0.74%) and lowest in IC565495 (0.29%). Major constituent in both species was 1,8-cineole, which was higher in *A. galanga* (47.5 to 67.3 %) than in *A. calcarata* (13 to 30.2 %).

Chandrakanthan *et al.* (2020) compared the essential oil composition of leaf and rhizome oil of *Alpinia calcarata* and they reported that the main constituents in the rhizome oil were 1,8-cineole, *α*-terpineol, and fenchyl acetate, however in the leaf oil, camphor's richness was highlighted together with 1,8-cineole and *α*-terpineol.

The chemical compositional variability of essential oils in rhizome and leaf oils of *Alpinia calcarata* and *A. zerumbet* was evaluated by Bhatt *et al.* (2021). They identified a total of thirty one compounds in both the species and aroma chemicals *viz.* 1,8-cineole, terpinen-4-ol, α-pinene, β-pinene, ocimene and fenchyl acetate were detected in essential oils from leaves and rhizomes of *A. calcarata and A. zerumbet.*

2.2.2 Oleoresin

Arambewela and Arawwawala (2010) evaluated the ethanol and hot water extract of *Alpinia calcarata* rhizome and found that the extractive yield of ethanol and hot water were 18.5 per cent and 15.6 per cent on dry weight basis respectively.

Dried rhizomes of *Alpinia galanga* were extracted by Kaur *et al.* (2010) using Soxhlet method with hexane, chloroform and ethyl acetate and they observed that the yield of hexane, chloroform, ethyl acetate and methanol extracts were 2.7, 0.8, 0.6 and 1.2 per cent respectively.

Raj *et al.* (2011) extracted the dried rhizome of *Alpinia calcarata* using Soxhlet extraction method and they acquired an extractive yield of 19.5 per cent on dry weight basis.

The powdered rhizome of *Alpinia galanga* was successively extracted using a Soxhlet apparatus with hexane and chloroform to provide 1.98 per cent and 0.66 per cent of extracts, respectively (Roy *et al.*, 2012).

The percentage of extractable yield of oleoresin in various solvents of *Alpinia calcarata* was evaluated by (Mohanasundari and Suja, 2015) and found that oleoresin content was highest in ethanol (2.64%) whereas hexane, petroleum ether, chloroform, ethyl acetate and water extract give less recovery.

2.2.3 Total phenols and flavonoids

The total phenolic contents of Indian gooseberry and galangal extracts, as

determined by Mayachiew and Devahastin (2008), were 290.47 and 40.97 $mg.g^{-1}$ plant extract (in gallic acid equivalent (GAE)), respectively and they reported that Indian gooseberry extract possessed much higher total phenolic contents than galangal extract.

Mahae and Chaiseri (2009) compared the total phenolic and flavonoid content in the water extract, ethanolic extract and essential oil of *Alpinia galanga* and they stated that ethanolic extract had the highest phenolic (31.49 ± 4.09 mg $GAE.g^{-1}$ extract) and flavonoid content (13.78 ± 0.60 mg catechin $equivalent.g^{-1}$ extract). But, lowest content in essential oil.

Kambar *et al.* (2014) estimated the total phenolic and flavonoid contents of leaf and rhizome extract of *Alpinia galanga* and they reported that the content of total phenolics and flavonoids was high in rhizome extract when compared to leaf extract.

Nampoothiri *et al.* (2015) estimated the total phenolic content in *Alpinia galanga* and *A. calcarata* and they observed that the phenolic content was higher in *A. galanga* (320 $mg.kg^{-1}$) than in *A. calcarata* (75 $mg.kg^{-1}$). Due to this, *A. galanga* showed better antioxidant activity.

Total phenolic and flavonoid content in ethanolic extract of *Alpinia calcarata* was evaluated by Ramya *et al.* (2015) and they found that phenolic content of 100.7 ± 0.36 $mg.g^{-1}$ of gallic acid equivalent in the extract and flavonoid content of 24.2 ± 0.40 $mg.g^{-1}$ of quercetin equivalent in the ethanolic plant extract.

The total phenolic and flavonoid content of *Alpinia galanga* and *Eryngium foetidum* was studied using different solvents (methanol, ethanol and water) by Malik *et al*. (2016). It was observed that methanolic extracts of both plants had higher phenolic content than ethanolic and aqueous extracts. However, ethanolic extract showed higher flavonoid content.

Triapti and Swain (2016) compared the total phenol and flavonoid content in three different (ethanolic, methanolic and aqueous) extracts of *Alpinia calcarata* rhizomes. They

reported that methanolic extract had greatest phenolic content (21.08 mg.g^{-1} GAE) followed by ethanolic (17.52 mg.g^{-1} GAE) and aqueous extract (8.91 mg.g^{-1} GAE). Similarly, the flavonoid content ranged between 60.7 mg rutin.g^{-1} (methanolic extract) and 14.3 mg ruitn.g^{-1} (aqueous extract).

The biochemical composition of ethanolic extract of *Alpinia calcarata* rhizome was investigated by Islam *et al.* (2017). They discovered that total phenolic content was 222.99±0.41 mg of GAE.g^{-1} of dry extract, whereas total flavonoid content was 162.07±0.79 mg of CAE.g^{-1} of dry extract.

Ferdous *et al.* (2018) evaluated total phenolic and flavonoid content in methanolic extracts of leaves of *Alpinia calcarata* and they observed high phenolic content in the leaf extract (102.33 ± 0.25 mg GAE.g^{-1}), which is responsible for the antioxidant activity and also found a considerable amount of flavonoid content (66.25 mg QE.g^{-1}) in the leaf extract.

Tang *et al.* (2018) compared the total phenolic content of greater galangal flower with its rhizome and they found galangal flowers had a threefold higher total phenols content than had rhizomes (10.5 vs. 3.33 mg GAE.g^{-1} powder).

Total phenolic content of crude methanol extracts of the leaves of *Alpinia calcarata* was investigated by Sahoo *et al.* (2020) and they found that phenol content of the leaf extract of *A. calcarata* was found to be 59.25 mg Gallic acid equivalent per gram of extract, which might be responsible for antioxidant properties of the crude extract.

Samarasinghe *et al.* (2020) evaluated the total phenolic content of rhizomes and leaves of six *Alpinia* species available in Sri Lanka, *A. calcarata, A. galanga, A. nigra, A. malaccensis* and *A. purpurata a*nd they found that the dried leaves of *A. calcarata* had the highest phenolic content among the all species tested.

Singh *et al.* (2020) analysed different bioactive constituents present in the methanolic leaf and rhizome extracts of *Alpinia calcarata* and *A. galanga*. They discovered that phenolic content of the leaf extract of *A. calcarata* was found to be higher

(59.25±0.92mg GAE.g^{-1}) than that of rhizome extract (37.75±0.95mg GAE.g^{-1}). Similarly, in case of *A. galanga*, leaf extract had greater than rhizome extract. However, flavonoid content was higher in leaf extract of *A. galanga* (64.69±1.12 mg Quercetin equivalent.g^{-1} of extract) than *A. calcarata* extract.

Total phenolic content and total flavonoid content of rhizome and leaf of *Alpinia calcarata* in a different solvent (petroleum ether, methanol, acetone and aqueous) were compared by Jisha *et al.* (2021). The highest phenolic content was found in acetone extract of leaves and highest flavonoid content was found in the methanol extracts of leaves.

2.2.4 Crude fibre

Indrayan *et al.* (2009) conducted a study on nutritive value of rhizomes of certain Ginger like species, viz. *Alpinia officinarum, A. galanga, A. calcarata, A. zerumbet* and *Kaempferia galanga.* The rhizomes of *A. galanga* (18.6%) had the greatest percentage of crude fibre followed by *A. officinarum* (17%) and *Kaempferia galanga* (9%). Crude fibre percentage was found at the least level in *A. calcarata* (7.25%).

According to Lin *et al*. (2015) the percentage of crude fibre in the rhizome of *Alpinia officinarum* was 1.3±0.0 per cent.

Nohir *et al.* (2019) examined the biochemical composition of the dried rhizome of *Alpinia galanga* and found that crude fibre percentage was 9.86 ± 0.29 g.$100g^{-1}$ on dry weight basis.

The biochemical makeup of *Alpinia galanga* was studied by Afra and Ghannam (2022) and they observed the crude fibre percentage (14.00%) of rhizome on dry weight basis.

2.2.5 Starch

Ponmozhi and Kalaiselvi (2011) analysed the morphological, histochemical and biochemical characters in four *Alpinia spp*. (*A. calcarata, A. galanga, A. officinarum* and

Kampferia galanga). They reported that the starch content ranged from 11.5 ± 0.26 to 93.1 ± 0.11 $mg.g^{-1}$ and highest starch content was recorded in *A. galanga* (93.1 ± 0.11 $mg.g^{-1}$) followed by *Kampferia galanga* (60.3 ± 0.12 $mg.g^{-1}$), *A. calcarata* (38.2 ± 0.65 $mg.g^{-1}$) and *A. officinarum* (11.5 ± 0.26 $mg.g^{-1}$).

Comparative powder microscopy of *Alpinia calcarata* and *A. galanga* was conducted by Wijayasiriwardena and Premakumara (2012). They found that *A. calcarata* powder consisted of plenty of simple and compound starch grains and most of them were round and oval shaped but some of them were muller shaped, some were triangular and pear shaped. In case of *A. galanga*, a smaller number of simple starch grains and mostly round and oval shaped but very few pear shaped, were also found.

Girija and Rema (2014) compared the morphological, anatomical and histological characterization of the source plants of the ayurvedic drug Rasna (*Alpinia calcarata, A. galanga* and *Pluchea lanceolata*). They observed that the shape of the starch grains is specific in these crops. In case of *A. calcarata,* starch grains are simple, rounded or oval shaped but less in number.

The total starch content of four *Curcuma* species *viz, Curcuma amada, C. aromatica, C. caesia,* and *C. xanthorrhiza* varied from 45.24±0.25 to 48.48±0.3 per cent (Sajitha and Sasikumar, 2015).

Mathew *et al.* (2014) conducted a comparative powder microscopical screening of the rhizome and leaf of *Alpinia calcarata* and *A. galanga.* They observed the presence of simple starch grains in both species. The result showed that *A. calcarata* consisted of round and oval shaped starch grains, whereas in case of *A. galanga*, some of the starch grains were muller shaped, triangular and most of them were round and oval shaped.

2.2.4 Total Terpenoids

Phytochemical screening of the ethanolic extract of *Alpinia calcarata* rhizomes was conducted by Raj *et al.* (2011) for the detection of various phytoconstituents. They

reported the presence of flavonoids, triterpenoids and reducing sugars in the ethanolic extract.

Phytochemical evaluation of the rhizome of *Alpinia calcarata* was carried out by Ramya *et al.* (2015). They observed that most of the secondary metabolites like terpenoid, steroid, flavonoid, etc were found in the ethanolic extract of *A. calcarata.*

Singh *et al.* (2015) evaluated total terpenoid content in *Curcuma amada* and reported total terpenoid content of 5.89 per cent from the dried rhizome.

Phytochemical analysis of leaf extracts of three medicinal plants (*Alpinia calcarata, Bauhinia tomentosa* and *Curcuma zedoaria*) revealed the presence of phenols, glycosides, steroids, terpenoids, tannins, flavonoids, saponins and alkaloids (Vijayanand and Khumlianlal, 2015).

Phytochemical screening of methanolic rhizome extracts of *A. calcarata* showed that rhizomes were rich in terpenoids, sterols, phenols, etc. (Mathew and Victoria, 2020).

2.3 Anti-microbial properties

Habsah *et al.* (2000) evaluated the anti-microbial activity of dichloromethane and methanol extracts of thirteen Zingiberaceae species from the *Alpinia, Costus* and *Zingiber* genera against six microorganisms and they found that dichloromethane extracts were much stronger in anti-microbial activity than the methanol extracts.

Methanol extracts of *Alpinia galanga, A. purpurata, A. zerumbet,* and *A. zerumbet variegata* were tested against some bacteria and pathogenic fungi by Wong *et al.* (2009) and reported flower extracts from *A. galanga* and *A. purpurata* showed the largest zone of inhibition of *Micrococcus luteus*. Only the rhizome extract of *A. galanga* had anti-fungal activity towards *Aspergillus niger.*

Arambewala *et al.* (2010) investigated the antifungal activities of essential oil of *Alpinia calcarata* rhizomes against plant pathogens *Curvularia spp.* and *Colletotrichum*

spp. They found that its antifungal activity appears to be more pronounced against *Curvularia spp.* and the effect was better than that of positive control, Daconil.

Kochuthressia *et al.* (2010) compared the antimicrobial activity of three solvent extracts (ethanol, petroleum ether & chloroform) of leaves, roots and rhizomes of *Alpinia purpurata* against six bacterial strains and four pathogenic fungi. Ethanolic extracts of rhizomes exhibited a wide spectrum of activity against all tested bacteria, but no notable inhibition against any fungi except *Candida albicans.*

Kambar *et al.* (2014) evaluated the antimicrobial activity of leaf and rhizome extracts of *Alpinia galanga* against 15 clinical isolates of bacteria (from burn, dental caries and urinary tract infection) and two fungi (*Candida albicans* and *Cryptococcus neoformans*). The rhizome extract exhibited stronger inhibitory potential when compared to leaf extract.

Antimicrobial activity of the essential oil from the leaves, pseudostems, rhizomes and fruits of *Alpinia rafflesiana* against seven microorganisms was conducted by Jusoh *et al*. (2013) and they found moderate to weak inhibition against the tested microorganisms. The leaf oil was the most active and inhibited both *Staphylococcus aureus* and *Escherichia coli.*

Ethanolic and water extracts of *Alpinia galanga* were investigated by Pillai *et al.* (2019) for their antimicrobial activities against eight bacterial and two fungal isolates. They observed disparate activities ranging from nil, weak (< 12 mm), moderate (12 to ≤ 20 mm) and strong (≥ 20 mm) inhibitory effects.

Methanolic extracts of *Alpinia calcarata* rhizomes were tested against five important species of pathogenic fungi such as *Aspergillus flavus, A. niger, A. fumigatus, Rhizopus stolonifera,* and *Candida albicans* by Mathew and Victorio (2020) and recorded significant antifungal activity against all pathogens tested.

Jisha *et al.* (2021) reported that methanolic extract of *A. calcarata* leaf showed considerably good inhibition against *Klebsiella pneumoniae, Staphylococcus aureus* and

Candida albicans respectively and leaf extracts were more efficient inhibitors than rhizome extracts.

2.4.1 Percentage of inhibition for *Rhizoctonia* spp.

Antifungal activity of essential oils from roots and rhizomes of *Alpinia galanga, A. calcarata* and *Kaempferia galanga* was studied by Prasad *et al.* (2016) against *Rhizoctonia solani* and they found that *A. galanga* root oil had best antifungal efficacy at 500 ppm.

Choudhury *et al*. (2017) tested the effect of eleven plant extracts against the plant pathogen, *Rhizoctonia solani* and revealed that the rhizome of ginger exhibited the greatest anti-fungal activity against the pathogen.

Rajput *et al.* (2018) compared the anti-fungal property of 103 medicinal plants against *Rhizoctonia solani.* They reported that twelve crude methanolic extracts showed strong anti-fungal activities and *Glycyrrhiza uralensis* exhibited highest inhibitory effect against the pathogen.

Bhagat *et al.* (2019) evaluated the methanol and aqueous extracts of leaves and rhizome of three different medicinal plants *viz. Azadirachta indica, Lantana camara* and *Curcuma longa* against plant pathogen *Rhizoctonia solani*. They observed that rhizome extract of *Curcuma longa* showed maximum zone of inhibition against pathogen at concentration of 200 ppm when compared to other extracts.

2.4.2 Percentage of inhibition for *Colletotrichum* spp.

The anti-fungal property of *Alpinia calcarata* rhizome was investigated by Arambewela *et al.* (2010) against plant pathogen *Colletorichum* spp. and found that percentage growth inhibition of 38.67 at 1000 ppm concentration was the best treatment.

Choudhury *et al.* (2017) reported that methanolic extract of ginger rhizome showed antifungal property against *Colletotrichum musae* with a radial growth inhibition percentage of 55.41 per cent.

2.4.3 Percentage of inhibition for *Phytophthora* spp.

According to Pompimon *et al.* (2009), the extract of *Alpinia galanga* rhizome inhibited the mycelial growth of plant pathogen *Phytophthora capsici.*

Yanar *et al.* (2011) compared the anti-fungal activity of twenty six medicinal plant extract against *Phytophthora infestans* and reported that the minimum inhibitory concentration (MIC) of the extracts ranged between 2 and 8 per cent (w/v).

Abdelgaleil *et al.* (2019) evaluated the anti-fungal activity of *Curcuma longa* with different solvent extracts against plant pathogen *Phytophthora infestans*. They revealed that methanolic extract had the highest antifungal activity among the tested extracts.

Tongchure and Chanprapai (2022) investigated the antifungal activity of essential oils derived from the Zingiberaceae family, such as *Zingiber officinale, Alpinia officinarum* and *Curcuma longa* against *Phytophthora parasitica.* They found that the essential oil of *A. officinarum* demonstrated the highest antifungal activity.

2.4.4 Percentage of inhibition for *Fusarium* spp.

According to Handajani and Purwoko (2008), ethanolic extract of *Alpinia galanga* showed an anti-fungal property against *Fusarium moniliforme* with minimum growth inhibitory concentration of 1682 $mg.l^{-1}$.

The essential oil *Alpinia calcarata* rhizomes were evaluated by George (2014) for antibacterial and antifungal activity against ten pathogenic bacteria and seven fungi. The antimicrobial activity of the oil showed significant inhibitory activity against the human pathogenic bacteria, but no inhibition was observed against the fungi *Aspergillus aculeatus* and *Fusarium oxysporum*.

Abdelgaleil *et al.* (2019) examined the antifungal activity of three solvent extract (hexane, methylene chloride, and methanol) of rhizomes of *Curcuma longa* against Fusarium spp. They reported that methanolic extract had highest antifungal properties

against *Fusarium* spp.

2.4.2 Antibacterial properties

Oonmetta-aree (2006) evaluated the ethanol extracts of the Zingiberaceae family (galangal, ginger, turmeric and *Boesenbergia pandurata*) for their antimicrobial activity on various microorganisms. The strongest inhibitory effect was observed in galangal extract against *Staphylococcus aureus* and there was no anti-bacterial property against some gram-negative bacteria: *Salmonella* spp., *Enterobacter aerogenes* and *Pseudomonas aeruginosa.*

Mayachiew and Devahastin (2008) compared the antimicrobial activities of Indian gooseberry (*Phyllanthus emblica*) and galangal (*Alpinia galanga*) extracts against *Staphylococcus aureus*. The minimum inhibitory concentration (MIC) values of Indian gooseberry and galangal extracts were found to be 13.97 and 0.78 $mg.ml^{-1}$ and the minimum biocidal concentration (MBC) values were 13.97 and 2.34 $mg.ml^{-1}$, respectively.

The potential antimicrobial activity of greater galangal (*Alpinia galanga*) flower was observed by Hsu *et al.* (2010) against *Salmonella, Escherichia coli, Listeria monocytogenes, Staphylococcus aureus*, and *Shigella.* They reported that it was most effective against *S. aureus* with inhibition zone of about 26 to 31 mm and no antimicrobial activity was observed on *E. coli* and *Salmonella.*

Methanol, acetone and diethyl ether extracts of *Alpinia galanga* have been evaluated by Rao *et al.* (2010) against a combination of gram-positive and gram-negative pathogenic bacteria strains. Methanolic extracts had shown excellent activity towards all the pathogens with minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values ranging from 0.04 to 1.28 $mg.ml^{-1}$ and 0.08 to 2.56 $mg.ml^{-1}$, respectively.

Ethanol, methanol, petroleum ether, chloroform and aqueous extracts of *Kaempferia galanga* rhizome were evaluated against ten human pathogenic bacteria and four fungal pathogens. Ethanolic extract recorded highest inhibition zone against *Staphylococcus aureus* (Kochuthressia *et al.,* 2012).

Prakatthagomol *et al.* (2012) compared the antibacterial activity among the rhizomes of *Alpinia galanga*, *Curcuma longa*, and *Zingiber cassumunar* against food-borne bacteria and they reported that the extract and essential oil of *A. galanga* were the more effective than others.

According to Malik *et al.* (2016), methanolic, ethanolic and aqueous extracts from rhizome of *Alpinia galanga* and *Eryngium foetidum* showed varying antimicrobial properties against eight pathogenic bacteria strains. Methanolic and ethanolic extracts of both plants were found to be good antibacterial potential than the aqueous extracts.

Ferdous *et al*. (2018) conducted a disc-diffusion method to evaluate the antibacterial activity of the methanolic extract of *Alpinia calcarata* leaves against two gram-positive (*Staphylococcus aureus* and *Micrococcus luteus*) and two gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and found that none of the concentrations of methanolic extract showed antibacterial activity.

The antimicrobial activity of the greater galangal flower was tested against the food borne pathogens *Staphylococcus aureus* and *Listeria monocytogene*. Flowers exhibited the strongest antimicrobial activity, with minimum inhibitory concentration (MIC) values of 34 $\mu g.ml^{-1}$ against *Staphylococcus aureus* and 68 $\mu g.ml^{-1}$ against *Listeria monocytogenes* (Tang *et al.* 2018).

Seven different solvents extract of *Alpinia officinarum* rhizome showed the best effective antimicrobial activity against *Bacillus subtilis* and *Escherichia coli*. The ethanolic extract showed the highest inhibitory zone of 28 mm on *Bacillus subtilis* (Naing *et al.* 2020).

Syamsir *et al.* (2020) studied the antimicrobial property of the essential oils of leaf and rhizome of *Alpinia scabra* and *A. murdochii* against five strains of *Staphylococcus aureus*. The lowest minimum inhibitory concentration (MIC) values were recorded for the rhizome essential oil of both species with MIC values ranging from 0.04 $mg.ml^{-1}$ to 2.50 $mg.ml^{-1}$ and the rhizome oils also showed a broad spectrum of anti-microbial activity as

compared to the leaf oils.

Bhatt *et al.* (2021) evaluated the antimicrobial activity of leaf and rhizome essential oils of *Alpinia calcarata* against eight pathogenic bacteria strains. The antibacterial assay showed that leaf oil had good activity against *Streptococcus mutans*, whereas its rhizome oil expressed good activity against *Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus*, and *Staphylococcus epidermidis.*

Materials and methods

3. MATERIALS AND METHODS

The study entitled "Variability studies in lesser galangal (*Alpinia calcarata* Roscoe)" was carried out at Department of Plantation, Spices, Medicinal and Aromatic crops, College of Agriculture, Vellanikkara and ICAR NBPGR Regional Station, Thrissur during 2021-2022.

3.1 Experimental materials

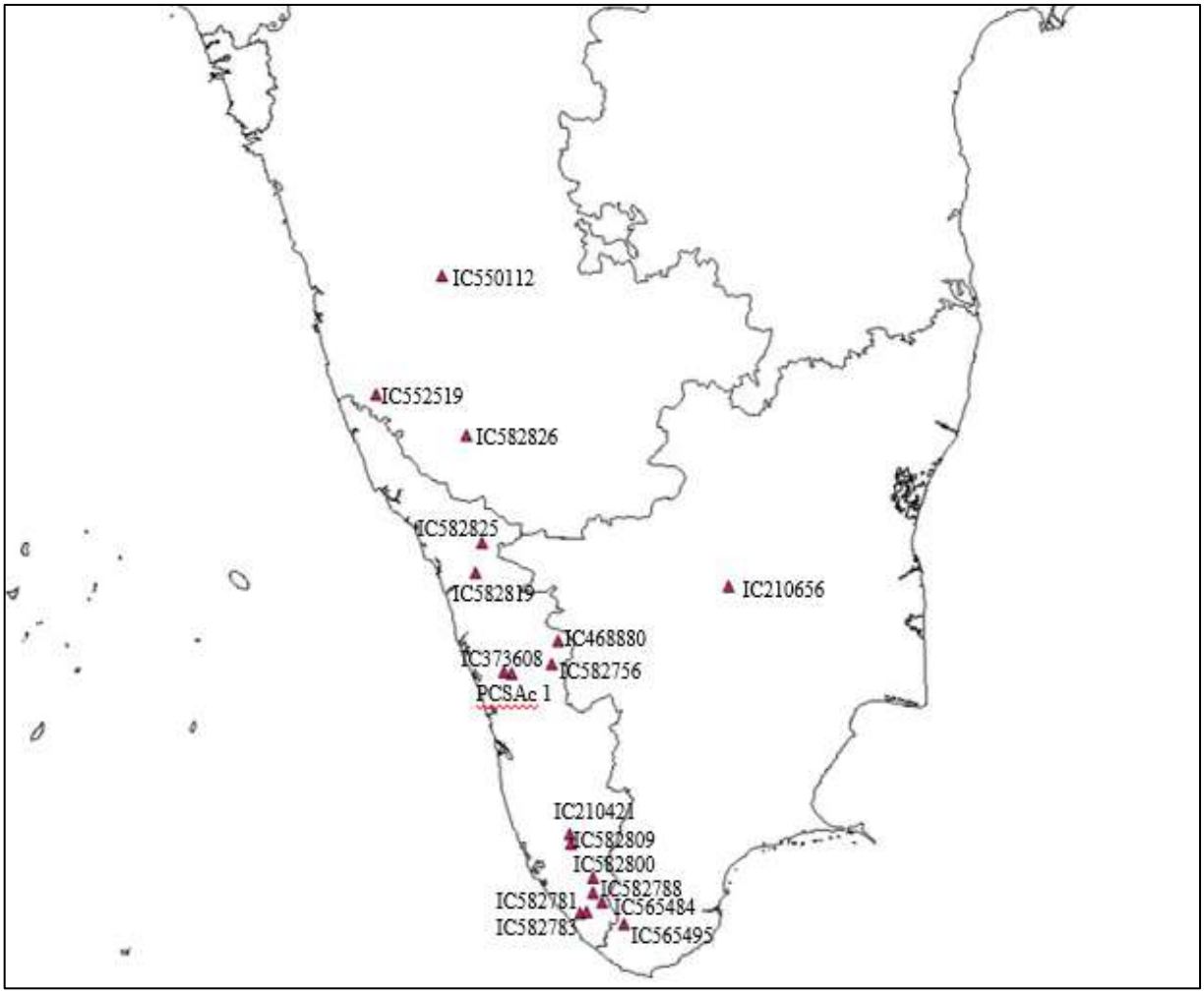
Seventeen accessions of *Alpinia calcarata* collected from South Indian states viz., Kerala, Tamil Nadu and Karnataka which are maintained at ICAR NBPGR Regional Station, Thrissur and one accession maintained at Plantation farm, under Department of Plantation, Spices, Medicinal and Aromatic crops, College of Agriculture, Vellanikkara were utilized for the present study. The details of accessions are given in the Table 3.1.

Table 3.1: Details of experimental materials

Sl. No.	Accessions	Place of collection
1.	IC210421	Pathanamthitta, Kerala
2.	IC210656	Namakkal, Tamil Nadu
3.	IC373608	Thrissur, Kerala
4.	IC468880	Palakkad, Kerala
5.	IC550112	Shimoga, Karnataka
6.	IC552519	Dakshin Kannad, Karnataka
7.	IC565484	Thiruvananthapuram, Kerala
8.	IC565495	Kanyakumari, Tamil Nadu
9.	IC582756	Palakkad, Kerala
10.	IC582781	Thiruvananthapuram, Kerala
11.	IC582783	Thiruvananthapuram, Kerala

12.	IC582788	Thiruvananthapuram, Kerala
13.	IC582800	Kollam, Kerala
14.	IC582809	Pathanamthitta, Kerala
15.	IC582819	Kozhikode, Kerala
16.	IC582825	Wayanad, Kerala
17.	IC582826	Kodagu, Karnataka
18.	PCSAc 1	Thrissur, Kerala

Figure 1: Collection sites of accessions



3.2 Details of experiment

3.2.1 Experiment 1: Morphological variation

The morphological observations of the above-mentioned accessions were recorded during the active vegetative period (December to January) and the yield data was taken during summer month when the plant started to wither (April). The morphological data were recorded from 10 samples from the existing plant population.

Statistical method: Sampling

Analysis: One-Way ANOVA

Replication: 10 for morphological parameters

5 for yield parameters

For floral characters, descriptive statistics method was used for data analysis.

3.2.1.1 Observations on morphological characters

3.2.1.1.1 Tiller characters

Length and diameter of randomly selected 10 tillers from each accession were recorded and expressed in cm.

3.2.1.1.2 Number of tillers

The number of aerial shoots arising around a single plant was counted and recorded.

3.2.1.1.3 Internodal length (cm)

Internodal length was measured from one node to adjacent node and was expressed in cm.

3.2.1.1.4 Number of leaves per tillers

Number of leaves produced per tillers was counted from each accession.

3.2.1.1.5 Leaf characters

The top fourth leaf was used for measuring leaf length, width and area. Leaf length was measured with a scale from the pseudo stem to the tip of leaf and leaf breadth, measured at its broadest section. Both are expressed in cm. Leaf area was measured using graph paper and expressed as square centimeters. Shape of leaves was also observed and recorded.

3.2.1.1.6 Petiole length (cm)

Petiole length was taken from base of the leaf and was expressed in cm.

3.2.1.1.7 Rhizome characters

Rhizome length, width and circumference were measured using vernier caliper and expressed in cm**.**

3.2.1.1.8 Rhizome yield (g per tiller)

Rhizome yield was determined by weighing the rhizome per tiller after harvesting, cleaning and removal of roots and expressed in gram per tiller (g per tiller).

3.2.1.1.9 Dry recovery (%)

Dry recovery of rhizome was determined on initial weight basis and expressed in percentage.

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

3.2.1.1.10 Season of flowering

The observations on months during which the inflorescence produced were

recorded.

3.2.1.1.11 Duration of flowering (days)

The time period between the emergence of flowers from the inflorescence and complete abscission of flowers was recorded.

3.2.1.12 Inflorescence length (cm)

Length of inflorescence measured from base to tip and expressed in cm.

3.2.1.1.13 Number of branches and flowers per inflorescence

Number of branches and flowers per inflorescence was counted and recorded.

3.2.1.1.14 Flower characters

Flower length and width was recorded from a randomly selected flower in an inflorescence and expressed in cm. The flower colour was observed using RHS (Royal Horticultural Society) colour chart.

3.2.1.1.15 Pollen studies

Pollen viability and germinability were tested using freshly collected pollen grains. The fertility of pollen was examined on the basis of stainability of pollen grains in 2 per cent aceto-carmine solution. Fertile pollen grains are those that had good stainability and others are sterile.

The pollen tube germination test was performed using nutrient medium. Pollen grains were collected from a freshly opened flower. Then, they were kept in a glass slide with fine painting brush and added a drop of previously prepared nutrient medium consisting of 10 per cent sucrose and 100 ppm boric acid. After 4 to 6 hours, the slide was observed under a light microscope.

3.2.1.1.16 Fruit set (%)

The inflorescence was regularly monitored for the formation of fruits and seeds.

3.2.1.1.17 Floral phenology

The reproductive development stages of ten lesser galangal inflorescences were observed daily from April to May. The principal growth stages of *Alpinia calcarata* inflorescence were identified and described using BBCH (Biologische Bundesanstalt, Bundessortenamt and Chemical industry) scale (Meier, 2001), which has ten growth stages described using two-digit BBCH coding systems. In a two-digit coding system, the first digit indicates the principal growth stages, which range from 0 to 9. The second digit describes precisely the secondary growth stages, which also fall within the range of 0 to 9.

3.2.1.1.18 Meteorological data

Weather data of the experimental site was collected from department of Agricultural Metrology, College of Agriculture, Vellanikkara and attached as appendix II.

3.2.2 Experiment 2: Quality characters of rhizomes

The biochemical parameters like essential oil, oleoresin, phenols, flavonoids, starch, crude fiber and total terpenoids were estimated in the rhizomes of 18 accessions. Rhizomes were collected at their maturity.

Design : CRD

Treatments : 18

Replication : 2

3.2.2.1 Observations on Quality characters of rhizomes

3.2.2.1.1 Essential oil (%)

The essential oil content in the dried rhizome was determined by hydro distillation of rhizome in Clevenger's apparatus (Guenther, 1972). After washing and cleaning, the rhizomes are chopped into small pieces and dried. The dried rhizome (100g) was taken into

a round bottom flask and added distilled water. The flask was fitted with Clevenger's apparatus and kept on a heating mantle for 5 to 6 hours. The oil that was formed in the uppermost layer was collected in eppendorf tubes. Oil content was measured as

$$\text{Essential oil (\%)} = \frac{\text{Volume of oil (ml)}}{\text{Weight of sample (g)}} \text{ X } 100$$

3.2.2.1.2 Oleoresin (%)

The oleoresin content in the dried rhizomes was determined by solvent extraction method using Soxhlet apparatus. Ten grams of dried rhizome powder were taken in thimble and kept in Soxhlet extractor. Then added 250 ml methanol to the flask and assembled the extraction apparatus. The extraction was carried out in a water bath. When the solvent became colourless, the extract was collected, desolvated and weighed. Oleoresin content was calculated as

$$\text{Oleoresin content (\%)} = \frac{\text{Weight of residue (g)}}{\text{Weight of sample (g)}} \text{ X } 100$$

3.2.2.1.3 Total phenols (mg gallic acid equivalent.g^{-1})

The total phenol content was calculated based on the method proposed by Mallik and Singh (1980). One mg of dried rhizome sample was taken and homogenized with 10 ml of 80 per cent ethanol using mortar and pestle. The supernatant was saved after centrifugation. One ml of the extract was taken and diluted with 7.5 ml of water. To the extract, 1 ml of Folin-Ciocalteau reagent was added and incubated for 5 minutes at room temperature. Then, 800µl of 20 per cent Na_2CO_3 was added and kept for 2 hours in dark. The absorbance was read at 725 nm using spectrophotometer. A standard curve was created using different concentrations of gallic acid. The result was expressed in mg gallic acid equivalent.g^{-1}.

3.2.2.1.4 Total flavonoids (mg quercetin equivalent.g^{-1})

The total flavonoid content was estimated as described by Sereena (2011). five mg of rhizome sample was weighed and homogenized with 80 per cent methanol. It was centrifuged at 10000 rpm for 20 minutes. The supernatant solutions were collected and pooled and made up to 50 ml. One ml of extract was taken and added 0.3 ml of 5 per cent $NaNO_2$ to it and waited for 3 minutes at room temperature. Further, 0.3 ml of 10 per cent $AlCl_3$ was added to it and incubated for another 2 minutes. To the aforementioned mixture, 2.4 ml of NaOH was added and kept for 10 minutes at room temperature. The absorbance was read at 510 nm against blank in spectrophotometer. A standard curve was created using a different concentration of quercetin. The Result was expressed in mg quercetin equivalent.g^{-1}.

3.2.2.1.5 Starch (mg.g^{-1})

The starch content was estimated by Anthrone method (Hedge and Hofreiter, 1962). The dried rhizome sample of 0.1 g was homogenized in hot 80 per cent ethanol. It was centrifuged at 3000 rpm for 20 minutes and the residue was retained. The procedure was repeated till the washings did not give color. Then the residue was dried using water bath. To the residue, 5 ml of water was added, followed by 6.5 ml of 52 per cent perchloric acid. This was cooled and centrifuged. The supernatant was saved. Using fresh perchloric acid, the extraction was repeated and then centrifuged. The supernatant was pooled and made up to 100 ml. One ml aliquot of the sample was taken and added 4 ml of anthrone reagent. The mixture was heated for 8 minutes in boiling water bath. After cooling, read the intensity of green to dark green colour at 630 nm in a spectrophotometer. Glucose was used as standard. The starch content was expressed in mg.g^{-1} dry weight.

3.2.2.1.6 Crude fibre (%)

The crude fibre content was estimated as described by Sadasivam and Manickam (1992). Two grams of dried rhizome powder were weighed into a conical flask and boiled with 200 ml of sulphuric acid for 30 minutes. Then filtered using muslin cloth and washed

with boiling water to remove acid residue completely. The filtrate was again boiled with 200 ml of sodium hydroxide solution for 30 minutes. The sample was filtered through muslin cloth and washed using boiling water until washings are no longer alkaline. The residue was collected in a pre-weighed ashing dish (W_1) and dried in hot air oven at a temperature of 230^0C for two hours. The dish was cooled in a desiccator and weighed (W_2) and ignited in a muffle furnace for 30 minutes at 600^0 C. The dish was cooled and reweighed (W_3). The result was expressed in percentage.

$$\text{Crude fibre content} = \frac{\text{Loss in weight on ignition (W2-W1) - (W3-W1)}}{\text{Weight of the sample}} \text{ X } 100$$

3.2.2.1.7 Total terpenoids (%)

The total terpenoid content was analysed using Ferguson method (Ferguson, 1962). 100 mg dried rhizome powder was taken and soaked in 9 ml of alcohol (95% ethanol) for 24 hours. Then filtered it and the filtrate was extracted with 10 ml of petroleum ether using separating funnel. The ether extract was collected in pre-weighed glass vials and allowed for its complete drying. The ether extract was treated as total terpenoids. The result was expressed in percentage.

$$\text{Total terpenoid content (\%)} = \frac{\text{Weight of terpenoid extract (g)}}{\text{Weight of sample (g)}} \text{ x } 100$$

3.2.2.1.8 GCMSMS profile of essential oil

The essential oil after extraction was analysed by Triple quadruple GCMSMS (Model TSQ 8000 MSMS). TG5M5 column of 30 mm x 0.25 mmx 0.25 µm dimension was used as stationary phase. The oven temperature increased from 60°C to 240°C with a constant rate of 3°C/min. Helium was used as carrier gas with flow rate of 1 ml/min. one microliter of the essential oil was injected by Finnigan Autoinjector A13000 with split ratio

of 10:1. MS was conducted by electron impact positive mode at 70 electron volts. The chemical constituents were analysed by comparing mass spectra and retention time indices with NIST MS Search 2.0 Library. Peak area was expressed in percentage.

3.2.3 Experiment 3: Anti- microbial properties

The efficacy of extracts of leaves and rhizomes for anti-bacterial and anti-fungal properties against the plant pathogens *Phytophthora, Colletotrichum, Rhizoctonia, Fusarium* and *Ralstonia* were studied under in vitro conditions.

Design : CRD

Treatments : 18

Replication : 3

3.2.3.1 Observations on anti-microbial properties

3.2.3.1.1 Anti-fungal property

The efficacy of extracts of leaves and rhizomes for anti-fungal properties against the plant pathogens *Phytophthora, Colletotrichum, Rhizoctonia* and *Fusarium* were studied under *in vitro* conditions by using poison-food technique (Svecova *et al.,* 2017). Methanolic extract of leaves and rhizomes of 1000 ppm was added to sterilized PDA (Potato dextrose agar) medium in a conical flask and mixed thoroughly. Twenty milli litre of media containing extract was separately poured into Petri plates, allowed to cool and solidify. After solidification of the medium, a 5 mm diameter of actively growing mycelium disc of the pathogen was placed in the center of the Petri plates. The Petri plates containing media devoid of the extract served as control. Three replicates were maintained for each treatment. Plates were incubated at 27°C up to the day on which control plates reached full growth. The per cent inhibition of the fungus in treatments was calculated as

Percentage of inhibition = $\frac{C-T}{C}$ x 100

Where,

C = Average mycelial growth in control plate (cm)

T = Average mycelial growth in treatment plate (cm)

3.2.3.1.2 Anti-bacterial property

The anti-bacterial property of methanolic extract of leaves and rhizomes were estimated against *Ralstonia solanacearum* using agar well diffusion method (Valgas *et al.*, 2007). Nutrient agar medium was poured into sterile Petri plates and 100 µl of bacterial inoculum (1.0 x 10 8 cfu.ml^{-1}) was evenly spread onto the medium. A well of 5.0 mm diameter was made in each agar plate using a sterilized cork borer. 50 µl of rhizome and leaf extracts were loaded into the wells in Petri plates separately. Sterile distilled water was used as control check. The Petri plates were incubated at 27°C temperature for 48 to72 hours. Observations were recorded periodically on inhibitory zones (diameter in mm) produced by the extract.

Results

4. RESULTS

The present study entitled “Variability studies in lesser galangal (*Alpinia calcarata* Roscoe)” was carried out at Department of Plantation, Spices, Medicinal and Aromatic crops, College of Agriculture, Vellanikkara and ICAR- NBPGR Regional Station, Thrissur with the objective to assess the variability in morphological, yield and quality parameters of lesser galangal germplasm collected from South Indian states. The results of the study are presented in this chapter.

4.1 Morphological variation

Observations on plant morphological features were made on existing plant population of *Alpinia calcarata* during September – October 2021. Yield of above-mentioned accessions were recorded during April 2022.

4.1.1 Tiller characters

Tiller characters including length of tillers, diameter of tillers, number of tillers and intermodal length of the 18 accessions are presented in the Table 4.1.

4.1.1.1 Length of tillers

Significant differences were observed for tiller length among accessions and accession IC582825 (180.34 cm) recorded highest tiller length however, IC582783 (178.39 cm), IC582800 (177.76 cm), IC582826 (176.83 cm), IC582809 (174.98 cm) showed on par values. The lowest length of tiller was observed in the accession IC565495 (119.38 cm).

4.1.1.2 Diameter of tillers (cm)

The mean diameter of tillers ranged from 2.18 cm to 3.21 cm. The highest tiller diameter was recorded in accession IC582809 (3.21 cm) followed by IC582825 (3.15 cm), IC582783 and IC582800 (3.14 cm). The lowest tiller diameter was in IC565495 (2.18 cm).

4.1.1.3 No. of tillers

Accession IC373608 (59 tillers) recorded highest number of tillers per plant and the lowest was recorded in accession IC210421 (26 tillers).

4.1.1.4 Internodal length (cm)

Among the eighteen accessions, IC582788 (11.35 cm) was observed for the highest internodal length which was on par with PCSAc1 (11.32 cm). The lowest internodal length was recorded in IC582819 (9.84 cm).

Table 4.1: Variation in tiller characters of *Alpinia calcarata* accessions

Accessions	Length of tillers (cm)	Diameter of tillers (cm)	Number of tillers	Internodal length (cm)
IC210421	121.71	2.60	26	10.44
IC210656	121.86	2.90	43	10.60
IC373608	138.61	2.51	**59**	10.39
IC468880	146.46	2.43	54	10.44
IC550112	149.00	2.57	29	10.11
IC552519	168.69	2.81	52	10.91
IC565484	140.47	2.50	38	10.96
IC565495	119.38	2.18	41	10.76
IC582756	162.50	2.97	48	10.55
IC582781	169.15	2.99	34	10.42
IC582783	178.39	3.14	41	11.07
IC582788	160.53	2.77	33	**11.35**
IC582800	177.76	3.14	45	10.97
IC582809	174.98	**3.21**	56	11.05
IC582819	126.93	2.27	38	9.84

IC582825	**180.34**	3.15	43	10.76
IC582826	176.83	3.05	43	10.66
PCSAc 1	143.97	2.22	43	11.32
Mean	**153.20**	**2.75**	**42.56**	**10.7**
CV (%)	**15.52**	**13.57**	**21**	**4.21**
CD (0.05)	**10.90**	**0.16**	**NA**	**0.23**

NA: Un-replicated data

4.1.2 Leaf characters

The results of number of leaves per tiller, leaf length, leaf width, leaf area and petiole length of the *Alpinia calcarata* accessions were statistically analysed and presented in the Table 4.2.

4.1.2.1 Number of leaves per tillers

The highest number of leaves per tiller was in the accession IC373608 (13.40) followed by IC210421 (12.40), IC210656 (12.10) and IC582826 (11.8) which were on par. Accession PCSAc 1 (8.10) had lowest number of leaves per tiller.

4.1.2.2 Leaf length (cm)

Significant difference was noticed among the accessions for leaf length. The maximum leaf length was recorded in IC582783 (55.23cm) followed by IC373608 (53.80 cm), IC582781 (53.66 cm) and IC582825 (53.63 cm) which were on par. The minimum leaf length (41.33 cm) was observed in IC210421.

4.1.2.3 Leaf width (cm)

The leaf width of different accessions ranged from 4.44 cm (IC582819) to 6.72 cm (IC582783). There was significant difference in leaf width among various accession. IC582783 (6.72cm) and IC373608 (6.47 cm) showed on par values.

4.1.2.4 Leaf area (cm^2)

The accession IC582783 (259.84 cm^2) recorded the highest leaf area IC373608 (242.94 cm^2) was on par. The lowest leaf area was observed in the accession IC582819 (130.01cm^2).

4.1.2.5 Petiole length (cm)

Significant difference was not observed for petiole length of leaves. However, accession IC210656 (0.75cm) had highest petiole length and lowest in IC582781 (0.57 cm).

Table 4.2: Variation in leaf characters of *Alpinia calcarata* accessions

Accessions	Number of leaves per tiller	Leaf length(cm)	Leaf width (cm)	Leaf area (cm^2)	Petiole length (cm)
IC210421	12.40	41.33	5.31	154.80	0.69
IC210656	12.10	44.05	5.35	164.89	**0.75**
IC373608	**13.40**	53.80	6.47	242.94	0.68
IC468880	10.50	48.48	6.27	212.84	0.72
IC550112	11.50	49.51	5.38	187.25	0.59
IC552519	10.30	49.12	5.92	204.59	0.63
IC565484	9.60	44.25	4.49	138.63	0.67
IC565495	10.60	41.83	4.52	132.92	0.71
IC582756	10.40	52.13	5.59	204.19	0.65
IC582781	10.80	53.66	5.67	213.76	0.57
IC582783	11.20	**55.23**	**6.72**	**259.84**	0.60
IC582788	11.00	49.98	4.99	156.81	0.58
IC582800	10.30	43.23	5.65	171.04	0.69
IC582809	10.10	52.27	5.70	208.77	0.72

IC582819	9.80	41.82	4.44	130.01	0.61
IC582825	11.50	53.63	6.05	226.97	0.60
IC582826	11.80	51.87	5.34	194.54	0.59
PCSAc 1	8.10	44.34	4.45	138.29	0.70
Mean	**10.86**	**48.36**	**5.43**	**185.73**	**0.65**
CV (%)	**19.23**	**11.02**	**12.7**	**23.17**	**9.90**
CD (0.05)	**1.80**	**2.53**	**0.32**	**20.00**	**NS**

4.1.3 Rhizome characters

The rhizome characters including rhizome length, width and circumference are presented in the Table 4.3.

4.1.3.1 Rhizome length (cm)

The mean rhizome length was highest in accession IC373608 (6.02 cm). Except IC468880 (5.28 cm) and IC582756 (4.98 cm), all other accessions recorded on par values.

4.1.3.2 Rhizome width (cm)

Accession IC373608 recorded the highest rhizome width of 2.4 cm, and it was on par with IC582825 (2.2 cm). The accession IC565484 was noted with lowest value of 1.02 cm.

4.1.3.3 Rhizome circumference (cm)

The mean rhizome circumference ranged from 3.34 cm to 7.5 cm. Accession IC373608 showed highest rhizome circumference of 7.5cm, which was on par with IC582825 (7.1 cm). The lowest value was noticed in IC565484 (3.34 cm).

Table 4.3: Variation in rhizome characters of *Alpinia calcarata* accessions

Accessions	Rhizome length (cm)	Rhizome width (cm)	Rhizome circumference (cm)
IC210421	5.64	1.72	5.38
IC210656	5.52	1.76	5.42
IC373608	**6.02**	**2.40**	**7.50**
IC468880	5.28	1.80	5.80
IC550112	5.68	1.76	5.56
IC552519	5.62	1.80	5.70
IC565484	5.82	1.02	3.34
IC565495	5.66	1.28	4.18
IC582756	4.98	1.24	3.90
IC582781	5.66	1.70	5.42
IC582783	5.84	1.36	4.42
IC582788	5.46	1.58	4.21
IC582800	5.44	1.48	5.02
IC582809	5.60	1.62	5.08
IC582819	5.64	1.70	5.28
IC582825	5.94	2.20	7.10
IC582826	5.80	1.65	5.00
PCSAc 1	5.52	1.70	5.46
Mean	**5.61**	**1.70**	**5.23**
CV (%)	**8.50**	**19.50**	**19.60**
CD (0.05)	**0.69**	**0.37**	**0.90**

4.1.4 Yield parameters

The prominent yield parameters like rhizome yield per tiller and dry recovery of fresh rhizomes are depicted in the Table 4.4.

4.1.4.1 Rhizome yield per tiller (g)

The highest mean rhizome yield was observed in accession IC373608 (14.63 g per tiller) and on par values were noted in IC582825 (13.47 g per tiller) and IC582826 (12.95 g per tiller). The lowest was observed in IC582756 and PCSAc1 with 9.74 g per tiller.

4.1.4.2 Dry recovery (per cent)

There was a significant difference among the accessions with respect to dry recovery. Maximum driage was recorded in accession IC373608 (33.26 per cent) followed by IC210421 (32.88 per cent) and IC582825 (31.04 per cent). Minimum dry recovery was recorded in IC565495 (19.82 per cent).

Table 4.4: Variation in yield parameters of *Alpinia calcarata* accessions

Accessions	Rhizome yield (g per tiller)	Dry recovery (%)
IC210421	12.68	32.88
IC210656	11.82	28.65
IC373608	**14.63**	**33.26**
IC468880	12.03	25.19
IC550112	11.98	26.26
IC552519	11.60	26.8
IC565484	10.19	20.33
IC565495	10.83	19.82
IC582756	9.74	22.77
IC582781	11.06	21.76
IC582783	11.30	25.59
IC582788	10.28	20.04
IC582800	11.12	25.62

IC582809	10.63	20.43
IC582819	12.39	30.61
IC582825	13.47	31.04
IC582826	12.95	27.54
PCSAc 1	9.74	19.90
Mean	**11.58**	**25.47**
CV (%)	**15.13**	**17.90**
CD (0.05)	**1.93**	**NA**

NA: Un-replicated data

4.1.5 Correlation of rhizome yield with other morphological characters in *Alpinia calcarata.*

Rhizome yield showed a highly significant and positive correlation with rhizome length (0.585*), rhizome width (0.784***), rhizome circumference (0.785***), dry recovery (0.887***) and number of leaves per tiller (0.752***) and also showed a positive interaction with leaf area (0.367) and tiller diameter (0.022). Rhizome yield also exhibited a negative correlation with tiller length (-0.088). Rhizome length showed a positive correlation with rhizome width (0.334), rhizome circumference (0.339), and dry recovery (0.357), number of leaves per tiller (0.398), leaf area (0.214) and tiller length (0.021); whereas a low negative correlation with tiller diameter (-0.004). Rhizome width had a significantly high positive correlation with rhizome circumference (0.974***), dry recovery (0.667**) and number of leaves per tiller (0.51*) and also positive correlation with leaf area (0.329), tiller length (0.019) and tiller diameter (0.017). Rhizome circumference exhibited a highly significant positive correlation with dry recovery (0.678**) and other parameters such as number of leaves per tiller (0.451), leaf area (0.344), tiller length (0.037) and tiller diameter (0.019) were also positively related to rhizome circumference. Dry recovery of rhizome and number of leaves per tiller exhibited a significant positive correlation with each other (0.679**) and also showed positive

correlation with leaf area (0.235) and tiller diameter (0.058) whereas negatively correlated with tiller length (-0.095). Number of leaves per tiller positively correlated with leaf area (0.456) and tiller diameter (0.249) and negatively correlated with tiller length (-0.095). Leaf area exhibited significant and positive correlation on tiller length (0.611**) and tiller diameter (0.583*). Tiller length showed a significantly high positive correlation with tiller diameter (0.795***)

Table 4.5: Coefficient of correlation between rhizome yield and morphological characters of *Alpinia calcarata* accessions

	Rhizome yield	Rhizome length	Rhizome width	Rhizome circumference	Dry recovery	Number of leaves per tiller	Leaf area	Tiller length	Tiller diameter
Rhizome yield	1								
Rhizome length	0.585*	1							
Rhizome width	0.784***	0.334	1						
Rhizome circumference	0.785***	0.339	0.974***	1					
Dry recovery	0.887***	0.357	0.667**	0.678**	1				
Number of leaves per tiller	0.752***	0.398	0.51*	0.451	0.679**	1			
Leaf area	0.367	0.214	0.329	0.344	0.235	0.456	1		
Tiller length	-0.088	0.021	0.019	0.037	-0.167	-0.095	0.611**	1	
Tiller diameter	0.022	-0.004	0.017	0.019	0.058	0.249	0.583*	0.795***	1

*** Correlation is significant at 0.001 level (two tailed)

** Correlation is significant at 0.01 level (two tailed)

* Correlation is significant at 0.05 level (two tailed)

4.1.6 Inflorescence characters

The data pertaining to inflorescence characters like season of flowering, duration of flowering, and inflorescence length were recorded and presented in the Table 4.6.

4.1.6.1 Flowering behaviour

The accessions IC210656, IC373608, IC550112, IC582819 and IC582825 showed two flowering seasons in the study period during April-May to November–December. However, all other accessions under investigation had flowered during April to May, which was considered as the season of flowering in *Alpinia calcarata*. During the course of investigation, there was no flowering observed in accessions IC582756, IC582800, IC582809 and IC582826.

Table 4.6: Flowering behaviour of *Alpinia calcarata* accessions

Sl. No.	Accessions	Flowering behavior
1	**210656, IC373608, IC550112, IC582819, IC582825**	Flowering during April to May and November to December
2	**IC210421, IC468880, IC552519, IC565484, IC565495, IC582781, IC582783, IC582788, PCSAc 1**	Flowering during April to May
3	**IC582756, IC582800, IC582809, IC582826**	No flowering

4.1.6.2 Duration of flowering (days)

Duration in days between emergence of first flower from the inflorescence and

complete abscission of flowers was noted, which ranged from 16.5 to 22.7 days. Accession IC210421 recorded maximum duration of flowering. It was closely followed by IC373608 (22.4 days), IC582825 (21.5 days) and IC210656 (21.3 days). Minimum duration of flowering was noticed in IC565484 (16.5 days).

4.1.6.3 Inflorescence length (cm)

The highest inflorescence length of 16.10 cm was observed in the accession IC373608 followed by IC210656 (15.57 cm) and IC210421 (15.16 cm). The lowest length of inflorescence was recorded in IC565484 (10.20 cm).

4.1.6.4 Branches per inflorescence

Maximum number of branches produced per inflorescence was noticed in IC373608 (16.20). However, the lowest number of branches per inflorescence was observed in IC552519 (13.10).

Table 4.7: Variation in inflorescence characters of *Alpinia calcarata* accessions

Accessions	Duration of flowering (days)	Inflorescence length (cm)	Branches per inflorescence
IC210421	**22.7**	15.16	15.20
IC210656	21.30	15.57	14.30
IC373608	22.40	**16.10**	**16.20**
IC468880	18.20	13.56	13.80
IC550112	19.60	14.23	14.60
IC552519	19.20	13.13	13.10
IC565484	16.50	10.20	13.50
IC565495	17.40	10.92	14.80
IC582781	16.90	11.95	13.60
IC582783	18.50	11.45	13.20

IC582788	20.20	13.85	14.60
IC582819	20.40	13.56	15.60
IC582825	21.50	14.58	16.10
PCSAc 1	17.50	12.52	14.30
Mean	**19.45**	**13.34**	**14.50**
CV (%)	**10.48**	**13.2**	**6.97**

4.1.7 Flower characters

Results of flower characters like number of flowers per inflorescence, flower length and width are depicted in the Table 4.8.

4.1.7.1 Number of flowers per inflorescences

Among the accessions, number of flowers per inflorescence ranged from 23.50 to 30.40. Accession IC468880 was recorded with maximum number of flowers of 30.40 per inflorescence. Minimum number of flowers of 23.50 was observed in IC582819.

4.1.7.2 Flower length (cm)

The maximum flower length of 3.6 cm was recorded in IC550112 and IC582819, followed by IC210656 (3.58 cm) and IC552519 (3.50cm). Accession IC582781 (2.8 cm) showed the lowest flower length.

4.1.7.3 Flower width (cm)

There was no significant variation among the accessions for flower width. However, IC210421 (2.50 cm) observed highest value and lowest in IC565484 (2.10 cm).

4.1.7.4 Pollen studies

Pollen of *Alpinia calcarata* was spherical in shape. Acetocarmine test was performed to check the viability of pollen grains. The pollen grains stained red in the acetocarmine (2%) and hence confirmed the viability of the pollen grains (Plate 1a). However, there was

no pollen tube formation observed during *in vitro* pollen germination test (Plate 1b).

4.1.7.5 Fruit set

At the end of flowering period, no fruit and seed set were observed among the accessions under study.

Table 4.8: Variation in flower characters of *Alpinia calcarata* accessions

Accessions	Number of flowers per inflorescences	Flower length (cm)	Flower width (cm)
IC210421	28.10	3.20	**2.50**
IC210656	27.50	3.58	2.22
IC373608	29.00	3.40	2.25
IC468880	**30.40**	2.84	2.03
IC550112	26.40	**3.60**	2.17
IC552519	28.40	3.50	2.30
IC565484	23.90	3.22	2.10
IC565495	25.80	3.30	2.35
IC582781	25.40	2.80	2.25
IC582783	24.30	3.10	2.35
IC582826	24.80	2.90	2.35
IC582819	23.50	**3.60**	2.46
IC582825	27.10	3.40	2.23
PCSAc 1	26.50	3.00	2.20
Mean	**26.51**	**3.25**	**2.27**
CV (%)	**7.70**	**8.74**	**5.70**

4.1.8 Floral phenology

Floral phenology is the study of the sequence of all periodical events in a floral life cycle. It helps to know the influence of weather dynamics on reproductive growth period thereby adopting better management of crops. In the present study, we identified the phenological stages of the *Alpinia calcarata* inflorescence as described in BBCH (Biologische Bundesanstalt, Bundessortenamt and Chemical industry) scale (Meier, 2001) (Plate 3).

4.1.8.1 Principal growth stage 5: Inflorescence Emergence

In *Alpinia calcarata*, inflorescence emerged terminally. Inflorescence development started with swelling of the reproductive bud and then it burst open to an inflorescence. Complete opening of inflorescence happened within 8-10 days.

Table 4.9: ***Principal growth stage 5: Inflorescence Emergence***

BBCH Code	Observations	Days taken after visibility of inflorescence
50	Inflorescence visible. It is protected by the sheath of leaves	0
51	Beginning of Inflorescence buds swelling	1-2
53	Inflorescence bud bursts and flower buds are visible	3-4
55	50 per cent inflorescence open. Flower bud are more visible between the sheaths of leaf	6-7
59	End of inflorescence development and fully open	8-10

4.1.8.2 Principal growth stage 6: Flowering

Flowering began after the complete emergence of inflorescence. In a panicle, about

25-30 flowers were present and 2-5 flowers per inflorescence opened daily. The longevity of a flower was one day. It took about 13-15 days for completion of flowering.

Table 4.10: ***Principal growth stage 6: Flowering***

BBCH code	Observations	Days taken after complete emergence of inflorescence
60	Inflorescence began to bloom	0
61	10 per cent of flowers on inflorescence were completely bloomed and began to fall off	1-2
63	30 per cent of flowers on inflorescence were completely bloomed and began to fall off	4-5
65	50 per cent of flowers on inflorescence were completely bloomed and began to fall off	7-9
68	80 per cent of flowers on terminal inflorescence were completely bloomed and fell off	11-13
69	End of flowering. All flowers on terminal inflorescence were completely bloomed and fell off	13-15

4.1.9 Qualitative morphological parameters of *Alpinia calcarata*

Qualitative morphological parameters of *Alpinia calcarata* are depicted in the Table 4.11and Plate 2.

There was no variation in the qualitative morphological parameters among the accessions. Leaves were dark green color with linear lanceolate in shape. The inflorescence was terminal panicle and flower was creamy yellow colour with red streak. The cylindrical rhizome was less branched bearing numerous root tubers. The skin colour of the rhizome was pale yellow and inner core was moderate yellow in colour.

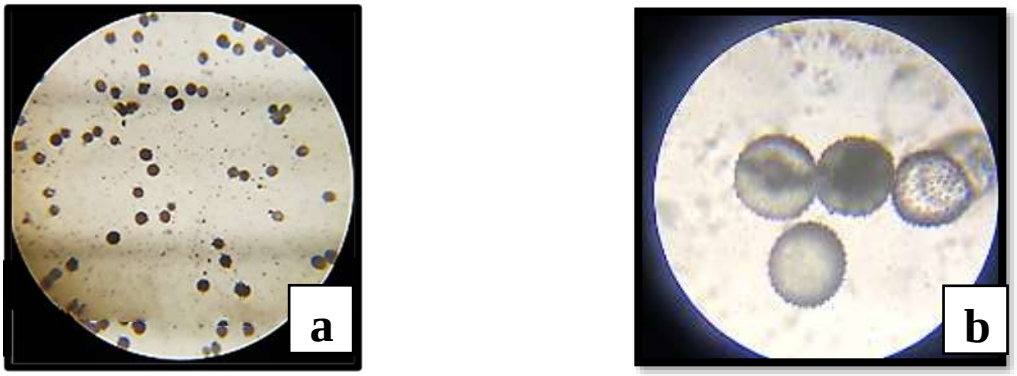
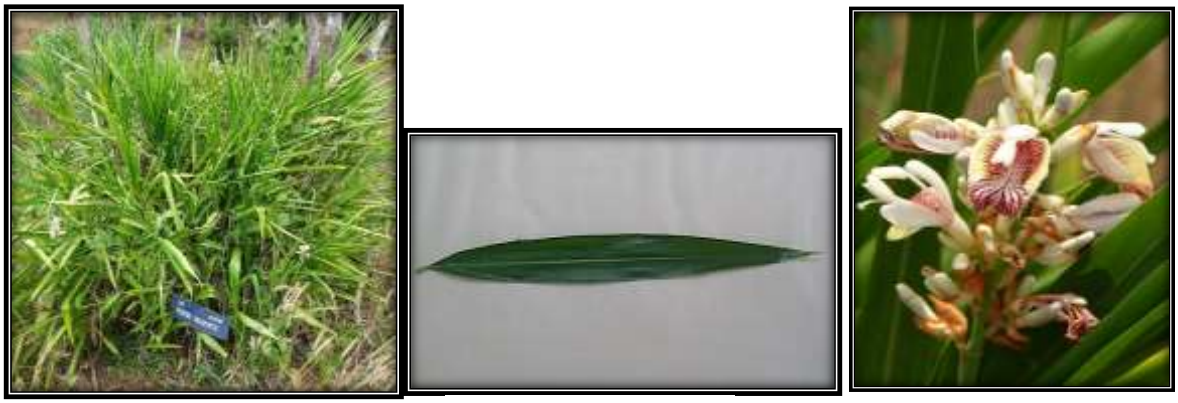


Plate 1: Pollen studies in *Alpinia calcarata* (a) Pollen grains stained red in pollen viability test (b) No formation of pollen tube in *in vitro* pollen germination test



Plant **Leaf** **Inflorescence**

Rhizome **Rhizome skin color – Pale yellow (RHS 158A)** **Rhizome core color –Moderate yellow (RHS 161A)**

Plate 2: Qualitative morphological characters of *A. calcarata*

Plate 3: Floral phenology of *A. calcarata* (BBCH scale)

Plate 4: Variability in rhizome characters of *A. calcarata* accessions

Table 4.11: Qualitative morphological features of *Alpinia calcarata*

Parameters	Features
Shape of leaf	Linear lanceolate
Leaf colour	Dark green
Leaf tip	Acuminate
Leaf base	Attenuate
Leaf lamina	Glabrous
Inflorescence	Terminal panicle
Flower colour	Creamy yellow colour with red streak
Rhizome	Less branched
Shape of rhizome	Cylindrical
Rhizome skin colour	Pale yellow (RHS 158A)
Rhizome core colour	Moderate yellow (RHS 161A)
Root tubers	Present

4.2 Quality characters of rhizome

The rhizomes of all accessions of *Alpinia calcarata* under study were subjected to biochemical analysis for estimating essential oil, oleoresin, total phenols, total flavonoids, starch, crude fibre and terpenoids. GC-MSMS analysis of essential oil of accession with superior performance in terms of yield and quality was also carried out.

4.2.1 Essential oil (%)

Significant difference was observed among the accessions for essential oil content in the rhizome (Table 4.12). The highest essential oil content was recorded in the accession IC468880 (0.75%). The lowest essential oil content was observed in IC565495 (0.30%).

4.2.2 Oleoresin (%)

The oleoresin content in rhizomes showed significant differences among the accessions. The accession IC373608 (34.20%) recorded the highest oleoresin content and accession IC565484 (20.20%) recorded the lowest value.

Table 4.12: Variation in essential oil and oleoresin content among accessions of *Alpinia calcarata*

Accessions	Essential oil (%)	Oleoresin (%)
IC210421	0.72^{b}	27.80^{bcd}
IC210656	0.72^{b}	24.80^{efgh}
IC373608	0.70^{b}	**34.20^{a}**
IC468880	**0.75^{a}**	25.80^{def}
IC550112	0.58^{e}	22.10^{ijk}
IC552519	0.48^{g}	24.10^{fghi}
IC565484	0.34^{ij}	20.20^{k}

IC565495	0.30^{k}	28.20^{bc}
IC582756	0.52^{f}	24.20^{fghi}
IC582781	0.62^{d}	22.70^{hij}
IC582783	0.46^{g}	28.50^{b}
IC582788	0.36^{i}	25.90^{cdef}
IC582800	0.32^{jk}	23.30^{ghi}
IC582809	0.42^{h}	25.50^{defg}
IC582819	0.64^{cd}	20.90^{jk}
IC582825	0.48^{g}	25.60^{defg}
IC582826	0.65^{c}	27.10^{bcde}
PCSAc 1	0.42^{h}	27.50^{bcd}
CV (%)	**2.57**	**3.94**
CD (0.05)	**0.03**	**2.11**

4.2.3 Total phenols (mg gallic acid equivalents.g^{-1}(mg GAE.g^{-1}))

The total phenols were the highest in accession IC373608 (115.25 mg GAE.g^{-1}), whereas, IC565484 (91.50 mg GAE.g^{-1}) had the lowest phenol content. The accessions IC565495 (92.75 mg GAE.g^{-1}) and IC582781 (94.37 mg GAE.g^{-1}) were on par with IC565484.

4.2.4 Total flavonoids (mg quercetin equivalents g^{-1}(mg QE.g^{-1}))

Among the accessions, the total flavonoids ranged from 30.30 to 55.65 mg QE.g^{-1} (Table 4.13). The maximum flavonoid content was recorded in IC582825 (55.65 mg QE.g^{-1}) and IC373608 (54.45 mg QE.g^{-1}) was on par. The minimum flavonoid content was observed in IC582756 (30.30 mg QE.g^{-1}) and IC565495 (30.40 mg QE.g^{-1}).

4.2.5 Starch (mg.g^{-1})

The starch content in the rhizomes differed significantly among the accessions. The highest starch was recorded in IC210656 (44.05 mg.g^{-1}) whereas, the minimum value of starch was recorded in IC582756 (27.65 mg.g^{-1}), IC565495 (27.75 mg.g^{-1}) and IC565484 (28.45 mg.g^{-1}) which were on par.

Table 4.13: Variation in biochemical characters of *Alpinia calcarata* accessions

Accessions	Total phenols (mg GAE.g^{-1})	Total flavonoids (mg QE.g^{-1})	Starch (mg.g^{-1})
IC210421	110.75^{bc}	50.75^{ab}	40.80^{c}
IC210656	112.80^{ab}	48.50^{abc}	**44.05^{a}**
IC373608	**115.25^{a}**	**54.45^{a}**	43.00^{b}
IC468880	95.20^{fgh}	47.10^{bc}	37.65^{e}
IC550112	107.90^{cd}	43.60^{bcde}	38.55^{de}
IC552519	102.75^{e}	42.35^{cde}	36.05^{f}
IC565484	91.50^{i}	34.30^{fg}	28.45^{k}
IC565495	92.75^{hi}	30.40^{g}	27.75^{k}
IC582756	95.25^{fgh}	30.30^{g}	27.65^{k}
IC582781	94.37^{ghi}	33.55^{fg}	30.50^{j}
IC582783	97.75^{f}	36.35^{efg}	31.00^{j}
IC582788	96.50^{fg}	30.90^{fg}	32.35^{i}
IC582800	106.15^{d}	38.25^{def}	32.30^{i}
IC582809	97.30^{fg}	38.15^{def}	33.20^{hi}
IC582819	102.75^{e}	44.95^{bcd}	33.75^{gh}
IC582825	105.60^{de}	**55.65^{a}**	39.40^{d}
IC582826	109.85^{bc}	44.10^{bcd}	34.65^{g}

PCSAc 1	95.50^{fgh}	32.30^{fg}	30.15^{j}
CV (%)	**1.40**	**4.18**	**1.25**
CD (0.05)	**2.99**	**3.59**	**0.91**

4.2.6 Crude fibre (%)

Significant difference was observed for crude fibre content among the accessions (Table 4.14). Lowest crude fibre content is considered as an appreciable character, the accession IC550112 (7.90%) registered the lowest value. Accessions IC552519 (8.60%), IC373608 (8.70%), IC210656 (8.80%) and IC582819 (9.02%) were on par with IC550112. The maximum crude fibre was recorded in the accessions IC582809 (15.42%), IC582788 (15.35%), IC582783 (14.40%), IC565484 (14.50%) and IC565495 (14.25%).

4.2.7 Total terpenoids (%)

Among the eighteen accessions, IC210421 (20.25%) showed the maximum value of total terpenoids. The accessions IC373608 (19.50%), IC550112 (19.00%), IC210656 (17.40%), IC582826 (16.50%) and IC582825 (16.20%) were on par with IC210421. The lowest terpenoid content was observed in IC565484 (6.70%).

Table 4.14: Variation in crude fibre and total terpenoids of *Alpinia calcarata* accessions

Accessions	Crude fibre (%)	Total terpenoids (%)
IC210421	9.70^{cd}	20.25^{a}
IC210656	8.80^{de}	17.40^{ab}
IC373608	8.70^{de}	19.50^{a}
IC468880	10.42^{c}	13.95^{bcd}
IC550112	7.90^{e}	19.00^{a}
IC552519	8.60^{de}	13.65^{bcd}

IC565484	14.50[a]	6.70[g]
IC565495	14.25[a]	8.95[efg]
IC582756	10.22[c]	8.45[efg]
IC582781	13.05[b]	11.00[def]
IC582783	14.40[a]	8.55[efg]
IC582788	15.35[a]	10.80[defg]
IC582800	12.12[b]	12.20[cde]
IC582809	15.42[a]	9.85[defg]
IC582819	9.02[de]	11.70[def]
IC582825	9.55[cd]	16.20[abc]
IC582826	12.00[b]	16.50[ab]
PCSAc 1	12.20[b]	7.55[fg]
CV (%)	**4.44**	**8.56**
CD (0.05)	**1.07**	**2.33**

4.2.8 GCMSMS profile of *Alpinia calcarata* essential oil

GC-MSMS profile with respect to major components of rhizome essential oil of *Alpinia calcarata* accession IC373608 which was identified superior in terms of yield and quality is given in the Table 4.15.

Table 4.15: GCMSMS profile of *A. calcarata* essential oil

Sl. No.	Compound name	RT	Molecular weight ($g.mol^{-1}$)	Area (%)
1	à-Pinene	5.54	136.23	1.54
2	Camphene	5.79	136.24	2.03

3	Bicyclo[2.2.1]heptane, 2,2-dimethyl-3-methylene-,	5.79	272.50	2.03
4	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	6.23	136.23	2.75
5	**Eucalyptol**	**7.08**	**154.25**	**19.17**
6	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)-	8.91	154.25	1.41
7	Camphor	8.91	152.23	1.41
8	à-Terpineol	9.67	154.25	1.49
9	L-à-Terpineol	9.67	154.25	1.49
10	Fenchyl acetate	10.17	196.29	3.25
11	á-copaene	13.35	204.36	1.25
12	Octadecanoic acid	22.76	284.48	1.01
13	Eicosanoic acid	24.61	312.53	0.60
14	Octadecanoic acid, 2-(2-hydroxyethoxy) ethyl ester	26.57	460.70	0.75
15	L-Ascorbic acid, 6-octadecanoate	27.99	442.59	0.64
16	Estra-1,3,5(10)-trien-17á-ol	28.66	334.40	1.02
17	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	31.88	568.90	0.90
18	1-Heptatriacotanol	32.68	537.00	0.68
19	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester, cis	33.21	444.60	0.64
20	Glycine, N-[(3à,5á,7à,12à)-24-oxo-3,7,12-tris[(trimethyl silyl)oxy]cholan-24-yl]-, methyl ester	35.46	291.61	5.04

21	5,8,11-Eicosatriynoic acid, tert-butyldimethylsilyl ester	36.86	414.70	6.62
22	Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17- yl)-	37.56	430.60	8.28
23	4aà,4bá-Gibbane-1à,10á-dicarboxylic acid, 4a-formyl-7-hydroxy-1-methyl-8-methylene-, dimethyl ester	37.90	330.41	4.28
24	Docosanoic acid, 1,2,3-propanetriyl ester	38.19	1059.80	1.32
25	Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate	38.34	498.78	0.65
26	7,8-Epoxylanostan-11-ol, 3-acetoxy	38.78	502.8	0.61
27	Ethyl iso-allocholate	39.08	436.60	15.06
28	1-Monolinoleoylglycerol trimethylsilyl ether	39.68	498.88	6.19

Twenty eight compounds were identified from essential oil of *Alpinia calcarata* rhizome. Major compounds were eucalyptol (1,8-cineole) (19.17%), ethyl iso-allocholate (15.06%), propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17- yl)- (8.28%), 5,8,11-Eicosatriynoic acid, tert-butyldimethylsilyl ester (6.62%) and 1-Monolinoleoylglycerol trimethylsilyl ether (6.19%).

4.2.8 Correlation of rhizome yield with quality characters of *Alpinia calcarata* accessions

The rhizome yield of *Alpinia calcarata* manifested a highly significant positive correlation with essential oil (0.626**), total phenols (0.781***), total flavonoids (0.907***), starch (0.781***) and total terpenoids (0.824***) and positive relation with oleoresin (0.43). The rhizome yield exhibited a negative correlation with crude fibre (-0.593). Essential oil possessed a significant positive correlation with total phenols (0.558*), total flavonoids (0.668**), starch (0.696**) and total terpenoids (0.695**) and a positive correlation with oleoresin (0.185). But the essential oil was negatively correlated with the crude fibre (-0.654). Oleoresin exhibited positive correlation with total phenols (0.331),

total flavonoids (0.274), starch (0.316) crude fibre (0.003) and total terpenoids (0.296). Total phenols had a significant positive correlation with total flavonoids (0.79***), starch (0.842***) and total terpenoids (0.888***) and a negative correlation with crude fibre (-0.668). Total flavonoids significantly and positively correlated with starch (0.905***) and total terpenoids (0.845***) and negatively correlated with crude fibre (-0.698). Starch had shown a significantly positive correlation with total terpenoids (0.908***) and a negative correlation with crude fibre (-0.689). Crude fibre and total terpenoids (-0.711) were found to be negatively correlated.

Table 4.16: Coefficient of correlation between rhizome yield and rhizome quality characters of *Alpinia calcarata*

	Rhizome yield	Essential oil	Oleoresin	Total phenol	Total flavonoid	Starch	Crude fibre	Total terpenoids
Rhizome yield	1							
Essential oil	0.626**	1						
Oleoresin	0.43	0.185	1					
Total phenol	0.781***	0.558*	0.331	1				
Total flavonoid	0.907***	0.668**	0.274	0.79***	1			
Starch	0.781***	0.696**	0.316	0.842***	0.905***	1		
Crude fibre	-0.593**	-0.654**	0.003	-0.668**	-0.698**	-0.689**	1	
Total terpenoids	0.824***	0.695**	0.296	0.888***	0.845***	0.908***	-0.711***	1

*** Correlation is significant at 0.001 level (two tailed)

** Correlation is significant at 0.01 level (two tailed)

* Correlation is significant at 0.05 level (two tailed)

4.3 Anti- microbial Properties

Under *in vitro* conditions, anti-microbial activities of methanolic extracts of rhizomes and leaves of *Alpinia calcarata* were evaluated at 1000 ppm against five plant pathogens *Phytophthora, Colletotrichum, Rhizoctonia, Fusarium* and *Ralstonia.* The anti-bacterial property was estimated using Agar well diffusion method and the anti-fungal properties by poison-food technique.

4.3.1 Percentage of inhibition for *Rhizoctonia* spp.

Inhibition of mycelial growth of *Rhizoctonia* varied significantly with methanolic extract of rhizomes and leaves and the data is depicted in the Table 4.17.

4.3.1.1 Rhizome extract

The results revealed that rhizome extract of the accessions IC210421 (63.50%), IC210656 (62.10%) and IC373608 (60.90%) recorded the maximum percentage of inhibition for *Rhizoctonia* (Plate 5). The lowest percentage inhibition was observed in PCSAc1 (31.70%).

4.3.1.2 Leaf extract

The highest percentage of inhibition of *Rhizoctonia* was achieved by IC210656 (64.60%). Accession IC565484 inhibited *Rhizoctonia* with a minimum percentage of 30.80% (Plate 6).

Table 4.17: *In vitro* evaluation of methanolic extract of rhizome and leaf against *Rhizoctonia* spp.

Accessions	Percentage of inhibition (%)	
	Rhizome extract	**Leaf extract**
IC210421	**63.50^{a}**	58.70^{bc}
IC210656	**62.10^{a}**	**64.60^{a}**
IC373608	**60.90^{a}**	62.50^{ab}

IC468880	47.90^{bcde}	56.70^{cd}
IC550112	55.80^{ab}	60.00^{abc}
IC552519	49.60^{bcd}	58.30^{bc}
IC565484	37.50^{fg}	30.80^{i}
IC565495	42.50^{def}	49.60^{ef}
IC582756	42.10^{def}	42.90^{g}
IC582781	45.40^{cdef}	52.10^{de}
IC582783	45.00^{cdef}	55.80^{cd}
IC582788	39.60^{ef}	52.90^{de}
IC582800	45.40^{cdef}	52.90^{de}
IC582809	47.50^{bcde}	46.20^{fg}
IC582819	51.20^{bc}	55.00^{cd}
IC582825	47.20^{cde}	60.00^{abc}
IC582826	49.60^{bcd}	56.70^{cd}
PCSAc 1	31.70^{g}	36.20^{h}
CV (%)	9.30	5.22
CD (0.05)	7.40	4.57

4.3.2 Percentage of inhibition for *Colletotrichum* spp.

From the data represented in the Table 4.18, it is evident that the rhizome and leaf extract showed appreciable level of inhibition of mycelial growth of *Colletotrichum.*

4.3.2.1 Rhizome extract

The pathogen was tested with the methanolic rhizome extract of all the eighteen accessions (Table 4.18 and Plate 7). The results revealed that the extent of percentage inhibition ranged from 25.40 to 40.90 per cent. The accession IC373608 (40.90%) recorded maximum inhibition whereas IC565484 (25.40%) gave minimum inhibition. Accessions

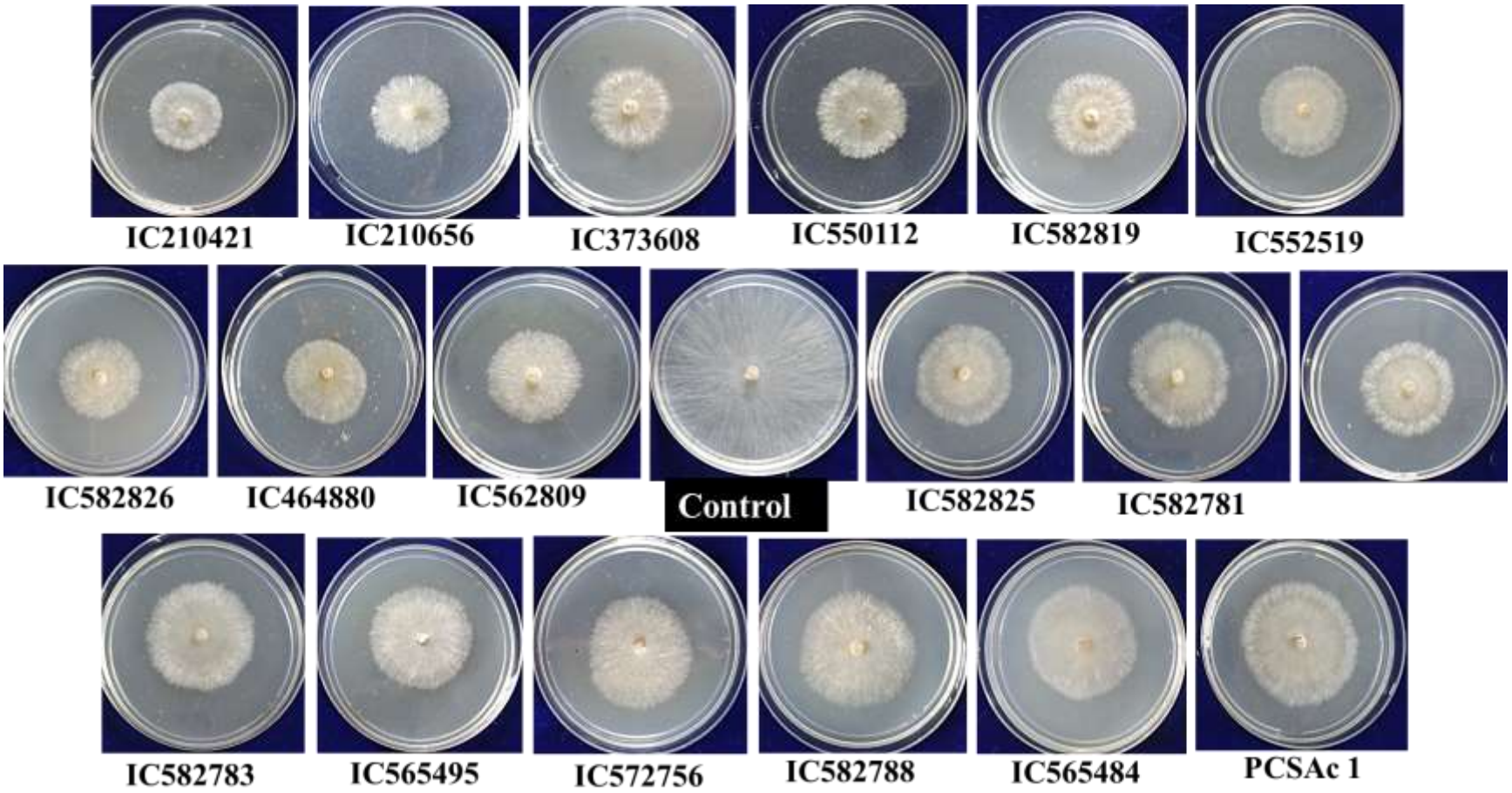


Plate 5: Percentage of inhibition for *Rhizoctonia* spp. – Rhizome extract

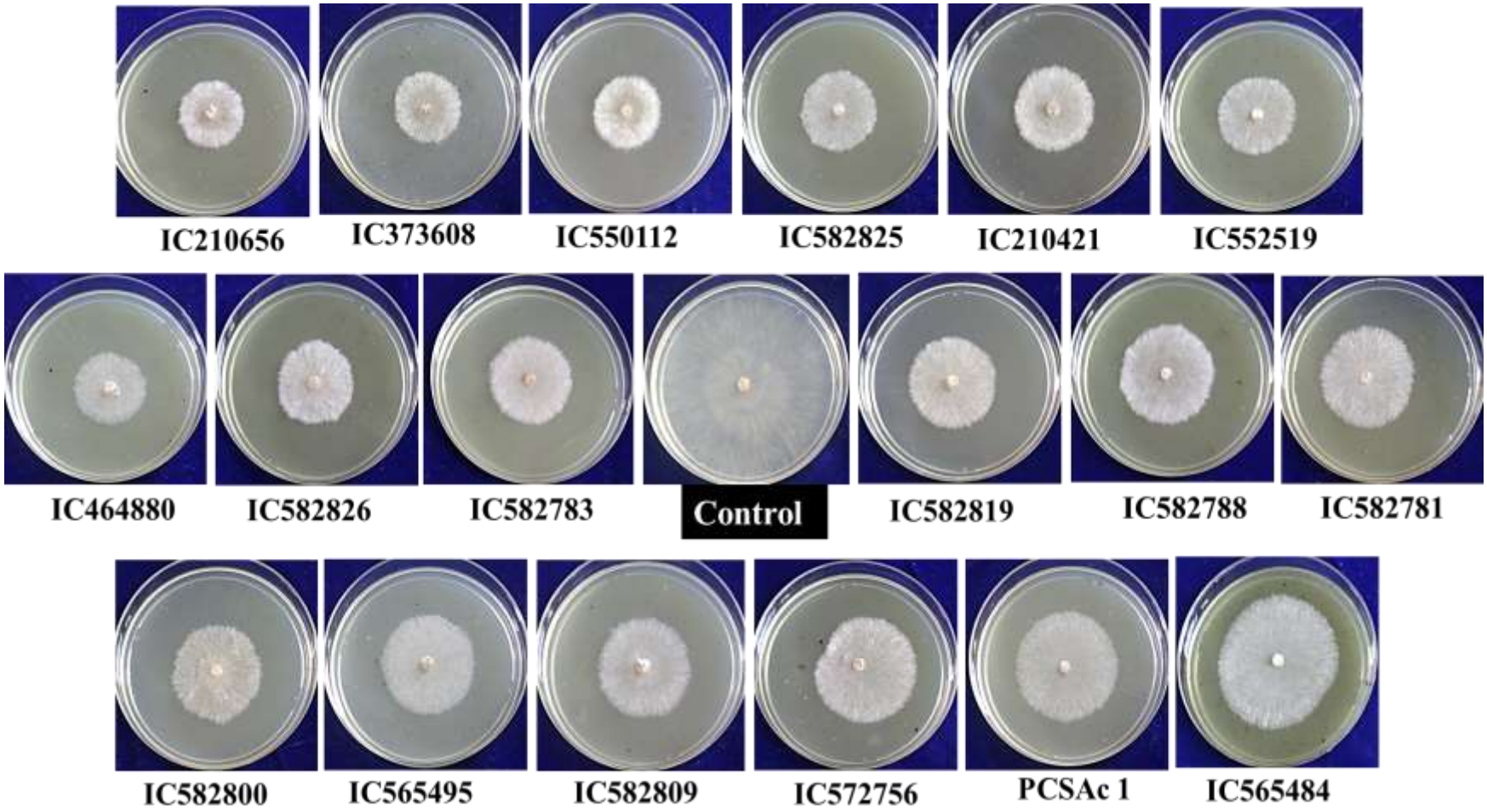


Plate 6: Percentage of inhibition for *Rhizoctonia* spp. – Leaf extract

IC210421(40.40%), IC550112 (40.00%), IC552519 (39.60%), IC210656 (37.10%), IC468880 (37.10%) and IC582826 (37.10%) were on par with IC373608.

4.3.2.2 Leaf extract

Among the accessions, IC373608 (38.70%) recorded the highest percentage inhibition against *Colletotrichum* (Plate 8). The minimum percentage of inhibition was noticed in IC565484 (23.70%) and IC582809 (25.40%).

Table 4.18: *In vitro* evaluation of methanolic extract of rhizome and leaf against *Colletotrichum* spp.

Accessions	Percentage of inhibition (%)	
	Rhizome extract	**Leaf extract**
IC210421	40.40^{ab}	33.30^{cd}
IC210656	37.10^{abc}	32.50^{cde}
IC373608	**40.90^{a}**	**38.70^{a}**
IC468880	37.10^{abc}	32.10^{cdef}
IC550112	40.00^{ab}	36.20^{b}
IC552519	39.60^{ab}	32.90^{cde}
IC565484	25.40^{f}	23.70^{j}
IC565495	32.50^{cd}	28.70^{ghi}
IC582756	31.70^{cde}	28.30^{hi}
IC582781	27.90^{def}	29.60^{fgh}
IC582783	33.40^{cd}	28.30^{hi}
IC582788	33.00^{cd}	31.20^{defg}
IC582800	35.90^{abc}	30.40^{efgh}
IC582809	31.30^{cde}	25.40^{j}

IC582819	36.70^{abc}	32.50^{cde}
IC582825	34.60^{bc}	34.60^{bc}
IC582826	37.10^{abc}	31.20^{defg}
PCSAc 1	25.90^{ef}	26.20^{ij}
CV (%)	**9.37**	**4.86**
CD (0.05)	**5.35**	**2.49**

4.3.3 Percentage of inhibition for *Phytophthora* spp.

The results of the percentage of inhibition for *Phytophthora* with methanolic extract of rhizome and leaf is furnished in the Table 4.19.

4.3.3.1 Rhizome extract

Rhizome extract of accession IC550112 (35.00%) recorded the maximum percentage of inhibition for *Phytophthora* spp. (Plate 9). The accessions IC373608 (33.70%), IC468880 (33.70%), IC210421 (32.90%), IC582826 (32.90%) and IC210656 (32.10%) were on par with IC550112. The lowest percentage of inhibition for *Phytophthora* was shown in PCSAc 1 (25.8%).

4.3.3.2 Leaf extract

Among the all accessions, leaf extract of IC210656 and IC373608 showed maximum inhibition of 37.10 per cent. This was closely followed by IC552519 (36.20%). Accession IC565495 (26.20 %) recorded the lowest percentage of inhibition (Plate 10).

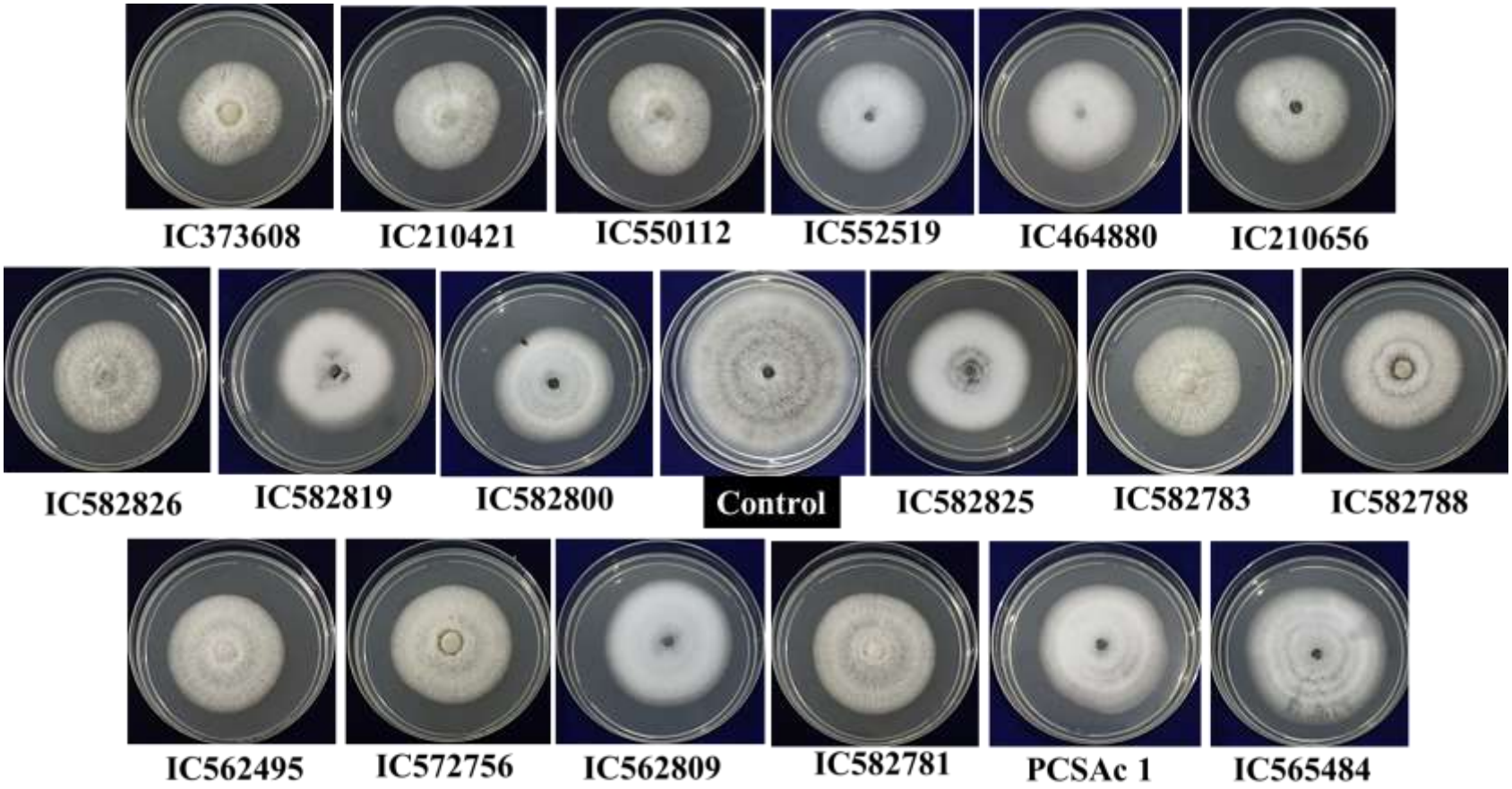


Plate 7: Percentage inhibition for *Colletotrichum* spp. – Rhizome extract

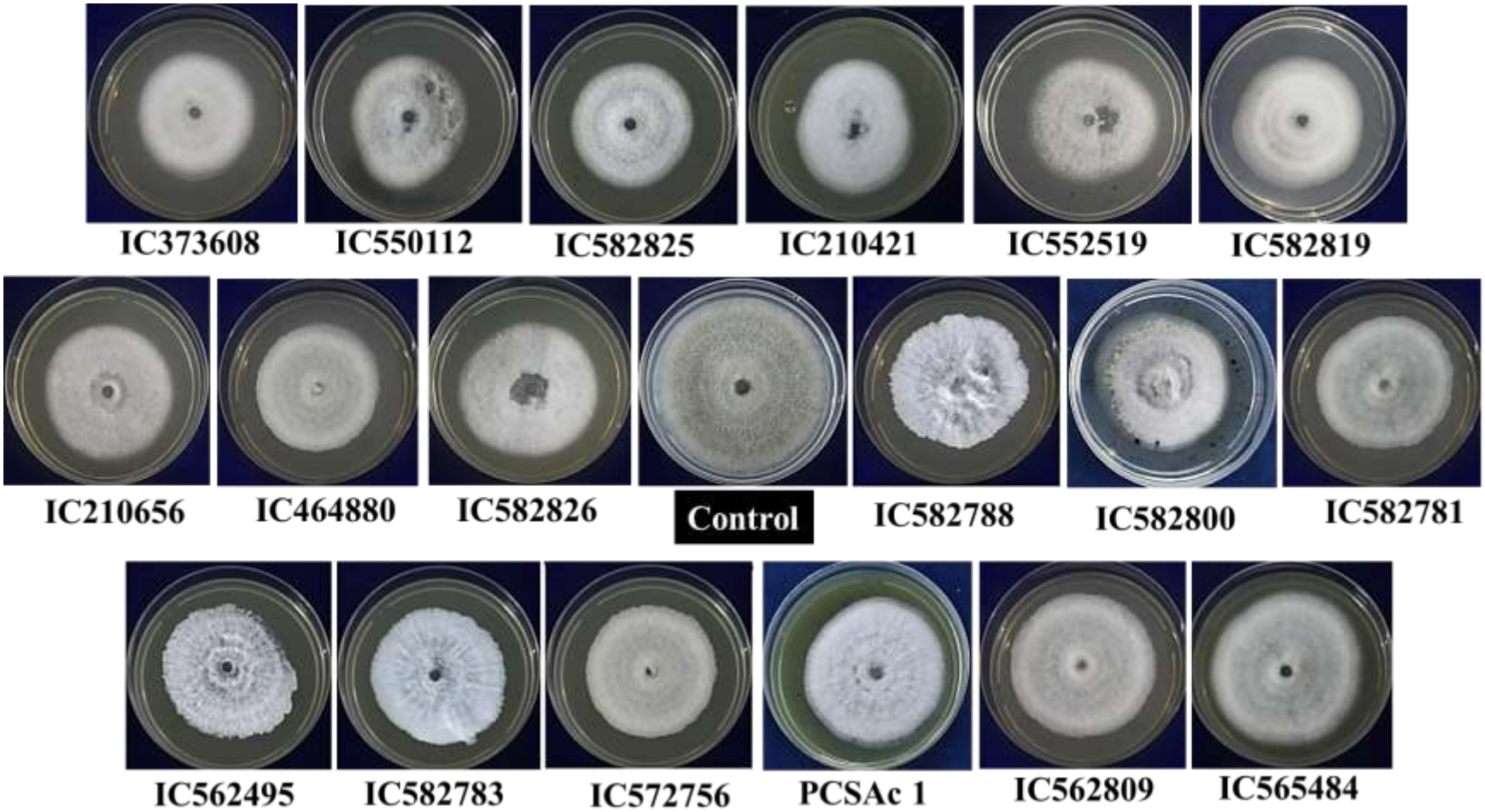


Plate 8: Percentage inhibition for *Colletotrichum* spp. – Leaf extract

Table 4.19: *In vitro* evaluation of methanolic extract of rhizome and leaf against *Phytophthora* spp.

Accession	Percentage of inhibition (%)	
	Rhizome extract	**Leaf extract**
IC210421	32.90^{abc}	32.90^{cd}
IC210656	32.10^{abcd}	**37.10^{a}**
IC373608	33.70^{ab}	**37.10^{a}**
IC468880	33.70^{ab}	30.40^{efg}
IC550112	**35.00^{a}**	31.70^{de}
IC552519	31.30^{abcd}	36.20^{ab}
IC565484	26.20^{ef}	27.90^{hi}
IC565495	28.70^{def}	26.20^{i}
IC582756	26.20^{ef}	28.30^{ghi}
IC582781	31.30^{abcd}	31.20^{de}
IC582783	29.60^{cdef}	29.60^{efgh}
IC582788	31.20^{abcd}	31.20^{de}
IC582800	29.20^{cdef}	30.80^{def}
IC582809	28.70^{def}	30.00^{efgh}
IC582819	31.20^{abcd}	31.20^{de}
IC582825	30.00^{bcde}	31.70^{de}
IC582826	32.90^{abc}	34.60^{bc}
PCSAc 1	25.80^{f}	28.70^{fgh}
CV (%)	**6.61**	**3.93**
CD (0.05)	**3.34**	**2.05**

4.3.4 Percentage of inhibition for *Fusarium* spp.

The methanolic extract of rhizome and leaf of *Alpinia calcarata* showed inhibition

of mycelial growth of *Fusarium* and the data is given in the Table 4.20.

4.3.4.1 Rhizome extract

Percentage of inhibition for *Fusarium* spp. by using methanolic extract of the rhizomes differed significantly among the accessions under study. IC550112 showed the maximum inhibition of 30.00 per cent (Plate 11). The inhibition of 29.20 per cent and 28.10 per cent was recorded in IC552519 and IC373608 respectively and were on par with IC550112. The lowest percentage inhibition was observed in IC582783 (21.20%).

4.3.4.2 Leaf extract

Maximum percentage inhibition for *Fusarium* among the accessions was recorded in methanolic leaf extract of IC550112 (32.10%) which was on par with IC210421 (31.20%) and IC373608 (29.60%). Minimum percentage inhibition was noted in IC582809 (22.90%) (Plate 12).

Table 4.20: *In vitro* evaluation of methanolic extract of rhizome and leaf against *Fusarium* spp.

Accession	Percentage of inhibition (%)	
	Rhizome extract	**Leaf extract**
IC210421	27.90^{abc}	31.20^{ab}
IC210656	27.50^{abc}	27.90^{cde}
IC373608	28.30^{abc}	29.60^{abc}
IC468880	26.70^{bcde}	28.30^{cde}
IC550112	**30.00^{a}**	**32.10^{a}**
IC552519	29.20^{ab}	27.10^{cdef}
IC565484	25.80^{cde}	25.80^{defg}
IC565495	23.70^{ef}	25.40^{efgh}

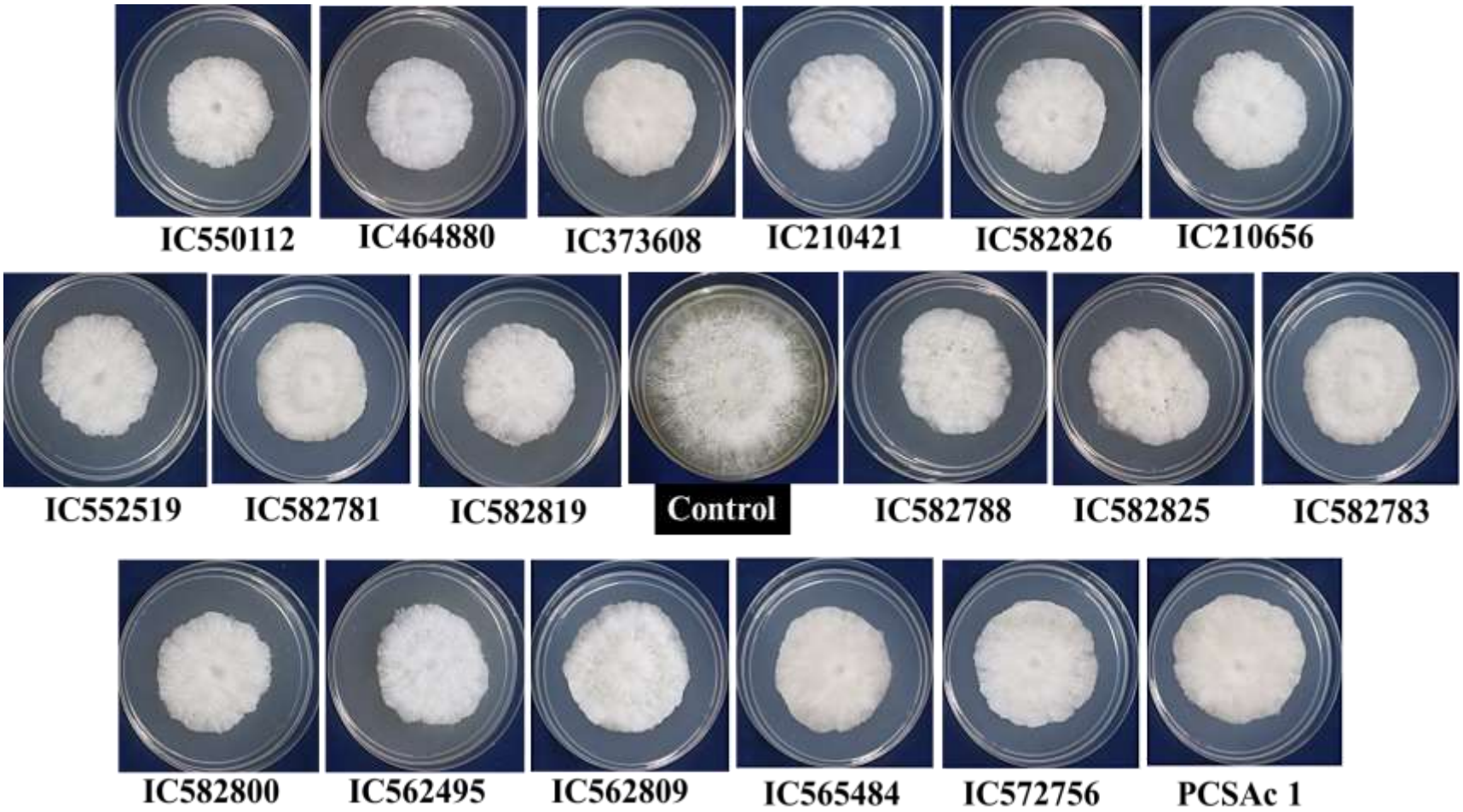


Plate 9: Percentage inhibition for *Phytophthora* spp. – Rhizome extract

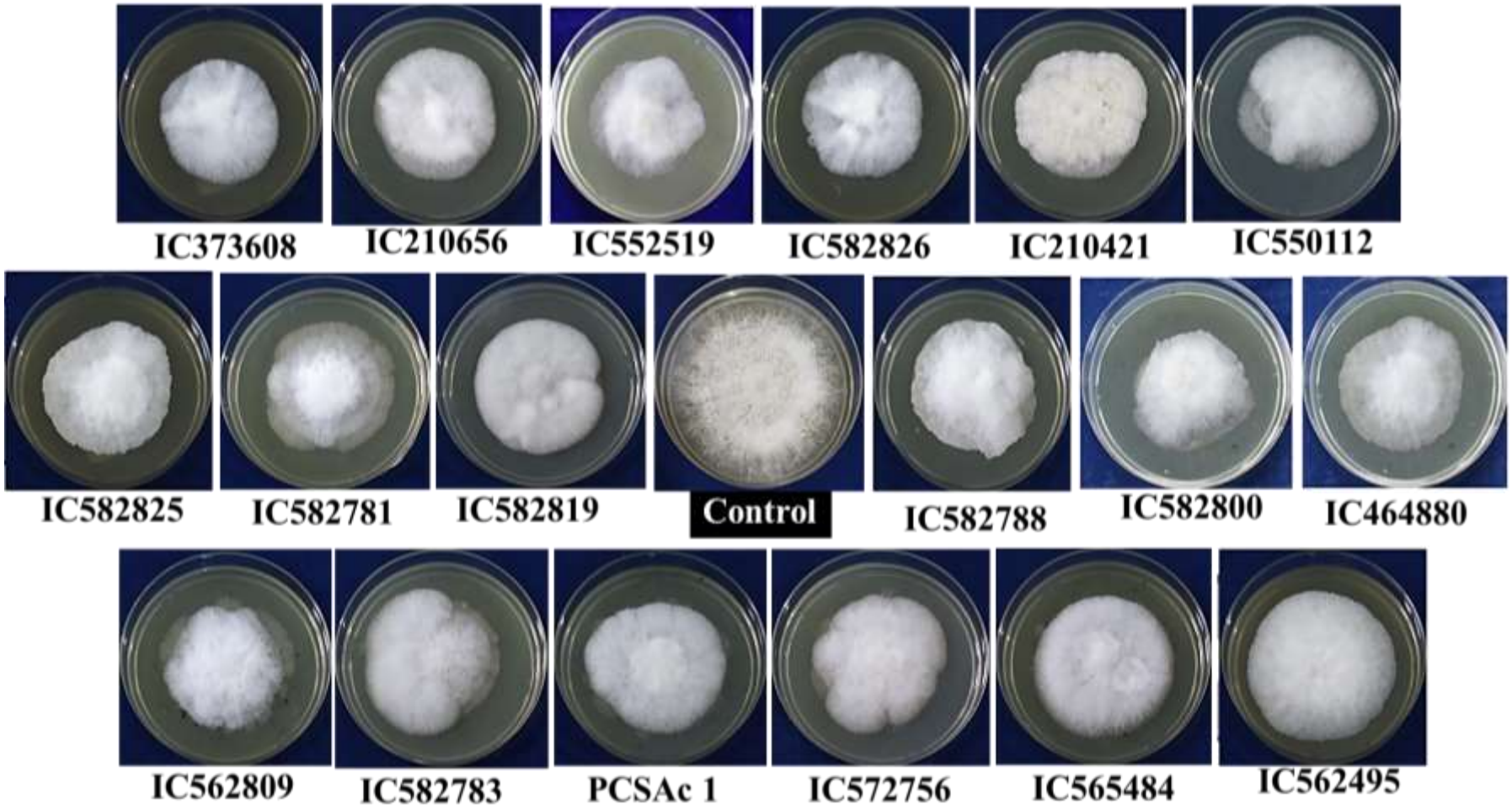


Plate 10: Percentage inhibition for *Phytophthora* spp. – Leaf extract

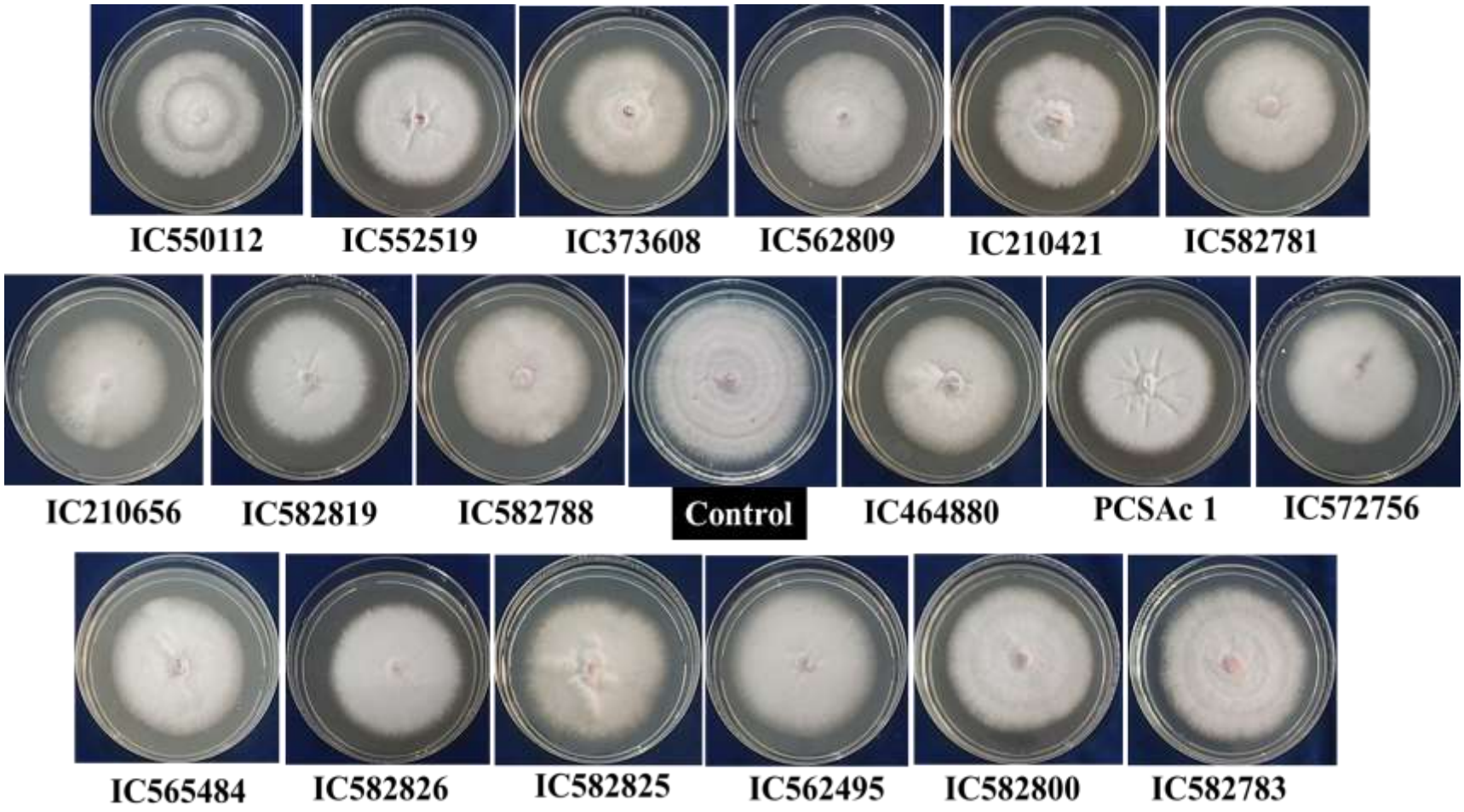


Plate 11: Percentage inhibition for *Fusarium* spp. – Rhizome extract

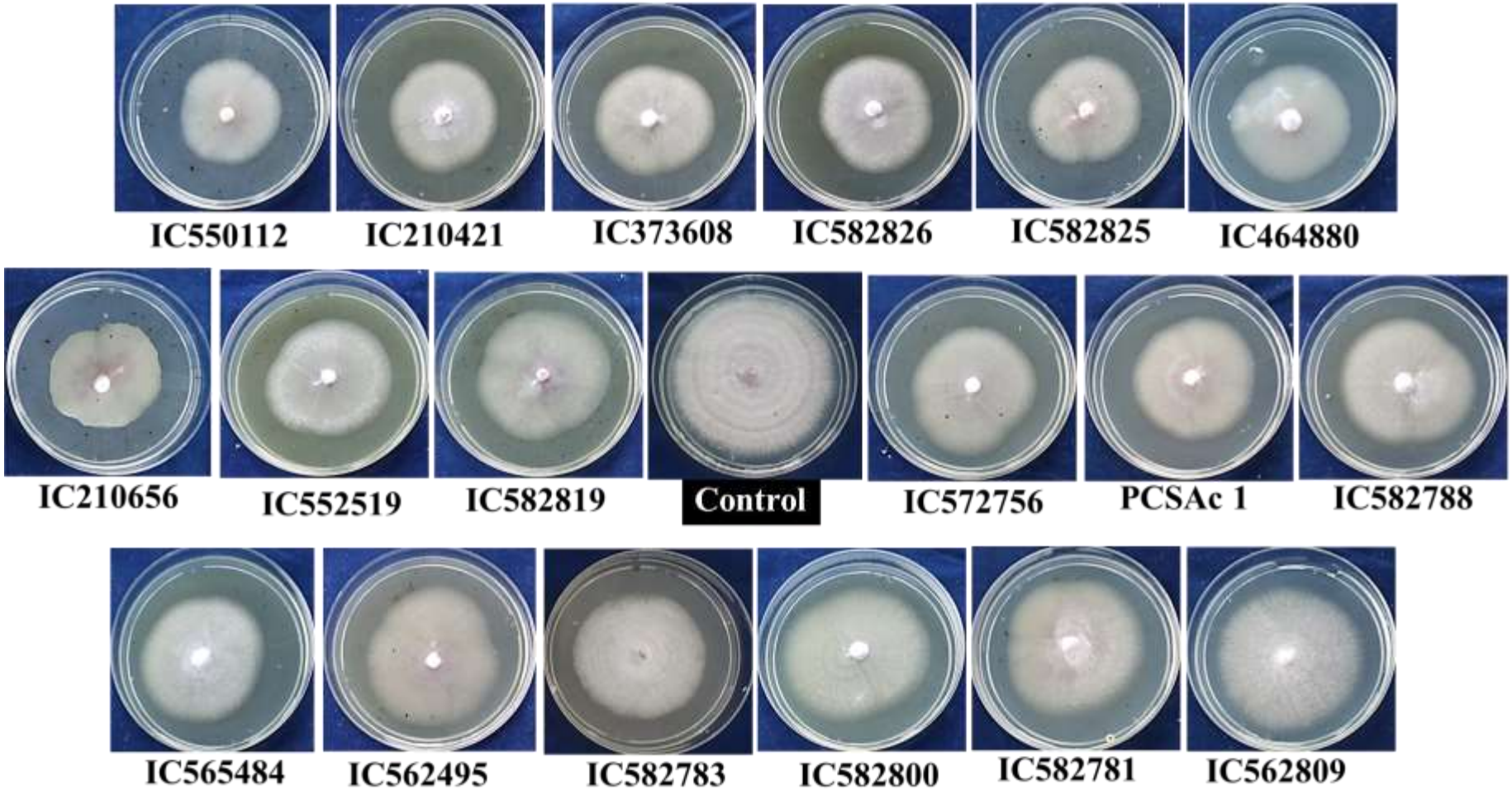


Plate 12: Percentage inhibition for *Fusarium* spp. – Leaf extract

IC582756	25.80^{cde}	26.70^{def}
IC582781	27.50^{abc}	23.70^{gh}
IC582783	21.20^{f}	25.40^{efgh}
IC582788	27.10^{abcd}	26.20^{defg}
IC582800	23.70^{ef}	24.60^{fgh}
IC582809	28.30^{abc}	22.90^{h}
IC582819	27.10^{abcd}	26.70^{def}
IC582825	24.10^{def}	28.30^{cde}
IC582826	25.00^{cde}	28.70^{bcd}
PCSAc 1	25.80^{cde}	26.20^{defg}
CV (%)	**6.48**	**5.59**
CD (0.05)	**2.83**	**2.50**

4.3.5 Zone of inhibition for *Ralstonia solanacearum*

The efficacy of methanolic extract of rhizome and leaves of *Alpinia calcarata* was evaluated against *Ralstonia solanacearum* under *in vitro* condition. The protocol adopted for the study was agar well diffusion method. It was found that none of the accessions showed any zone of inhibition for phytopathological bacteria *R. solanacearum* with leaf and rhizome extract (Plate 13 and 14).

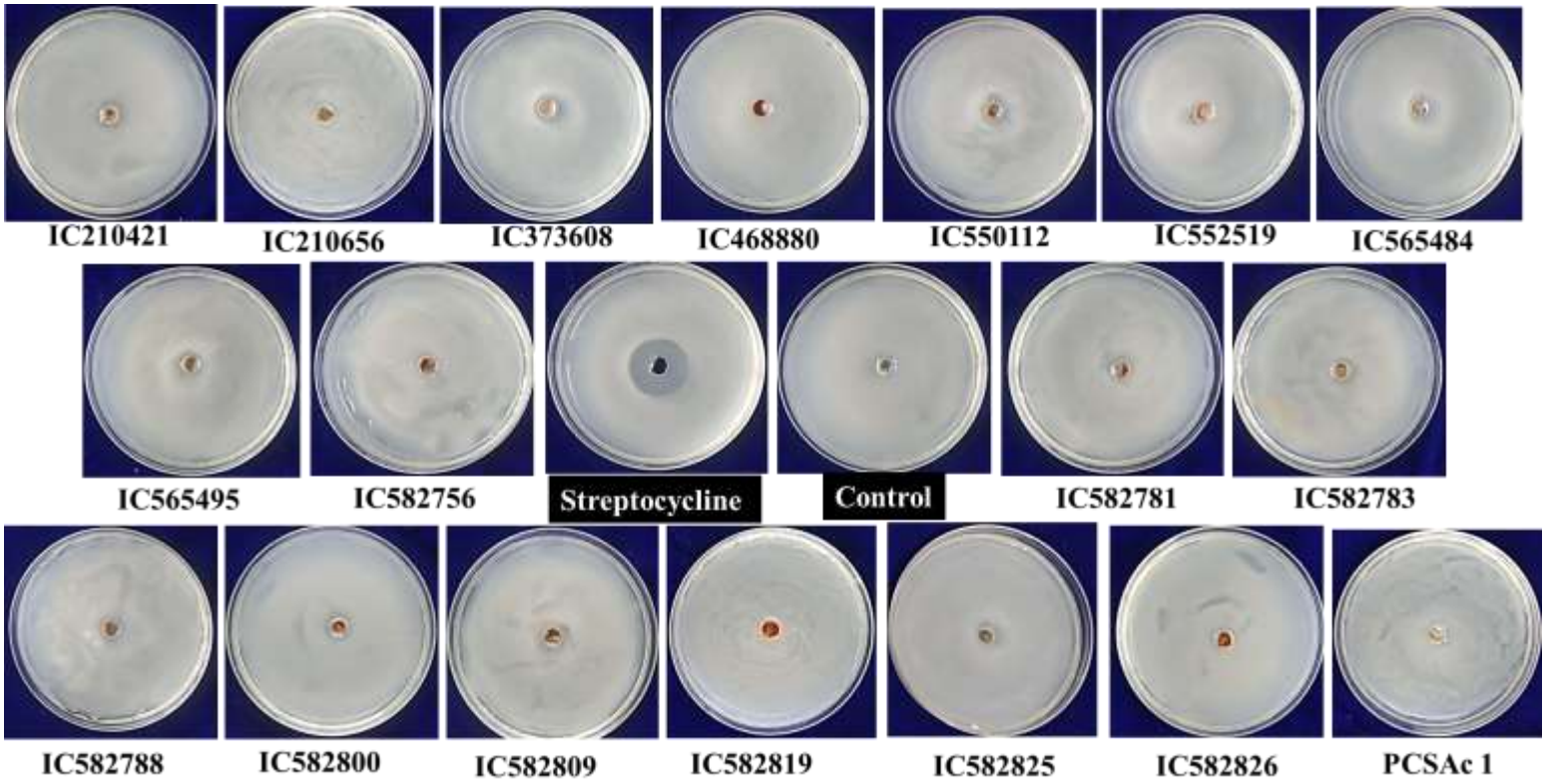


Plate 13: Zone of inhibition for *Ralstonia solanacearum* - Rhizome extract

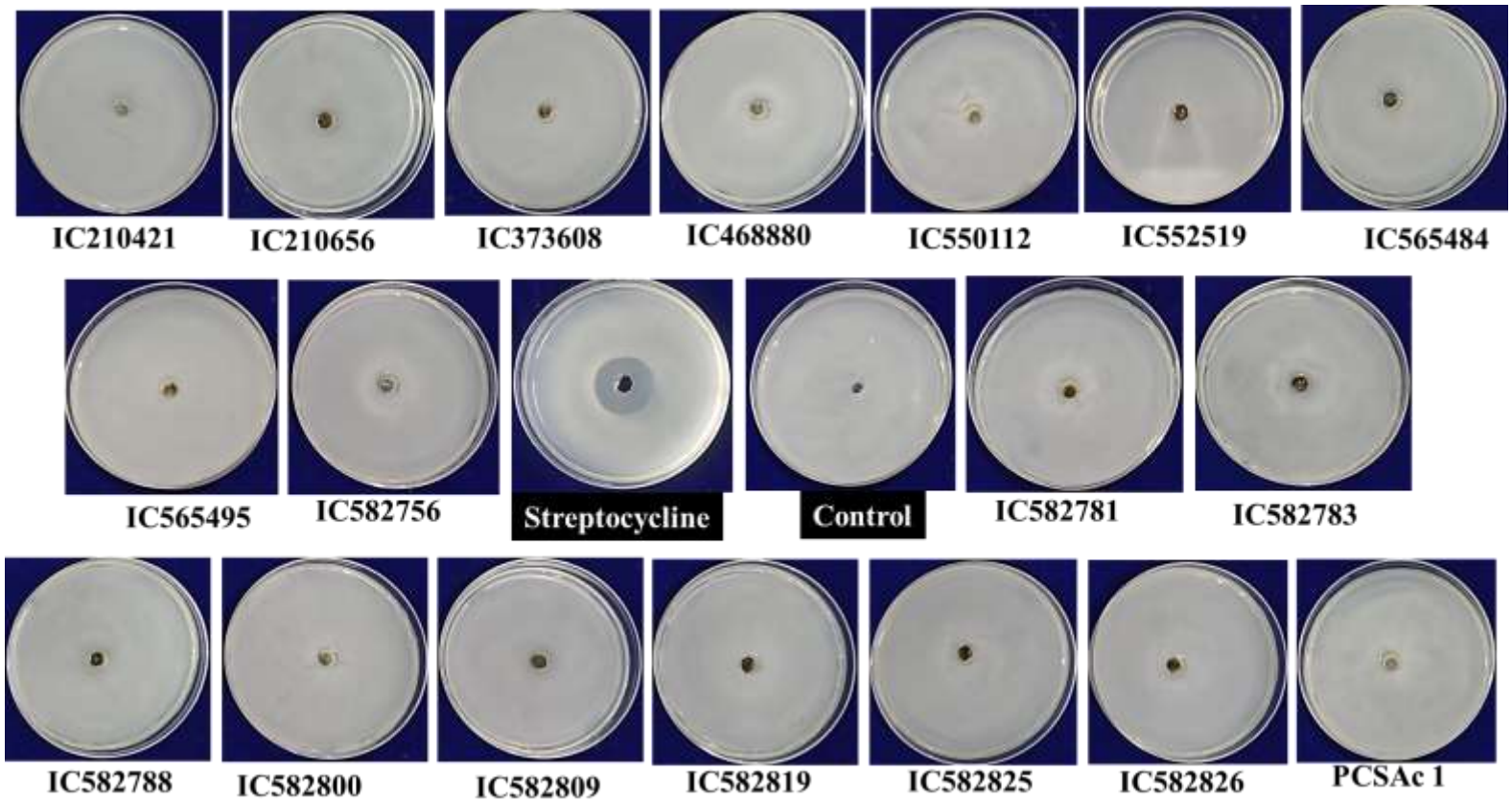


Plate 14: Zone of inhibition for *Ralstonia solanacearum* - Leaf extract

Discussion

5. DISCUSSION

The present study entitled “Variability studies in lesser galangal (*Alpinia calcarata* Roscoe)” was carried out at Department of Plantation, Spices, Medicinal and Aromatic crops, College of Agriculture, Vellanikkara and ICAR NBPGR Regional Station, Thrissur during the year 2021 to 2022. The salient findings of the study discussed in this chapter based on available literature.

5.1 Morphological variation

The eighteen accessions of *Alpinia calcarata* were evaluated in the present study.

5.1.1 Tiller characters

The results of tiller characters like tiller length and diameter, number of tillers per plant and internodal length are discussed below.

5.1.1.1 Tiller length and diameter

Significant variation was noticed in length and diameter of tillers among the genotypes (Fig. 2). The highest tiller length was observed in accession IC582825 (180.34 cm) and tiller diameter was maximum in IC582809 (3.21 cm). The present results revealed the higher vegetative growth by the genotypes IC582825, IC582800 and IC582809.

Sabu (2006) recorded the mean tiller length of 100 to 150 cm in *Alpinia calcarata* while Dissanayake *et al.* (2013) verified the mean tiller length of 170 cm. Pooja *et al.* (2020) reported mean tiller length of *Alpinia galanga* was 187.36 cm. Trimanto *et al.* (2021) observed variation in tiller length (210 to 300 cm) and tiller diameter (1.5 to 3.0 cm) in *Alpinia galanga*.

5.1.1.2 Number of tillers

Tiller production per plant among the accessions under study showed considerable variation (Fig. 3). Accession IC373608 (59 tillers) recorded highest number of tillers per

plant and the lowest in IC210421 (26 tillers). Variation in tiller number might be due to genetic makeup and environment interaction of the genotypes. Lesser galangal plant produces on an average 24 tillers per clump per year (KAU, 2016).

5.1.1.2 Internodal length

Internodal length varied from 9.84 to 11.35 cm in various accessions studied. The highest internodal length (11.35 cm) was observed in IC582788. The results of our study supported by similar previous works. Dissanayake *et al.* (2013) reported mean internodal length of 12 cm in *Alpinia calcarata*. In *Alpinia warburgi*, internodal length varied from 3.5 to 5 cm (Trimanto and Hapsari, 2021).

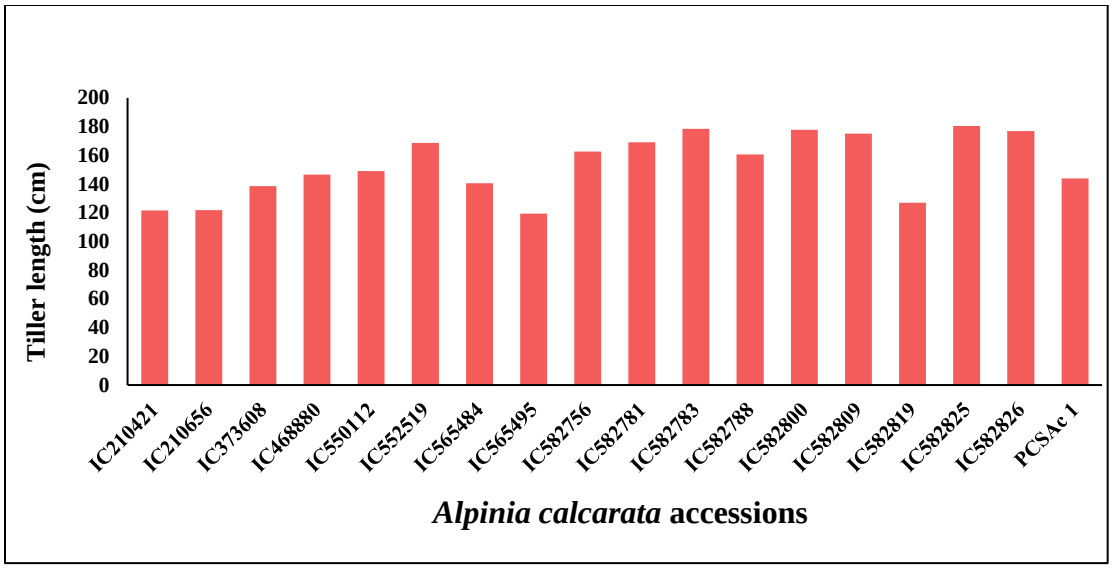


Figure 2: Variation in tiller length of *Alpinia calcarata* accessions

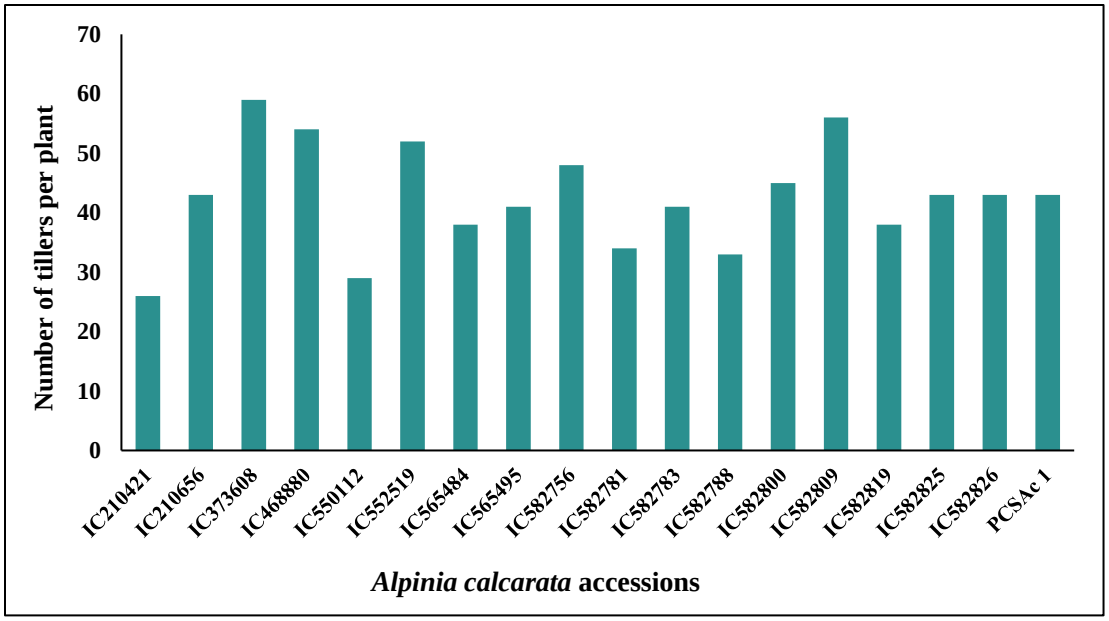


Figure 3: Variation in number of tillers per plant in *Alpinia calcarata* accessions

5.1.2 Leaf characters

Leaf characters include number of leaves per tiller, leaf length and width, leaf area and petiole length. The results of leaf characters are discussed here, based on similar available literatures.

5.1.2.1 Number of leaves per tiller

There was considerable variation among the accession under study for number of leaves per tiller and it ranged from 13.40 to 8.10 (Fig. 6). The indirect effects of plant height and the number of tillers might be the reason for the variation in number of leaves in ginger (Nybe, 1978). In present study, the highest number of leaves (13.40) and number of tillers (59) were observed in accession IC373608. Dissanayake *et al*. (2013) reported average number of leaves per tiller of 12 in *Alpinia calcarata. In Alpinia galanga,* Trimanto *et al.* (2021) observed 10 to 11 leaves per tiller. Trimanto and Hapsari (2021) observed 18 to 20 leaves per tiller in *Alpinia warburgi.*

5.1.2.2. Leaf length, width and leaf area

In the present study, the highest value of leaf length, width and leaf area was observed in accession IC582783 (55.23 cm, 6.72 cm and 259.84 cm^2 respectively) (Fig. 4 and 5). Leaf length and width of *Alpinia calcarata* were earlier reported to vary from 40 to 50 cm in length and 2.0 to 2.5 cm in breadth (Kasarkar and Kulkarni, 2011). Dissanayake *et al.* (2013) observed mean leaf length of 48.0 cm and mean width of 8.0 cm in *Alpinia calcarata*. According to Pooja *et al.* (2020), mean leaf length, width and leaf area in *Alpinia galanga* was 39.7 cm, 10.46 cm and 331.50 cm^2 respectively. Similar results in leaf area and leaf width had been reported in *A. galanga* by Trimanto *et al*. (2021). Inherent character of the genotypes might be the reason for the variability in length, width and leaf area among the accessions.

5.1.2.3 Petiole length

There was no significant difference among the accessions with respect to petiole length which ranged from 0.57 to 0.75 cm. Few reports on mean petiole length are available in *Alpinia galanga viz,* 0.63 cm (Pooja *et al.,* 2020), 0.30 cm (Trimanto *et al.,* 2021). In *Alpinia warburgi*, petiole length ranged between 1.5 to 2.0 cm (Trimanto and Hapsari, 2021).

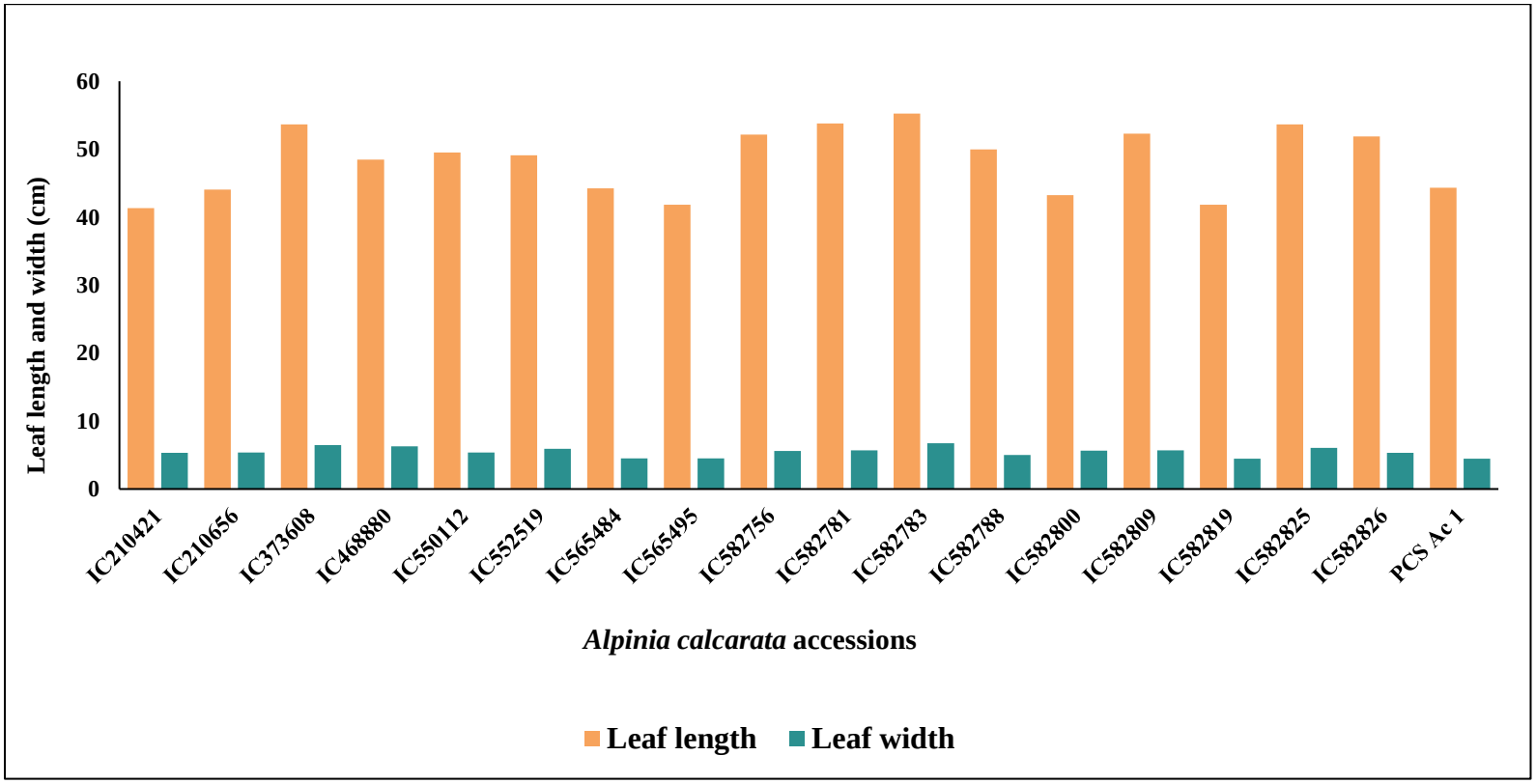


Figure 4: Variation in leaf length and width of *Alpinia calcarata* accessions

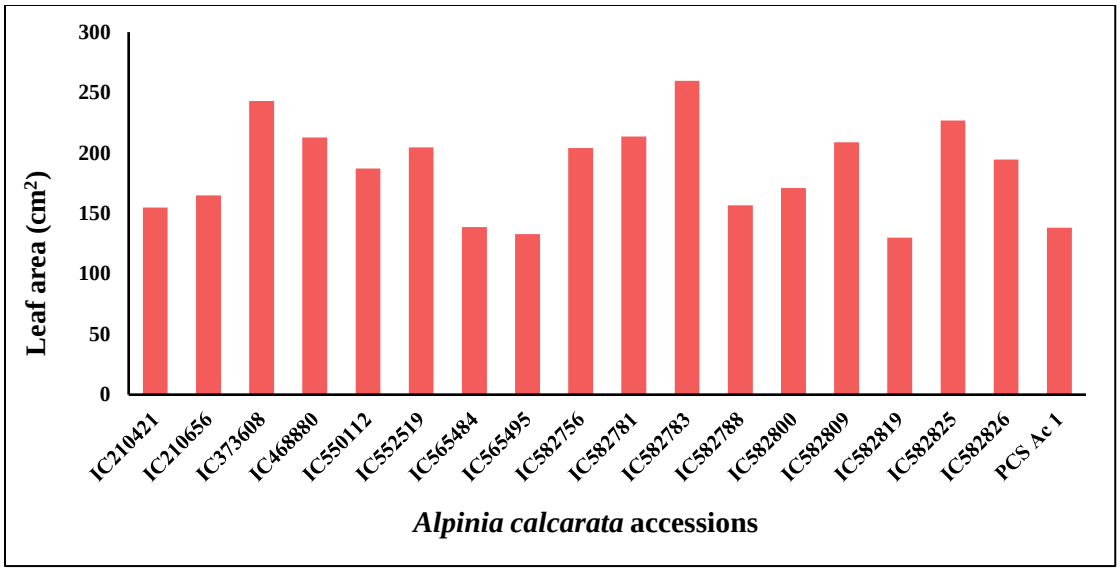


Figure 5: Variation in leaf area in *Alpinia calcarata* accessions

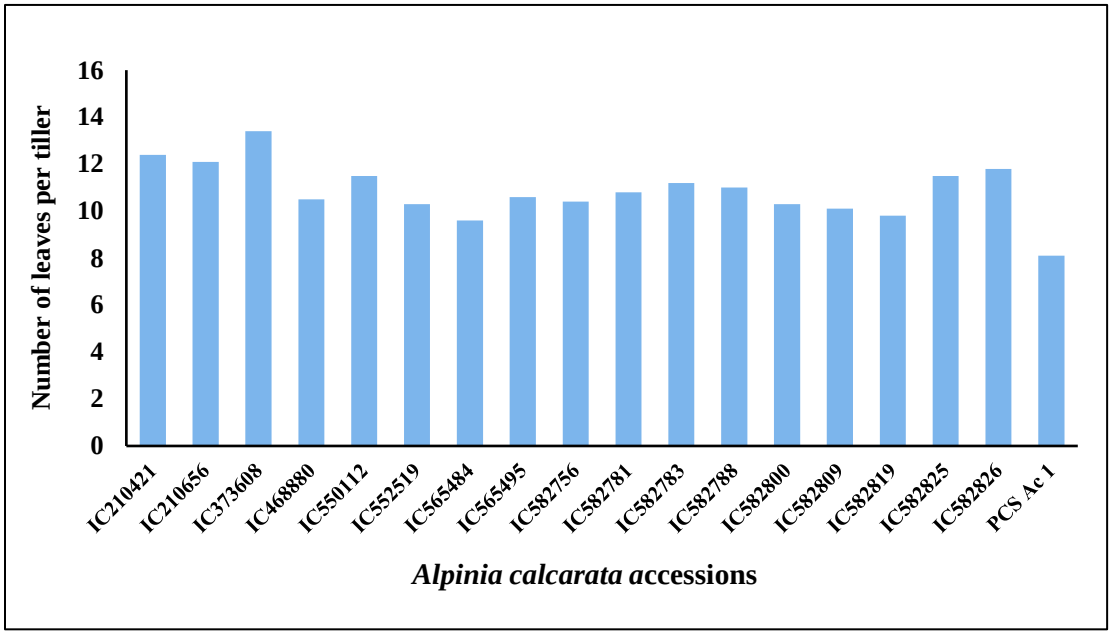


Figure 6: Variation in number of leaves per tiller in *Alpinia calcarata* accessions

5.1.3 Rhizome characters

The rhizomes were harvested when the aerial plant parts started drying and which indicated the maturity of rhizomes. The observations on rhizome characters were taken in fresh and dried rhizomes. The results of the rhizome characters are discussed here (Fig. 7).

5.1.3.1 Rhizome length, width and circumference

Since rhizomes are the main economic part of *Alpinia calcarata,* rhizome dimension has a significant impact on yield. The length, width and circumference of rhizome were differed impressively among the evaluated genotypes. Significantly higher value for dimensions of rhizome was recorded in the accession IC373608. Rhizome circumference of *Alpinia calcarata* was recorded as 5.0 cm by Dissanayake *et al.* (2013). Mathew *et al.* (2014) reported the fresh mean rhizome length of *Alpinia calcarata* as 2.0 to 6.0 cm and mean width as 2.0 cm. In *Alpinia galanga*, Trimanto *et al.* (2021) observed rhizome length of 6.0 to 7.0 cm and width of 2.0 to 4.5 cm. Trimanto and Hapsari (2021) have recorded mean rhizome diameter as 2.2 cm in *Alpinia warburgi*.

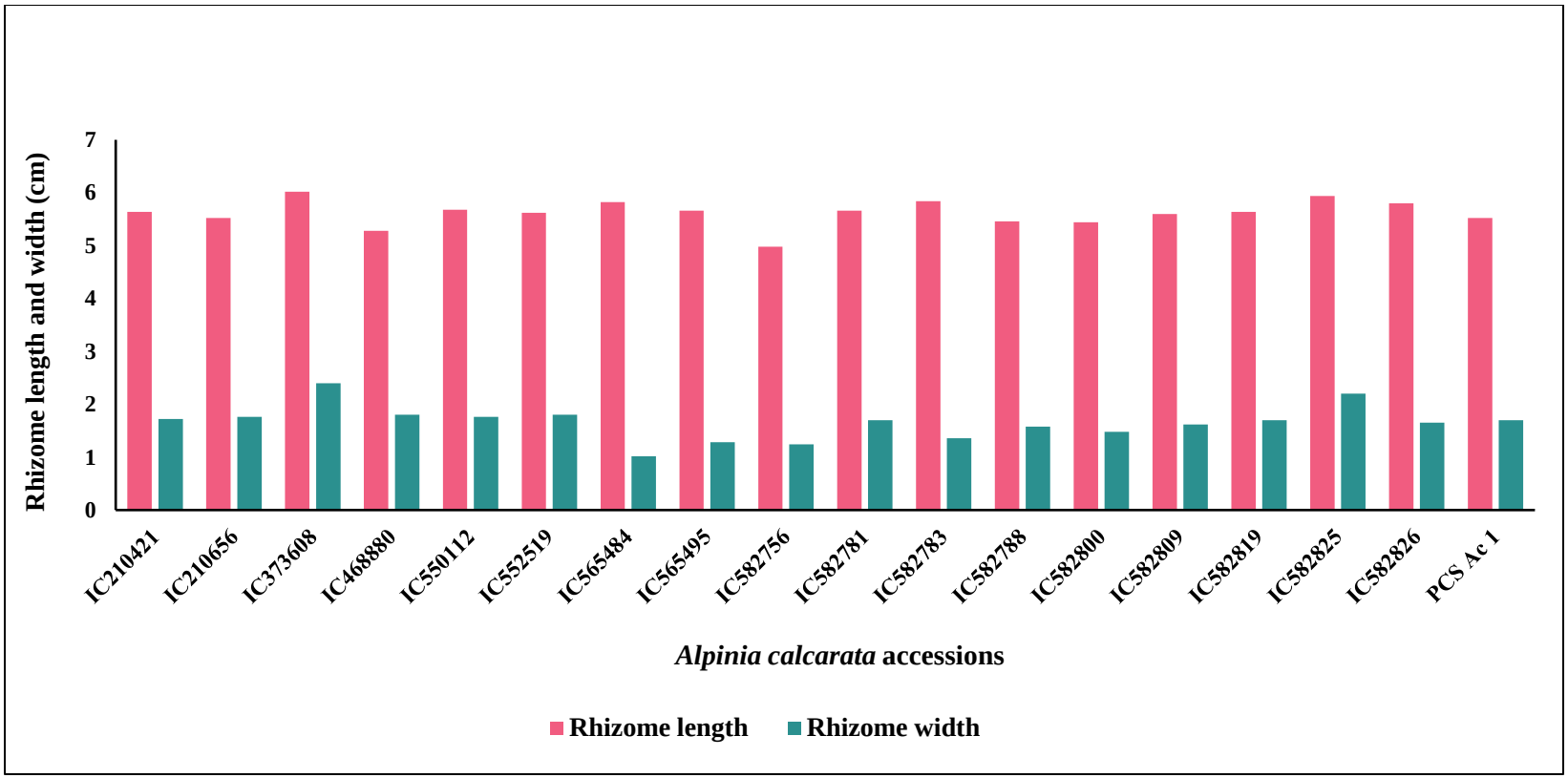


Figure 7: Variation in rhizome length and width in *Alpinia calcarata* accessions

5.1.4 Yield characters

The results of the prominent yield parameters including rhizome yield per tiller and dry recovery of fresh rhizome are discussed here.

5.1.4.1 Rhizome yield

Rhizome yield is the most important economic trait which determine the superiority of an accession. In the present study, yield of rhizome contrasted considerably among the accessions (Fig. 8). The highest yield of rhizome was obtained in accession IC373608 (14.63 g per tiller). The accession IC373608 had higher number of leaves per tiller and higher leaf area which might have resulted in higher photosynthesis and contributed to higher fresh weight of rhizome. Ponmozhi and Kalaiselvi (2011) reported the mean rhizome weight of *Alpinia calcarata* and *Alpinia officinarum* as 24.54 and 28.87 g per tiller respectively. The average rhizome yield of *Alpinia calcarata* as 23 tonnes per hectare (KAU, 2016). In *Alpinia galanga,* Pooja *et al.* (2020) recorded mean rhizome yield of 373.43 g per tiller.

5.1.4.2 Dry recovery

The dry recovery of rhizomes also showed significant variation among the accessions and it ranged from 19.82 to 33.26 per cent (Fig. 9). The accession IC373608 showed the highest dry recovery. The dry recovery of 27.98 per cent was observed in *Alpinia calcarata* by Joy *et al.* (2005). The dry recovery of *Alpinia calcarata* rhizome is 25 per cent as reported by KAU (2016).

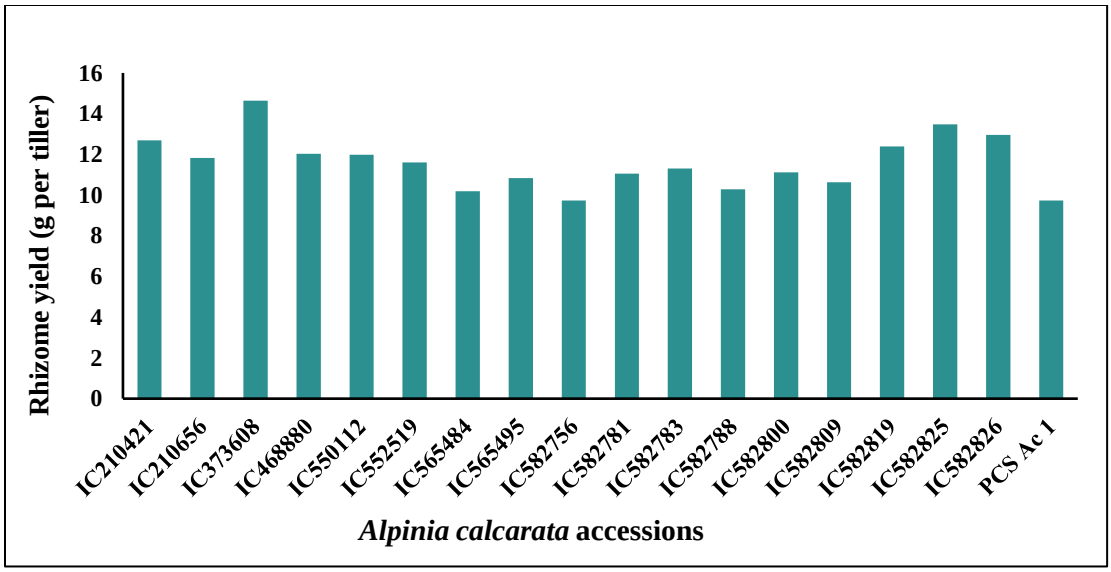


Figure 8: Variation in rhizome yield in *Alpinia calcarata* accessions

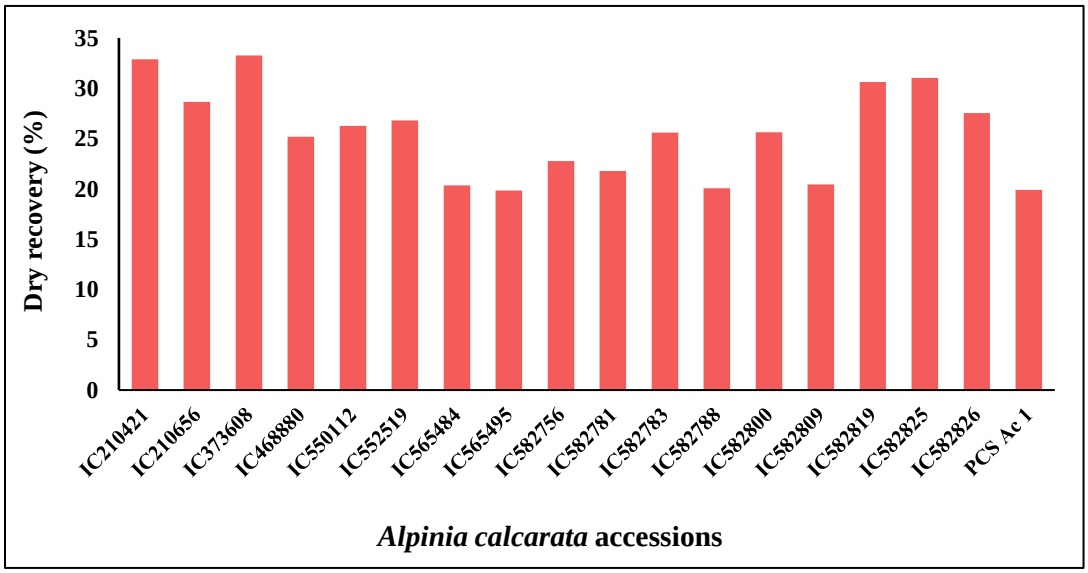


Figure 9: Variation in dry recovery of rhizomes in *Alpinia calcarata* accessions

5.1.5 Correlation of rhizome yield with other morphological characters in *Alpinia calcarata.*

Correlation studies had shown that rhizome yield is positively and significantly correlated with rhizome length, rhizome width, rhizome circumference, dry recovery percentage, number of leaves per tiller and also showed a positive relation with leaf area and tiller diameter. This finding suggested that rhizome yield per tiller can be enhanced by improving these traits. A negative correlation was obtained between yield and tiller length. The higher values of rhizome dimension contributed to higher fresh weight of rhizome. The maximum number of leaves per tiller and leaf area might have resulted in higher photosynthesis and contributed to the enhancement of rhizome yield. Similar results were obtained by Sasikumar *et al.* (1992) in ginger in which, rhizome yield is positively and significantly correlated with number of leaves per tiller, leaf length and width. Islam *et al.* (2008) also studied the correlation between rhizome yield and other quantitative plant morphological traits in ginger. According to them, rhizome yield per plant showed a significant and positive correlation with leaf length, leaves per tiller, number of primary, secondary and tertiary fingers per rhizome. According to Ravishanker *et al.* (2013), fresh yield of ginger per plant positively correlated with number of primary and secondary fingers, the length and diameter of primary fingers and dry matter content. Kumar *et al.* (2016) also found that rhizome yield of ginger exhibited a significant positive correlation with rhizome length, rhizome width, primary rhizome length and leaf width.

5.1.6 Inflorescence characters

4.1.6.1 Season of flowering

In the present study, fourteen accessions out of eighteen flowered from April to May. However, some of the genotypes had two flowering periods per year (April to May and November to December). Sabu (2006) reported the flowering time of *Alpinia calcarata* as May. Kasarkar and Kulkarni (2011) found two flowering seasons for *Alpinia calcarata* during October and April. Trimanto and Hapsari (2021) observed the flowering of *Alpinia*

warburgi from May to June.

4.1.6.2 Duration of flowering

Duration of flowering varied greatly among accessions studied and ranged from 16.50 to 22.50 days (Fig. 10). Accession IC210421 had the maximum duration of flowering. In *Alpinia blepharocalyx,* Zhang *et al.* (2003) reported that duration of flowering lasted about one month from late March to late April. Kasarkar and Kulkarni (2011) observed that nearly about 40 days are required to complete the development of inflorescence in *Alpinia calcarata.*

4.1.6.3 Inflorescence length

Among the accessions under study, the inflorescence length varied significantly and highest inflorescence length observed in IC373608 (16.10 cm) (Fig. 11). In *Alpinia calcarata,* inflorescence length was 10.0 to 15.0 cm (Sabu, 2006; Dissanayake *et al.,* 2013). Pooja *et al.* (2020) reported mean inflorescence length of 25.0 cm in *Alpinia galanga* while Trimanto *et al.* (2021) observed inflorescence length of 10.0 to 30.0 cm. The mean inflorescence length of *Alpinia warburgi* was recorded as 15.0 cm by Trimanto and Hapsari (2021).

5.1.7 Flower characters

5.1.7.1 Number of branches and flowers per inflorescences

In the present study, there was high variation with respect to number of branches and flowers per inflorescence (Fig. 12). Branches per inflorescence ranged from 13.10 to 16.20 and number of flowers per inflorescence ranged from 23.50 to 30.40. The highest number of flowers per inflorescence was observed in IC468880 (Fig. 8). Kasarkar and Kulkarni (2011) recorded 20 to 30 flowers per inflorescence in *Alpinia calcarata*. In case of A*lpinia warburgi,* 20 to 40 flowers were witnessed per inflorescence by Trimanto and Hapsari (2021).

5.1.7.2 Flower length and width

The length of flowers varied significantly among the accessions under investigation and it ranged from 2.80 to 3.60 cm. However, there was no significant difference with respect to flower width. The highest mean value of flower length was recorded in IC550112 (3.6 cm) and flower width in IC210421 (2.50 cm). According to Sabu (2006), *Alpinia calcarata* had flowers of 3.0 cm long and 1.5 to 1.8 cm wide. Dissanayake *et al.* (2013) also recorded the mean length of *Alpinia calcarata* flower as 5.6 cm. In *Alpinia galanga*, Trimanto *et al.* (2021) observed flower length of 2.5 to 4.0 cm. The mean flower length and width of *Alpinia warburgi* was 3.5 cm and 1.6 cm respectively (Trimanto and Hapsari, 2021).

5.1.7.3 Pollen studies

For pollen fertility studies, collected pollen grains were tested with acetocarmine (2%) and it was observed that all pollen grains stained with red. This result confirmed its viability. However, the pollen tube failed to germinate *in vitro* pollen germination test using nutrient medium. The findings of the study were supported by Adaniya and Shoda (1998) who reported 20.9 per cent pollen fertility and a very low germination rate (0.00 to 0.22 per cent) in ginger. Das *et al*. (1999) conducted pollen studies in ginger and found that very few pollen were fertile but large numbers were non-fertile and pollen grains failed to germinate in 2 to 15 per cent sucrose solution due to the absence of germinal apertures.

5.1.7.4 Fruit set

Among the eighteen accession, there was no fruit and seed set were observed at the end of flowering period. The failure of the development of the embryo-sac, and also pollen degenerations were the reason for no seed set in *Alpinia calcarata* (Raghavan and Venkatasubban, 1943). Kasarkar and Kulkarni (2011) verified that *Alpinia calcarata* failed to develop seeds and fruits.

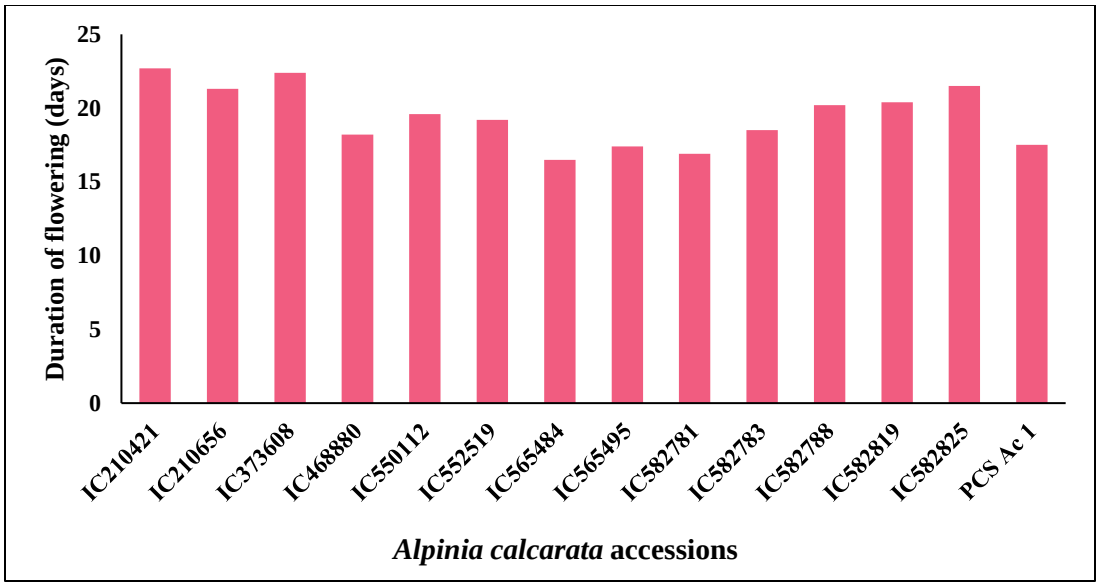


Figure 10: Variation in duration of flowering in *Alpinia calcarata* accessions

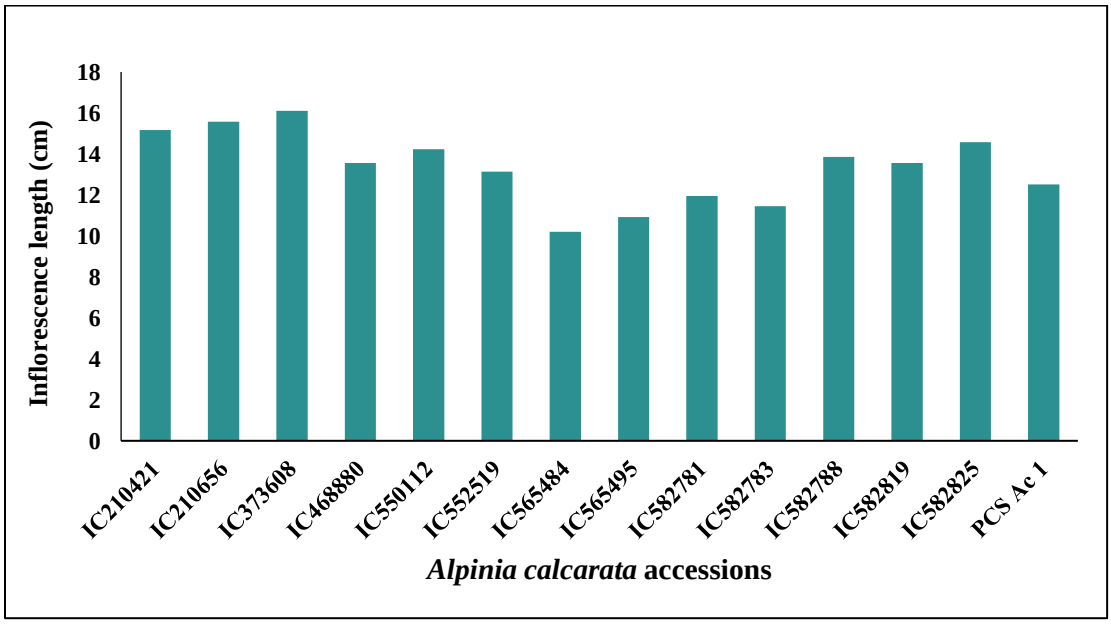


Figure 11: Variation in length of inflorescence in *Alpinia calcarata* accessions

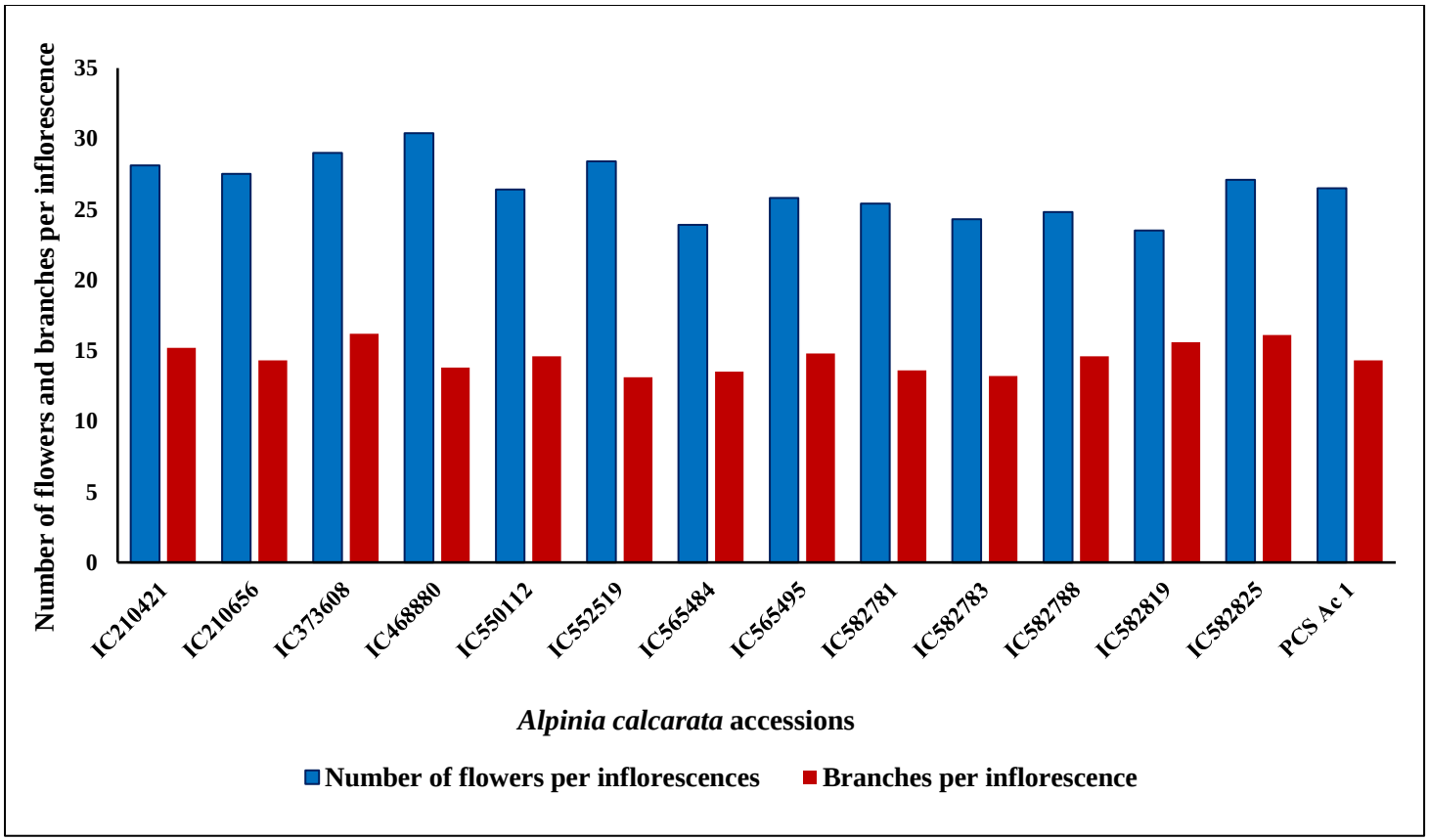


Figure 12: Variation in number of flowers and branches per inflorescence in *Alpinia calcarata* accessions

5.1.8 Floral phenology

Phenological investigations are important for a better understanding of ecological adaptations, interactions between different species, and germplasm conservation (Stern and Roche, 1974; Waser, 1979). These observations are of great use in plant multiplication and crop improvement programmes. In the present study, we identified and described the phenological stages of the *Alpinia calcarata* inflorescence from emergence to end of flowering. The morphological changes of the *A. calcarata* inflorescence was described in this study using the extended Biologische Bundesanstalt, Bundessortenamt, and Chemical industry (BBCH) scale (Meier, 2001), which has 10 principal growth stages, numbered from 0 to 9.

The reproductive development stage of lesser galangal includes two principal growth stages inflorescence emergence and flowering. The time period to complete each principal growth stage was also recorded. The principal growth stage 5- the inflorescence emergence lasted for 7 to 10 days and the principal growth stage 6- flowering extended for 10 to 15 days. In general, it required about 20 to 25 days to complete its reproductive stage. The flowers failed to develop into fruit and seed. The findings of this study provided a clear image of inflorescence development. Many researchers had discussed floral phenological studies in plants coming under the genus *Zingiber*, *Alpinia* (Kasarkar and Kulkarni, 2011) and in torch ginger (Choon *et al.,* 2016).

5.1.10 Qualitative morphological parameters

Significant variations were not observed in qualitative morphological characters *viz* leaf shape, flower colour, rhizome shape and colour. The leaves were linear lanceolate in shape and dark green colour. Flowers were creamy yellow colour with red streak and no seed set for the accessions under investigation. The fleshy rhizome was cylindrical shaped with pale yellowish outer skin and moderate yellowish inner core.

Sabu (2006) stated that in *A. calcarata* accessions leaves were sessile, linear lanceolate, narrowed towards the base and tip acuminate. Flowers were shortly pedicellate and variegated with dark purple and yellow colour. Mathew e*t al.* (2014) observed *Alpinia*

calcarata rhizomes with cylindrical shape and creamy yellow colour. Leaves were linear, glabrous with long acuminate apex. Namdeo and Kale (2015) described *Alpinia galanga* rhizome as cylindrical in shape, reddish brown colour externally and orange yellow colour internally. Trimanto *et al.* (2021) also reported cylindrical rhizome of *Alpinia galanga* having reddish brown outer skin colour and white inner core colour. Leaves were lanceolate, oblong and flowers were yellow white in colour.

5.2 Quality characters of rhizome

The results of biochemical characters like essential oil, oleoresin, phenols, flavonoids, starch, crude fibre and total terpenoids are discussed below based on available literature.

5.2.1 Essential oil

The essential oil of *Alpinia calcarata* has commercial importance which finds application in preparation of perfumes, room sprays, pharmaceuticals and cosmetic products. In the present study, essential oil recovery from dried rhizome ranged from 0.30 to 0.75 per cent and IC468880 showed the highest essential oil content of (0.75%). The lowest recorded oil content of 0.30 per cent was in IC565495 (Fig. 13). Raina and Abraham (2015) recorded the volatile oil content of 0.29 to 0.96 per cent from the dried rhizome of *Alpinia calcarata* and the highest volatile oil content was obtained in accession IC373610 (0.96%) followed by IC468880 (0.74%) and lowest in IC565495 (0.29%). It has been reported that the volatile oil content in dry rhizome was 0.93 per cent (Nampoothiri *et al.*, 2016).

5.2.2 Oleoresin

The rhizome oleoresin was extracted using Soxhlet apparatus which varied from 20.20 to 34.20 per cent (Fig. 14) and highest oleoresin content recorded in IC373608 (34.20%). Arambewela and Arawwawala (2010) evaluated the oleoresin content of *Alpinia calcarata* using hot water and ethanol as solvent and obtained oleoresin content of 18.5 and 15.6 per cent on dry weight basis respectively. Raj *et al.* (2011) reported that oleoresin content of 19.5 per cent from the dried rhizome of *Alpinia calcarata*.

5.2.3 Total phenols

Phenols are powerful antioxidants that will protect biomolecules from oxidative damage, which is linked to chronic diseases. Total phenols in rhizome varied significantly among accessions and a maximum value of 115.25 mg GAE.g^{-1} was recorded in the accession IC373608 (Fig. 15). The result of the present study was found to be in line with

similar previous works.

Ramya *et al.* (2015) reported a total phenolic content in ethanolic extract of *Alpinia calcarata* to be 100.7 ± 0.36 mg GAE.g^{-1}. According to Jisha *et al.* (2021), acetone extract of lesser galangal rhizome contained the highest phenol concentration (124.6 ± 0.73 mg GAE.g^{-1}) followed by methanolic extract (115.95 ± 1.26 mg GAE.g^{-1}) and the lowest in aqueous extract (25.2 ± 2.03 mg GAE.g^{-1}).

5.2.4 Total flavonoids

The total flavonoids ranged from 30.30 to 55.65 mg QE.g^{-1} (Fig. 16). Ramya *et al.* (2015) revealed that ethanolic extract of *Alpinia calcarata* rhizome yielded flavonoids content of 24.2 ± 0.40 mg QE.g^{-1} while Singh *et al*. (2020) reported total flavonoid content of 36.92±0.24 mg QE.g^{-1} of rhizome extract. According to Jisha *et al.* (2021), petroleum ether extract of rhizome contained more flavonoid content (29.17 ± 1.39 mg of rutin.g^{-1}) when compared to aqueous extract (15.30 ± 0.93 mg of rutin.g^{-1}).

5.2.5 Starch

The starch content varied considerably among the accessions under study. It ranged between 27.65 mg.g^{-1} (IC582756) and 44.05 mg.g^{-1} (IC210656) (Fig. 17). Ponmozhi and Kalaiselvi (2011) reported that the starch content in the rhizome of *Alpinia calcarata* was 38.2 ± 0.65 mg.g^{-1} and in the case of *Alpinia galanga*, it was 93.1 ± 0.11 mg.g^{-1}. According to Wijayasiriwardena and Premakumara (2012), *Alpinia calcarata* powder consisted of plenty of simple and compound starch grains and most of them were round and oval shaped but some of them were muller shaped, some were triangular and pear shaped. The total starch of *Curcuma* species varied from 45.24±0.25 to 48.48±0.3 per cent (Sajitha and Sasikumar, 2015).

5.2.6 Crude fibre

The eighteen accessions showed a significant difference in crude fibre and it ranged from 7.90 to 14.25 per cent. However, lowest crude fibre is an appreciable character and

IC550112 (7.90%) registered the lowest crude fibre content (Fig. 18). The results of the present study were in line with the reports of similar previous works.

Indrayan *et al.* (2009) conducted a study on the nutritive value of rhizomes of certain ginger like species, *viz. Alpinia officinarum, A. galanga, A. calcarata, A. zerumbet* and *Kaempferia galanga* and found that the lowest crude fibre percentage was in *A. calcarata* (7.25%). Nohir *et al.* (2019) reported the crude fibre percentage in dried rhizome of *A. galanga* was 9.86 ± 0.29 $g.100g^{-1}$ on dry weight basis, while Afra and Ghannam (2022) recorded crude fibre percentage (14.00%) of rhizome on dry weight basis in *Alpinia galanga*.

5.2.7 Total terpenoids

In the present study, the total terpenoids ranged from 6.70 to 20.25 per cent in the rhizomes. The total terpenoid varied greatly among the eighteen accessions, and the highest terpenoid content showed in IC210421 (Fig. 19). The presence of flavonoids, triterpenoids and reducing sugars was reported in the ethanolic extract of *Alpinia calcarata* rhizomes (Raj *et al.*, 2011). Singh *et al.* (2015) determined total terpenoid content of 5.89 per cent from the dried rhizome of *Curcuma amada*. Datta *et al.* (2018) reported the total terpenoids in turmeric was 12.36 ± 1.6 $mg.g^{-1}$. Mathew and Victoria, (2020) stated that rhizomes of *Alpinia calcarata* were very rich in terpenoids, sterols, flavonoids, etc.

5.2.8 GCMSMS profile of *Alpinia calcarata* essential oil

A total of twenty eight compounds were identified in the rhizome essential oil of *A. calcarata* accession IC373608 (Fig. 20). Eucalyptol (1,8-cineole) (19.17%) was the major compound identified followed by ethyl iso-allocholate (15.06%). The findings of the present study were in line with similar previous works.

Tewari *et al.* (1999) recorded thirty one constituents in rhizome oil of *A. calcarata* and l,8-cineole (41.4%) was the major constituent. Similar study was conducted by Arambewela *et al.* (2005) who reported eighteen compounds in rhizome oil of *A. calcarata* and major compound was 1,8-cineole (24.70%). Twenty three components were identified

in the essential oil of *Alpinia calcarata* rhizomes and the main constituents were eucalyptol (14.05%), L-camphor (7.90%) and borneol (5.67%) (Rahman *et al.*, 2012).Raina and Abraham (2015) identified main constituents of rhizome oil of *A. calcarata* germplasm from south India as that 1,8-cineole, α-fenchyl acetate, α-terpineol, camphor, terpinen-4-ol and borneol. Nampoothiri *et al*. (2016) determined fifty constituents from *A. calcarata* rhizome oil and main components were cubenol (15.0%), 1,8-cineole (12.1%), and α- and β-fenchyl acetates (12.9 and 9.7%, respectively).

5.2.9 Correlation of rhizome yield with quality characters of rhizome of *Alpinia calcarata*

In the present study, correlation studies showed that rhizome yield of lesser galangal had significant positive correlation with essential oil, total phenols, total flavonoids, total terpenoids and starch and positive relation with oleoresin. But the rhizome yield was negatively correlated with crude fibre. The essential oil possessed a significant positive correlation with total phenols, total flavonoids, starch and total terpenoids and a positive correlation with oleoresin. The essential oil negatively correlated with the crude fibre. The oleoresin exhibited a positive correlation with total phenols, flavonoids, starch, crude fibre and total terpenoids. The total phenols indicated a significant positive correlation with total flavonoids, starch and total terpenoids and a negative correlation with crude fibre. The total flavonoids positively correlated with starch and total terpenoids and negatively correlated with crude fibre. Starch had a significant positive correlation with total terpenoids and a negative correlation with crude fibre. The crude fibre and total terpenoids were found to be negatively correlated. A good quality rhizome is one that contains less crude fibre. These findings are in line with previous reports. Chandra and Govind (1999) reported a negative correlation with rhizome yield and crude fibre in ginger. Sanwal *et al.* (2012) revealed that the yield of ginger is positively correlated with starch. Das *et al.* (2022) found a positive correlation between yield and biochemical parameters such as essential oil and oleoresin in ginger. They also opined that essential oil was highly correlated with oleoresin but negatively correlated with crude fibre.

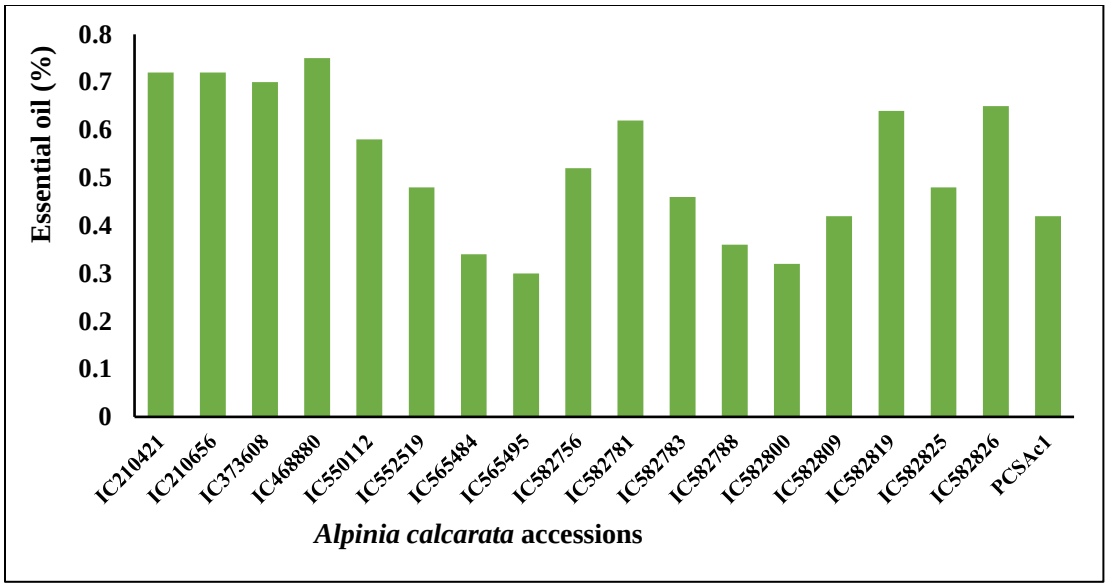


Figure 13: Variation in essential oil in *Alpinia calcarata* accessions

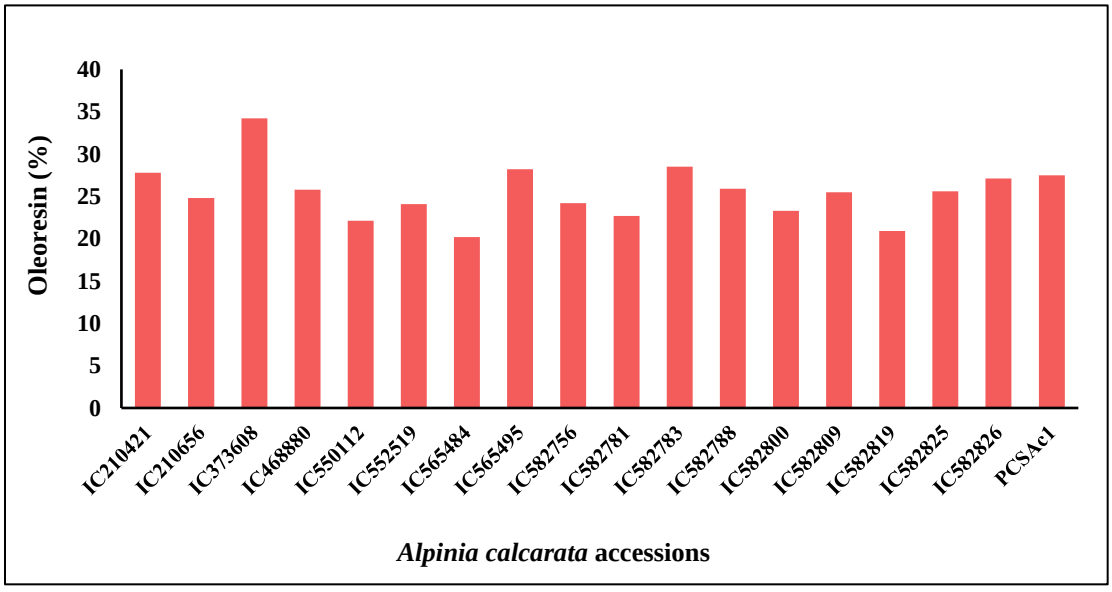


Figure 14: Variation in oleoresin content in *Alpinia calcarata* accessions

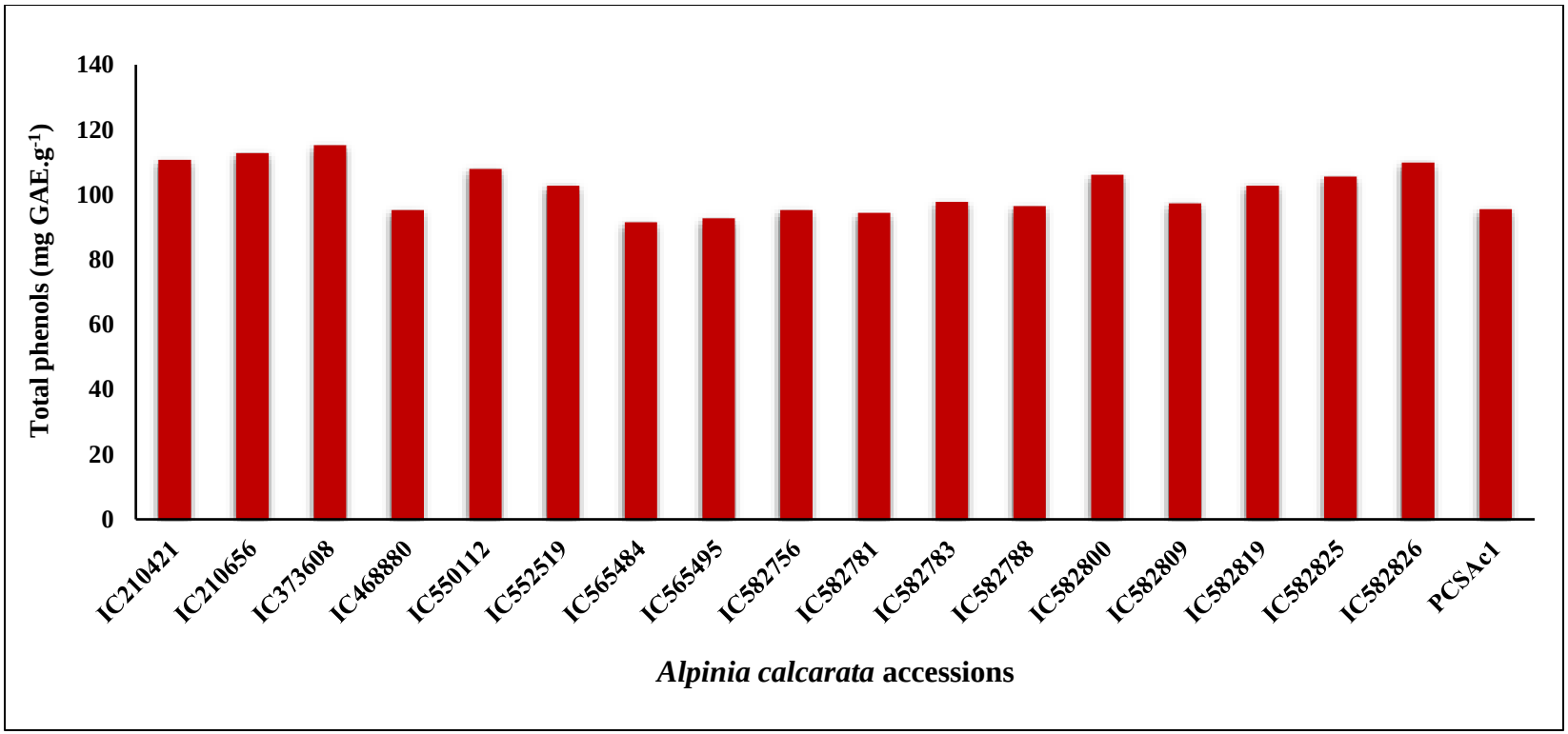


Figure 15: Variation in total phenols in *Alpinia calcarata* accessions

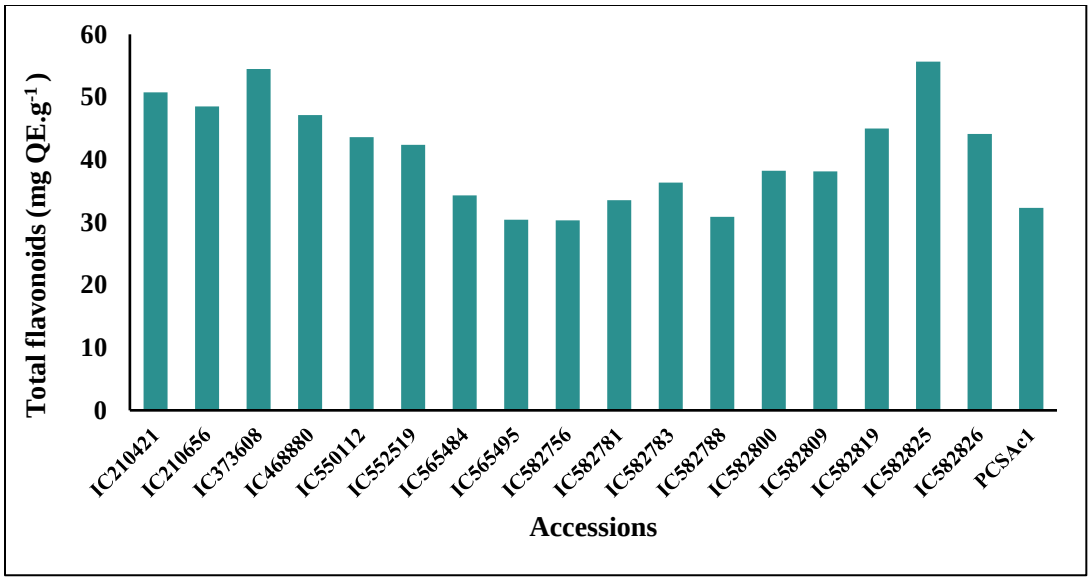


Figure 16: Variation in total flavonoids in *Alpinia calcarata* accessions

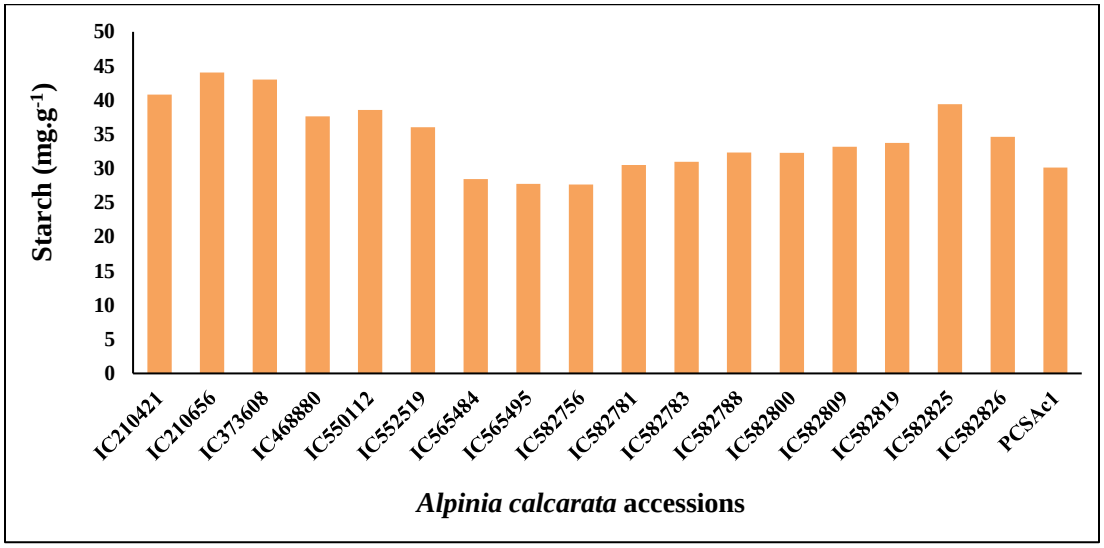


Figure 17: Variation in starch content in *Alpinia calcarata* accessions

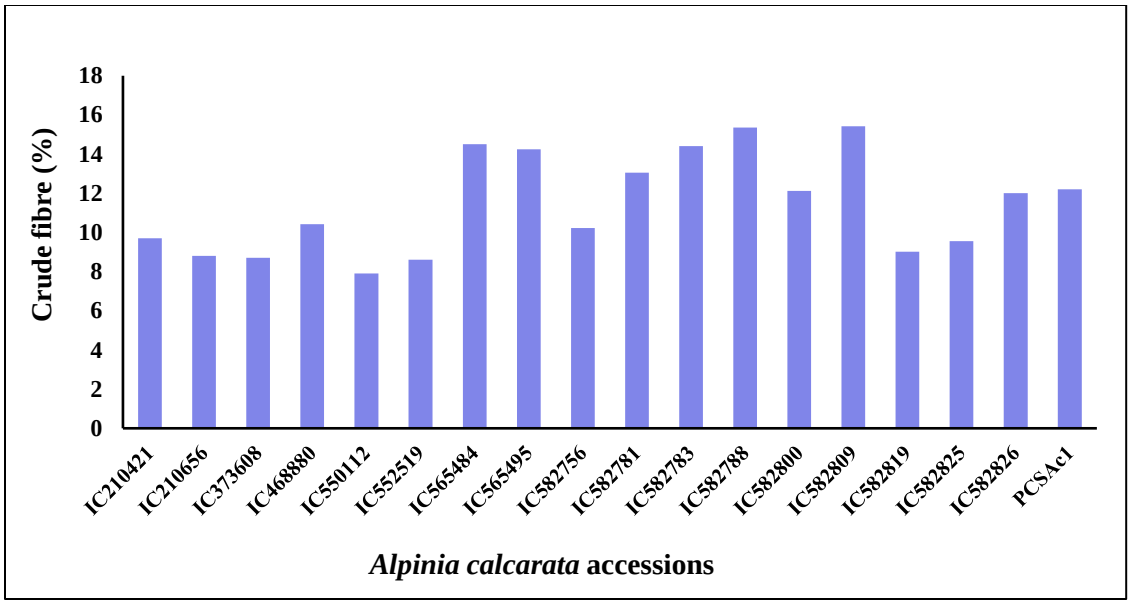


Figure 18: Variation in crude fibre content in *Alpinia calcarata* accessions

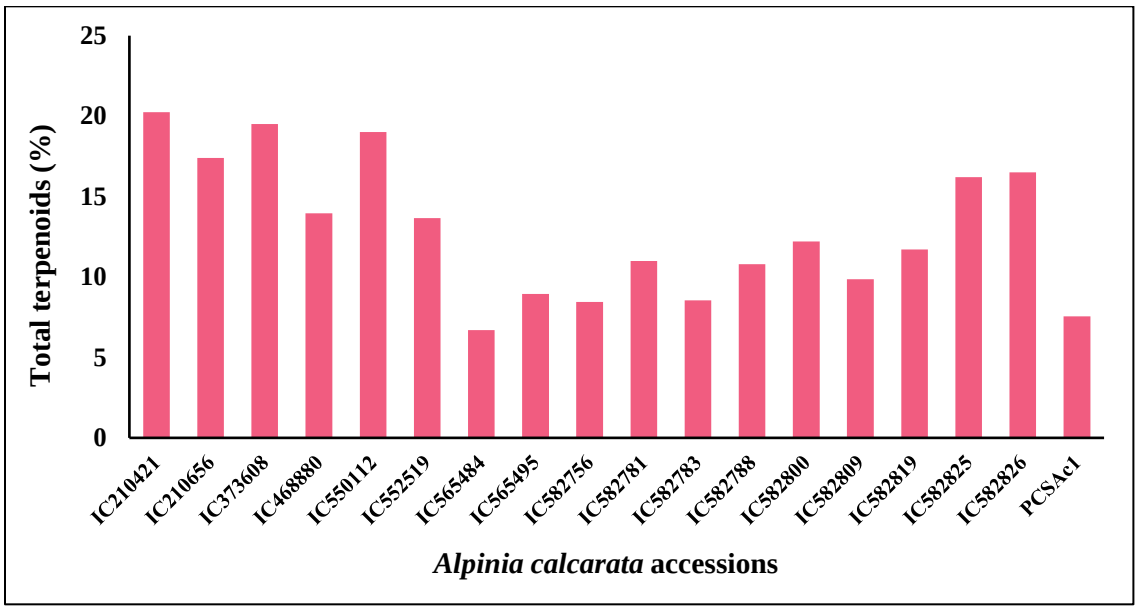


Figure 19: Variation in total terpenoids in *Alpinia calcarata* accessions

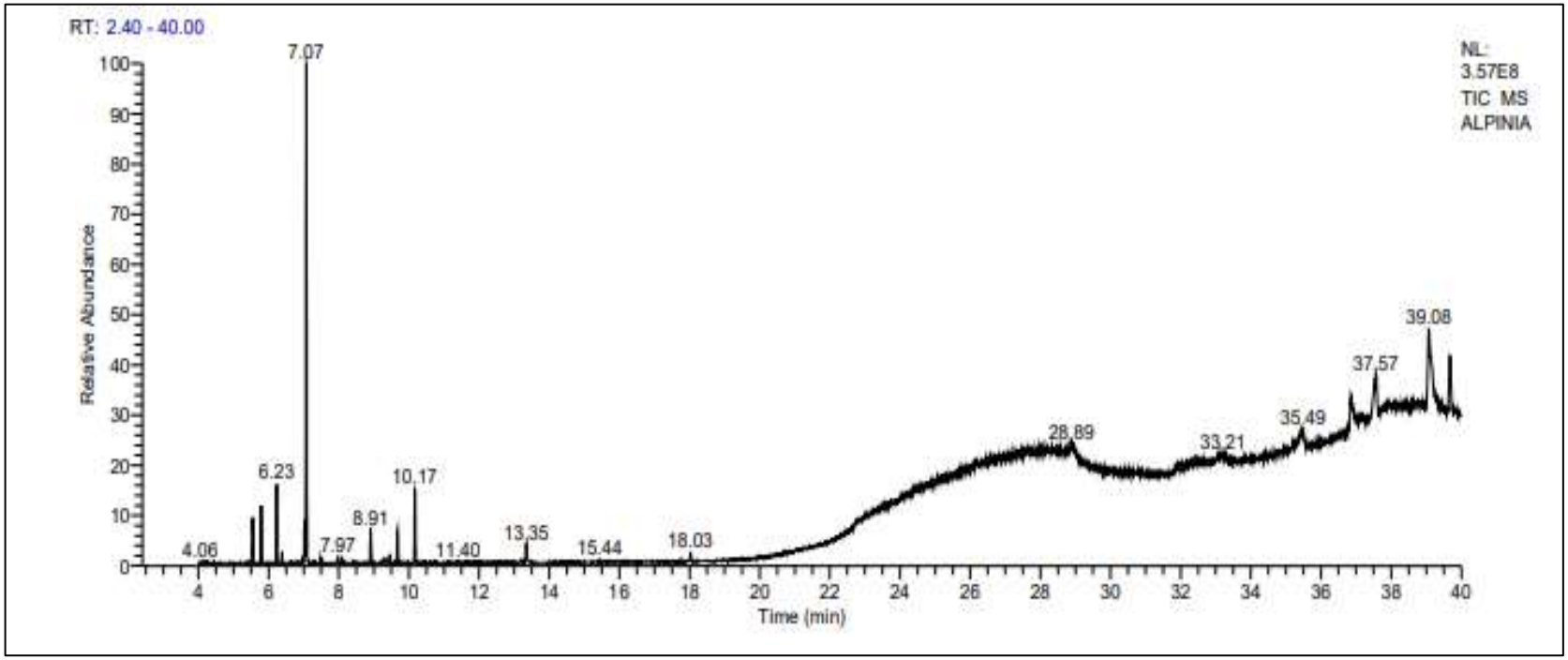


Figure 20: Chromatogram of GCMSMS profile of *A. calcarata* essential oil

5.3 Antimicrobial properties

Lesser galangal *(Alpinia calcarata*) is an under-exploited medicinal crop of India that is known for its antimicrobial activity (George and Pandalai, 1949; Robinson *et al.*, 2009). The phytochemical investigations on rhizomes of *Alpinia calcarata* revealed the presence of phenols, flavonoids, alkaloids, terpenoids, tannins, saponins, proteins and carbohydrates (Prabhu *et al.*, 2012). Due to the presence of these phytochemicals, the rhizome extract of *Alpinia calcarata* exhibited antimicrobial properties (Mathew and Victorio, 2020).

Rhizome and leaf extracts of *Alpinia calcarata* accessions were tested against five plant pathogens *viz., Rhizoctonia* spp., *Colletotrichum* spp., *Phytophthora* spp., *Fusarium* spp. and *Ralstonia solanacearum.*

5.3.1 Antifungal property

The anti-fungal property of methanolic extract of rhizome and leaf of *Alpinia calcarata* were tested against four phytopathogenic fungi *viz, Rhizoctonia* spp., *Colletotrichum* spp., *Fusarium* spp. and *Phytophthora* spp. using poisoned food technique. Similar studies were conducted by George (2014) who evaluated essential oil of *A. calcarata* for antifungal activity against seven fungi. The maximum inhibitory zones of 10 mm and 7 mm exhibited against *Aspergillus aculeatus* and *A. awomori,* respectively.

Arambewela *et al.* (2010) reported the anti-microbial property of essential oil of *Alpinia calcarata* against plant pathogens *Curvularia* spp. and *Colletorichum* spp. and found that it had a strong anti-fungal effect even sometimes better than the reference fungicide, Daconil.

5.3.1.1 Percentage of inhibition for *Rhizoctonia* spp.

The methanolic extract of rhizome and leaf of *Alpinia calcarata* exhibited significant anti-fungal properties on *Rhizoctonia* spp. The rhizome extract of accessions IC210421 (63.50%) and leaf extract of IC210656 (64.60%) recorded maximum percentage of inhibition for *Rhizoctonia* spp. (Fig. 21). The results obtained are in line with Prasad *et al.*

(2016) where they revealed that the essential oils isolated from roots and rhizomes of three plants belonging to family Zingiberaceae *viz., Alpinia calcarata, A. galanga*, *Kaempferia galanga* were found effective against *Rhizoctonia solani.* Choudhury *et al.* (2017) also revealed that the rhizome extract of ginger (*Zingiber officinale*) were found active against the plant pathogen *Rhizoctonia solani*. Timsina *et al*. (2022) demonstrated the antifungal activity of essential oil of ginger against *Rhizoctonia solani* in the 200 to 1000 ppm concentration range.

5.3.1.2 Percentage of inhibition for *Colletotrichum* spp.

The percentage inhibition of mycelial growth of *Colletotrichum* varied considerably with methanolic extract of rhizomes and leaves of *Alpinia calcarata* (Fig. 22). The rhizome extract of all the eighteen accessions showed per cent inhibition ranged between 40.90 to 25.40 per cent and leaf extracts ranged from 38.70 to 23.70 per cent. This result was consistent with Arambewela *et al.* (2010) who reported to e phytopathogen, *Colletorichum* spp. with percentage growth inhibition of 38.67 at 1000 ppm concentration. According to Choudhury *et al.* (2017), rhizome of ginger exhibited the antifungal property against *Colletotrichum musae.*

5.3.1.3 Percentage of inhibition for *Phytophthora* spp.

In the present study, variation was noted with respect to the anti-fungal property of rhizome and leaf extract of *Alpinia calcarata* against *Phytophthora.* Percentage of inhibition of rhizome extract ranged from 25.8 to 35.00 per cent with the highest value recorded in IC550112. In case of leaf extract, it was ranged from 26.20 to 37.10 per cent and maximum was observed in IC210656 and IC373608 (Fig. 23). The findings of the study were supported by Pompimon *et al.* (2009) who reported that the extract of *Alpinia galanga* inhibited the mycelial growth of *Phytophthora capsici.* Similar study was conducted by Tongchure and Chanprapai (2022) who compared the anti-fungal activity of the essential oil of *Zingiber officinale* and *Alpinia officinarum* against *Phytophthora parasitica* and found that the essential oil of *A. officinarum* exhibited the highest antifungal

activity against *Phytophthora parasitica.*

5.3.1.4 Percentage of inhibition for *Fusarium* spp.

Among the accessions under investigation, the percentage of inhibition for *Fusarium* using methanolic extract of *Alpinia calcarata* rhizomes and leaves gave significant results (Fig. 24). The rhizome and leaf extract of accessions IC550112 recorded the highest percentage inhibition for *Fusarium* (30.00 per cent and 32.10 per cent respectively). Handajani and Purwoko (2008) demonstrated that the antimicrobial activity of ethanol extract of *Alpinia galangal* rhizome against the pathogen *Fusarium moniliforme* with minimum growth inhibitory concentration of extracts was about 1,682 $mg.l^{-1}$. George (2014) reported that essential oil isolated from the rhizome of *Alpinia calcarata* showed anti-fungal activity against the plant pathogen *Fusarium oxysporum*.

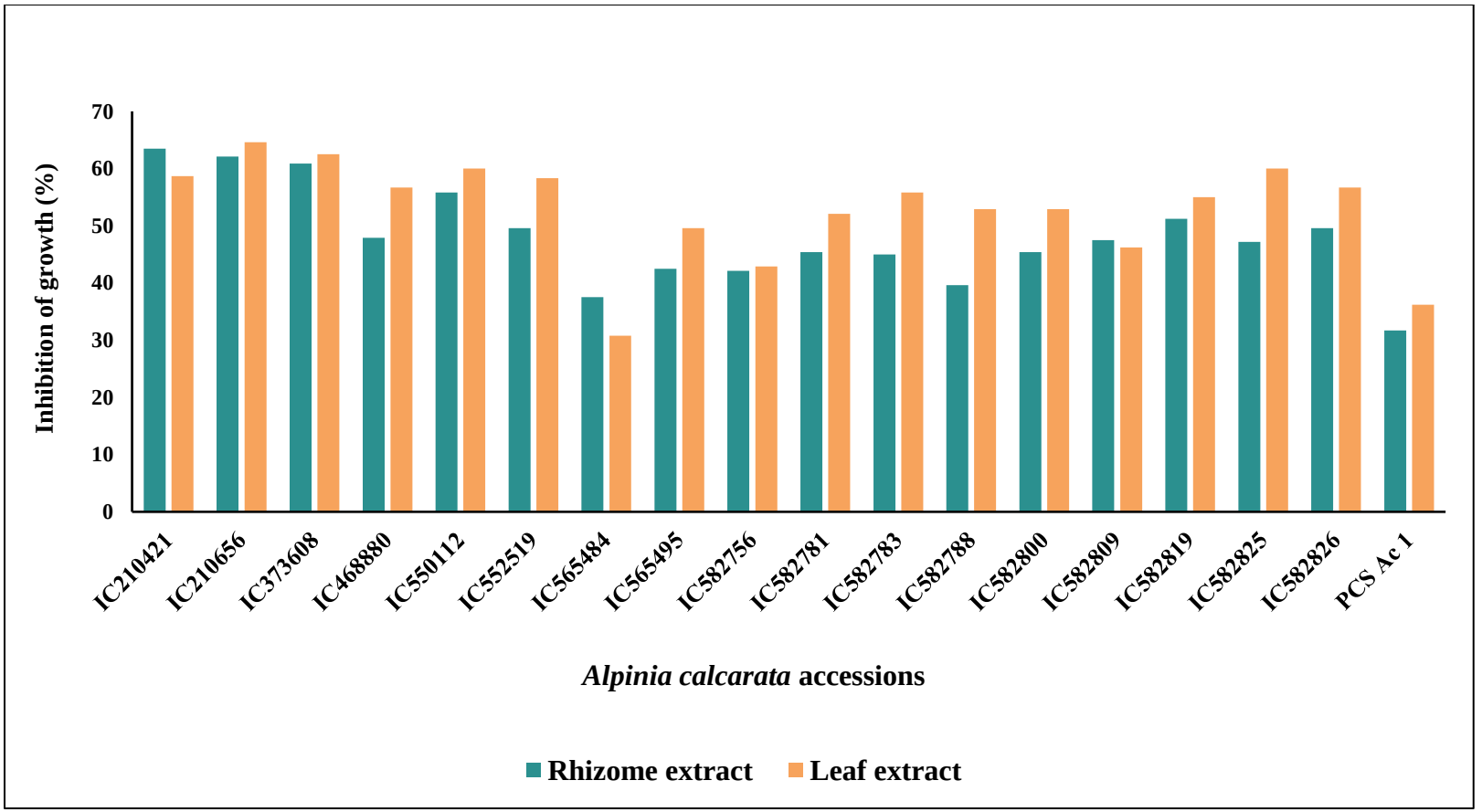


Figure 21: Percentage inhibition of methanolic extract of *Alpinia calcarata* rhizome and leaf against *Rhizoctonia* spp.

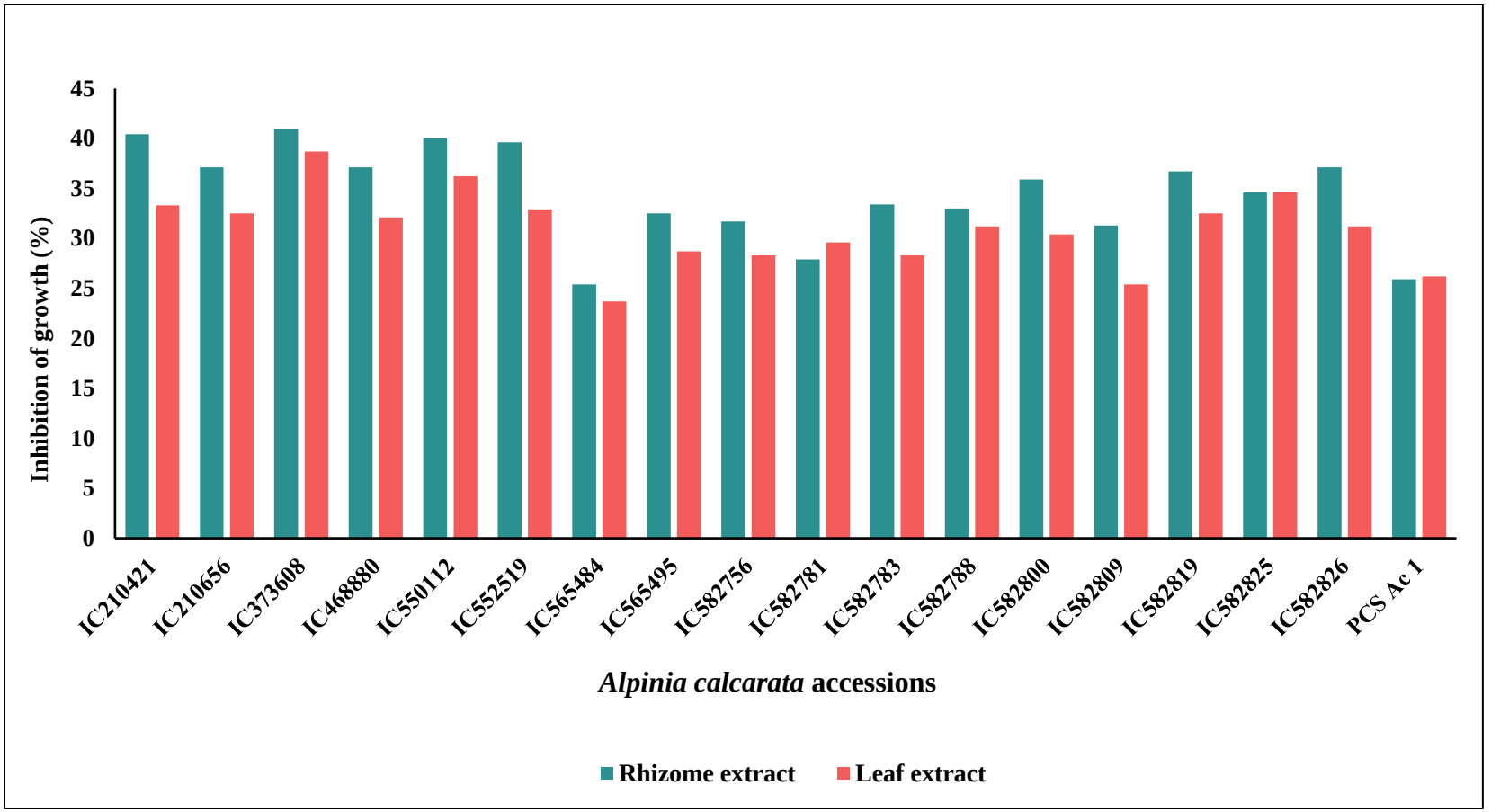


Figure 22: Percentage inhibition of methanolic extract of *Alpinia calcarata* rhizome and leaf against *Colletotrichum* spp.

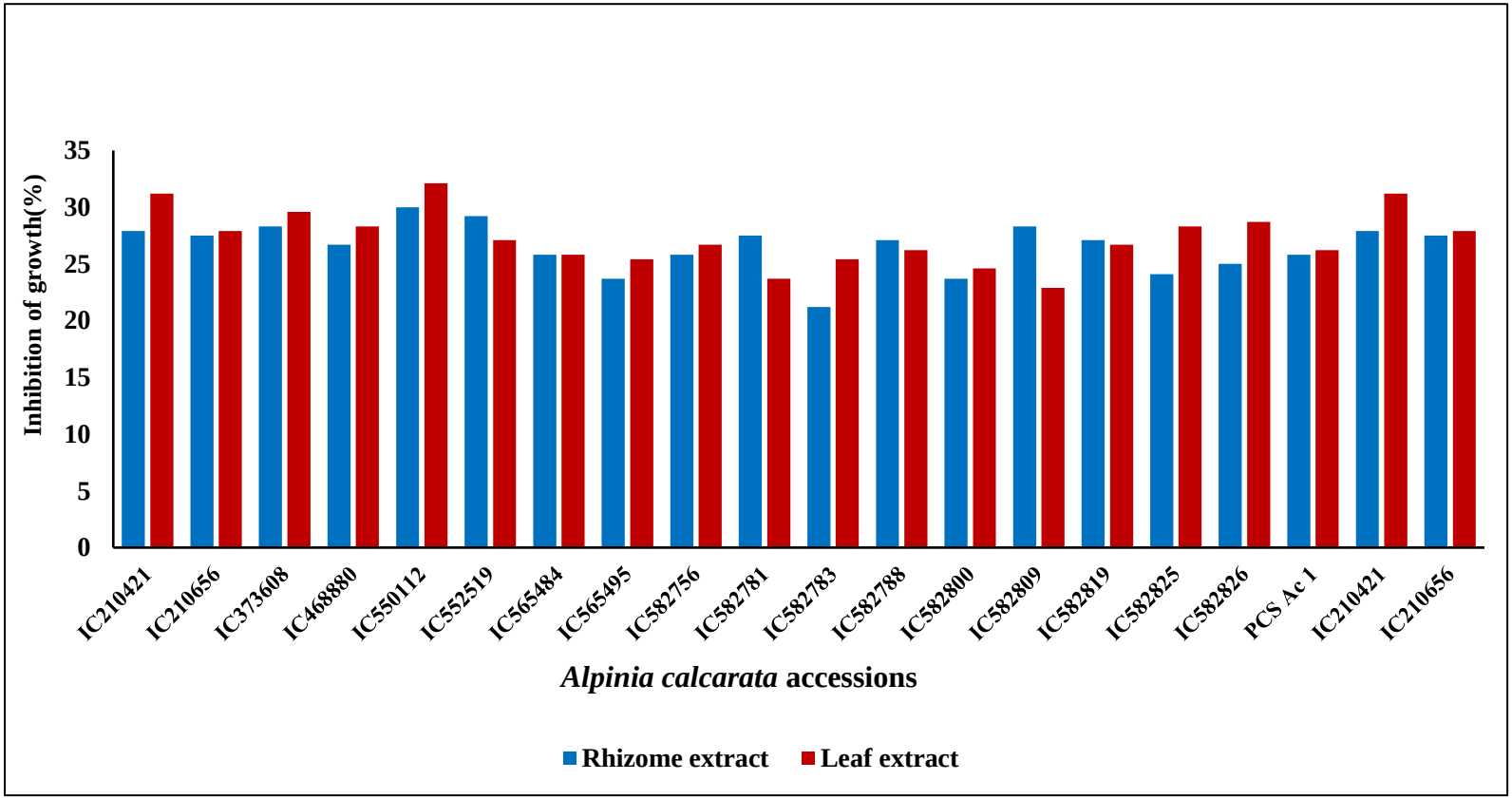


Figure 23: Percentage inhibition of methanolic extract of *Alpinia calcarata* rhizome and leaf against *Phytophthora* spp.

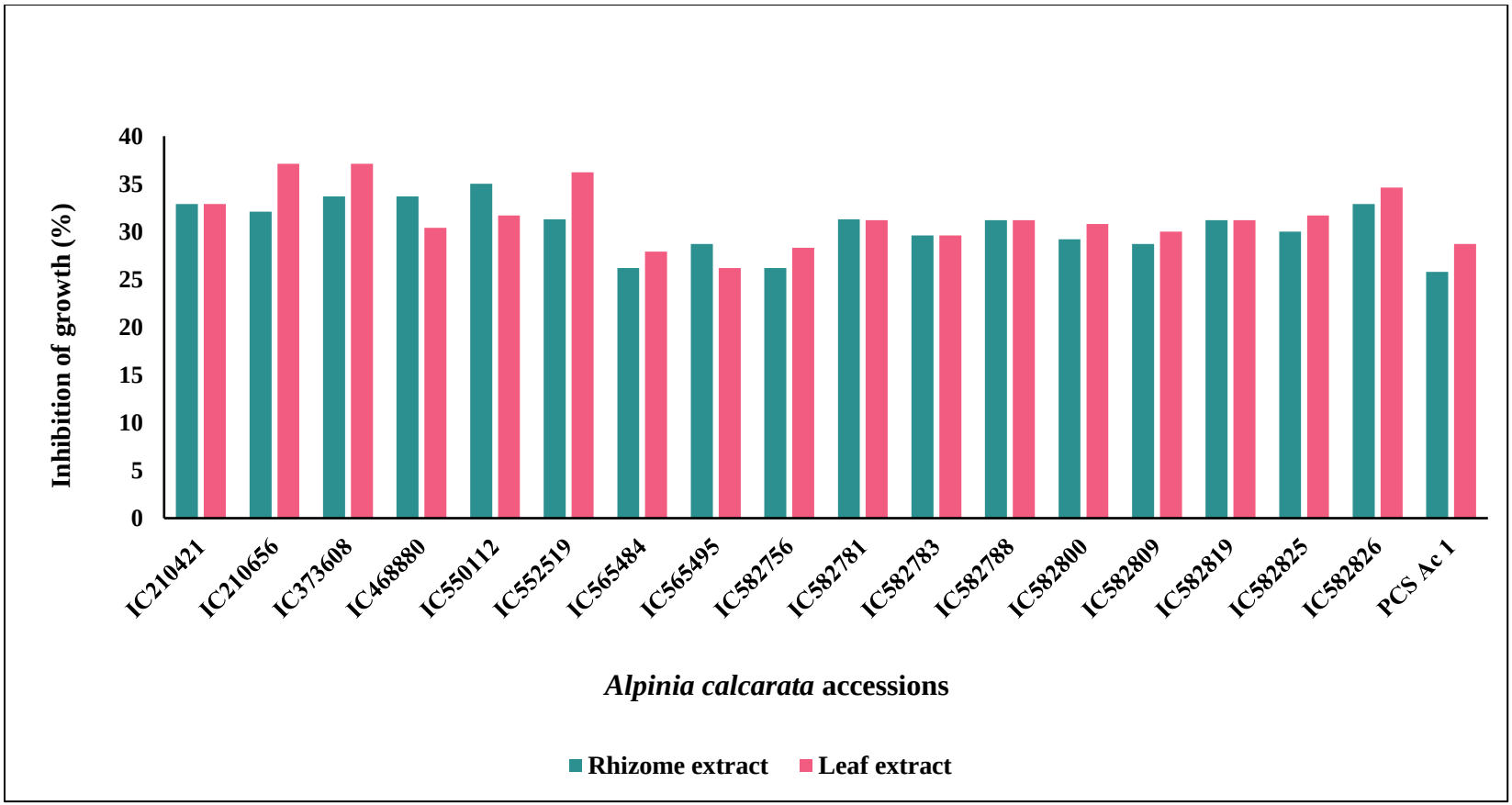


Figure 24: Percentage inhibition of methanolic extract of *Alpinia calcarata* rhizome and leaf against *Fusarium* spp.

5.3.2 Anti-bacterial property

The anti-bacterial property of methanolic extract of rhizome and leaf of *Alpinia calcarata* were tested against *Ralstonia solanacearum* using agar well diffusion method.

5.3.2.1 Zone of inhibition for *Ralstonia solanacearum*

In the present study, the methanolic extract of rhizome and leaf of *Alpinia calcarata* did not show any zone of inhibition for *Ralstonia solanacearum.* The results of our study were supported by similar previous works. Oonmetta-aree (2006) demonstrated that there is no anti-bacterial property of leaf extracts and fractions of *Alpinia galanga* against some Gram-negative bacteria: *Salmonella* spp., *Enterobacter aerogenes* and *Pseudomonas aeruginosa.* Similar findings have been reported by Chan (2011), observed that rhizome and leaf extracts of *Alpinia galanga* and *Curcuma long* did not show any antibacterial activity against *Staphylococcus aureus* and *Micrococcus luteus.* Ferdous *et al.* (2018) reported that methanolic leaf extract of *A. calcarata* did not exhibit antibacterial activity against two species of Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and two species of Gram-positive bacteria (*Micrococcus luteus* and *Staphylococcus aureus*).

Summary

6. SUMMARY

The present investigation entitled "Variability studies in lesser galangal (*Alpinia calcarata* Roscoe)" was carried out to evaluate various accessions of *Alpinia calcarata* which have been collected from Southern parts of India for genotypic variability and also to identify elite genotypes with desirable traits. The experiment was conducted at Department of Plantation, Spices, Medicinal and Aromatic crops, College of Agriculture, Vellanikkara and ICAR NBPGR Regional Station, Thrissur during 2021-2022.

Seventeen genotypes of *Alpinia calcarata* collected from South Indian states *viz.*, Kerala, Tamil Nadu and Karnataka which are maintained at ICAR NBPGR Regional Station, Thrissur and one genotype maintained at Plantation farm, under the Department of Plantation, Spices, Medicinal and Aromatic crops, College of Agriculture, Vellanikkara were evaluated in the study. The significant results of the research are outlined and presented below.

Morphological variation

Tiller characters including tiller length, diameter, tiller number and internodal length varied significantly among the accessions. The highest values for tiller characters were recorded in the accessions IC582825, IC373608 and IC582783. Significant variation was noticed among the accessions for leaf characters like number of leaves per tiller, leaf length, width and leaf area. The maximum values of leaf parameters were observed in accessions IC582783, IC373608 and IC582825. Rhizome characters like rhizome length, width and circumference varied greatly and accessions IC373608, IC582825 and IC468880 had shown the peak values. Yield parameters like rhizome yield per tiller and dry recovery showed significant variation among genotypes. The highest values were found in the genotypes IC373608, IC582825 and IC210421. Correlation studies of rhizome yield with other morphological characters in *Alpinia calcarata* exhibited that rhizome yield had positive and significant correlation with rhizome length, rhizome width, rhizome circumference, dry recovery, number of leaves per tiller, leaf area and tiller diameter.

Among the genotypes, inflorescence characters including duration of flowering, inflorescence length, and number of branches and flowers per inflorescence varied significantly and the highest values were recorded in IC373608, IC210421 and IC582825. While studying the reproductive phenological stages of lesser galangal, two principal growth stages were identified and described using the extended Biologische Bundesanstalt, Bundessortenamt, and Chemical industry (BBCH) scale. The principal growth stage 5- inflorescence emergence was of 7-10 days duration and the principal growth stage 6- flowering with 10-15 days. In general, it required around 20-25 days to complete its reproductive stage. However, the flowers did not produce fruit and seeds. The pollen viability and germination studies revealed that pollen grains were fertile but the pollen tubes failed to germinate. This might be the reason for failure of fruit development and non-seed setting in *Alpinia calcarata.*

The qualitative morphological parameter in *Alpinia calcarata* accessions under study represented no variation. The mature leaves were linear-lanceolate with dark green colour. The inflorescences were terminal panicle and the flowers had creamy yellow colour with red streak. The rhizomes were less branched bearing numerous root tubers. The cylindrical rhizome had a pale-yellow outer skin and a moderate yellow inner core.

Quality characters of rhizome

The rhizomes of all lesser galangal accessions were subjected to biochemical analysis for estimating the essential oil, oleoresin, phenols, flavonoids, starch, crude fibre and terpenoids content in it. Significant variation was noticed in these biochemical parameters among the genotypes. The highest essential oil content was documented in the accession IC468880 and oleoresin content was in IC373608. Significantly highest values for total phenols, total flavonoids and starch were observed in the genotypes IC373608, IC582825 and IC210656 respectively. The accession IC550112 had shown lowest crude fibre content which is desirable. The maximum total terpenoid content was found in the accession IC210421. A total of twenty eight compounds were identified from the essential oil of *A.* calcarata by GCMSMS analysis. The major compounds present in the essential oil were eucalyptol (19.17%) and ethyl iso-allocholate (15.06%). From these results, we can conclude that the genotypes

IC373608, IC210421, IC210656 and IC582825 were found to be superior with respect to biochemical properties of rhizomes.

Anti- microbial properties

The anti-microbial property of methanolic extract of rhizomes and leaves of *Alpinia calcarata* at 1000 ppm concentration were tested against five plant pathogens *viz, Rhizoctonia* spp., *Colletotrichum* spp., *Fusarium* spp., *Phytophthora* spp. and *Ralstonia solanacearum* under *in vitro* conditions. The studied accessions showed significant variation in percentage of inhibition of mycelial growth for four pathogens tested. The highest percentage of inhibition against *Rhizoctonia* spp. was noted in the rhizome extract of accession IC210421 and leaf extract of IC210656. The rhizome and leaf extract of accession IC373608 displayed the highest percentage of inhibition against *Colletotrichum* spp. The rhizome extract of IC550112 and leaf extract of IC373608 presented the maximum percentage of inhibition against *Phytophthora* spp. The highest percentage of inhibition against *Fusarium* spp. was noticed in rhizome and leaf extract of IC550112. The results revealed that the accessions IC550112, IC373608, IC210421 and IC210656 had the highest antifungal activity against the tested fungi. However, none of the accession exhibited any zone of inhibition for phyto pathological bacteria *R. solanacearum* with methanolic extract of leaf and rhizome.

The variability of *Alpinia calcarata* accessions was evaluated based on morphological characters, yield parameters, biochemical characters and antimicrobial properties. The genotypes IC373608, IC582825, IC210421, IC550112, and IC210656 were found as superior with desirable characteristics and can be utilized in future crop improvement programmes.

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Appendices

APPENDIX I

Abbreviations

%	: Per cent
µl	: Microlitres
µm	: Micrometres
ANOVA	: Analysis of Variance
BBCH	: Biologische Bundesanstalt, Bundessortenamt and Chemical industry
$cfu.ml^{-1}$	: Colony forming units per minute
cm	: Centimetres
cm^2	: Square centimetres
CRD	: Completely randomized design
et al.	: et alia (and associates)
etc.	: et cetera
g	: Gram
hrs	: Hours
ICAR	: Indian Council of Agricultural Reseach
l	: Litres
mg	: Milligrams
mg $GAE.g^{-1}$	:Milligrams gallic acid equivalent per gram
mg $QE.g^{-1}$	:Milligrams quercetin equivalent per gram
$mg.g^{-1}$	: Milligrams per gram
ml	:Millilitres
ml/min	: Millilitres per minute
mm	: Millimetres
NBPGR	: National Bureau of Plant Genetic Resourses
nm	: Nanometres

^{o}C	: Degree Celsius
^{o}C/min	: Degree Celsius per minute
PDA	: Potato dextrose agar
ppm	: Parts per million
RH	: Relative humidity
RHS	: Royal Horticultural Society
rpm	: Revolutions per minute
spp	: Multiple species
viz	: Videlicet

APPENDIX II

Meteorological data during the period of observation from November 2021 to October 2022

Month	Max temperature (^{0}C)	Min temperature (^{0}C)	Mean RH (%)	Rainfall (mm)	Mean evaporation (mm/day)	Mean sunshine (hrs/day)
November 2021	31.0	23.4	81	13.0	3.4	3.6
December 2021	32.5	23.3	67	1.0	3.8	8.2
January 2022	33.3	22.6	64	0.0	4.3	9.1
February 2022	34.8	23.3	58	0.0	5.1	8.3
March 2022	36.1	24.7	74	1.7	5.0	6.9
April 2022	34.2	25.1	77	84.3	3.2	5.9
May 2022	31.1	24	85	422.0	2.7	3.0
June 2022	31.3	23.6	84	391.8	3.2	4.5
July 2022	29.3	23.5	88	628.8	2.2	1.8
August 2022	29.9	23.6	84	563.7	2.9	4.3
September 2022	31.1	23.7	81	167.5	3.1	5.4
October 2022	32.0	23.6	77	69.6	3.1	5.9

APPENDIX III

Composition of media for isolation of pathogens (fungi and bacteria)

1. Potato dextrose agar (PDA)

Potato – 200 g

Dextrose – 20 g

Agar – 20 g

Distilled water – 1000 ml

2. Nutrient agar (NA)

Peptone – 20 g

Beef extract – 1g

NaCl – 5 g

Agar – 20 g

Distilled water – 1000 ml

pH – 6.5 – 7.5

APPENDIX IV

Composition of media for cultural characterization of bacteria

Tetrazolium chloride (TZC) medium

TZC Basal medium-

Peptone

Casein hydrolysate

Glucose

Agar

Distilled water

pH

Dissolve 1 g of TZC in 100 ml of distilled water separately and placed in a light proof capped bottle and autoclaved for eight minutes. From this stock solution, 5 ml was added to each 1000 ml basal medium.

VARIABILITY STUDIES IN LESSER GALANGAL

(*Alpinia calcarata* Roscoe)

By

SHIBILA K.

(2020-12-010)

ABSTRACT OF THE THESIS

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VARIABILITY STUDIES IN LESSER GALANGAL (*Alpinia calcarata* Roscoe)

ABSTRACT

Alpinia calcarata Rosc. (Family: Zingiberaceae) commonly known as lesser galangal is a widespread rhizomatous perennial herb, commonly used in Indian and Sri Lankan traditional medicine systems. Rhizomes form the economic part, which is a major constituent of many formulations of indigenous system of medicine for relieving throat inflammation, bronchitis, respiratory ailments, purifying blood, improving voice, asthma and arthritis. However, it is categorized as an endangered species due to excessive collection from the wild. This necessitated the evaluation of lesser galangal accessions for the identification of suitable varieties for commercial cultivation, in order to meet rising demand. In this context, the present study "Variability studies in lesser galangal (*Alpinia calcarata* Roscoe)" was carried out in the Department of Plantation, Spices, Medicinal and Aromatic crops, College of Agriculture, Vellanikkara and ICAR NBPGR Regional Station, Thrissur with the broad objective to assess the variability in morphological, yield and quality parameters of lesser galangal germplasm collected from South Indian states. Seventeen accessions of *Alpinia calcarata* are maintained at ICAR NBPGR Regional Station, Thrissur and one accession maintained at Plantation farm, under the Department of Plantation, Spices, Medicinal and Aromatic crops, College of Agriculture, Vellanikkara were evaluated.

Morphological and yield parameters varied significantly among the evaluated accessions of lesser galangal. Accessions IC582825, IC373608 and IC582783 recorded highest values for morphological characters like plant height, tiller diameter, number of tillers per plant, number of leaves per plant, leaf area and inflorescence characters. The highest values for rhizome dimensions and yield parameters like rhizome yield per tiller and dry recovery were recorded in accessions IC373608, IC582825 and IC210421. The correlation study implies that rhizome yield had a positive and significant

correlation with rhizome dimensions, dry recovery, number of leaves per tiller, leaf area and tiller diameter.

In the reproductive stages of *Alpinia calcarata,* two principal growth stages and twelve secondary growth stages were identified and described using the extended Biologische Bundesanstalt, Bundessortenamt, and Chemical industry (BBCH) scale (Meier, 2001). The principal growth stage 5-inflorescence emergence lasted 7-10 days and the principal growth stage 6-flowering recorded 10-15 days. It took approximately 20-25 days to complete its reproductive stage. However, the flower failed to develop fruit and seed. Pollen viability and germination tests confirmed that the pollen grains were viable, however, the pollen tube did not germinate. This could be the cause of failure in fruit development and non-seed setting. Among the genotypes, there were no significant variations observed for the qualitative morphological parameters including leaf color and shape, flower color and rhizome shape and colour.

The quality characters of rhizomes including essential oil, oleoresin, total phenols, total flavonoids, starch, crude fibre and terpenoids varied significantly among accessions. A total of 28 compounds were identified in the essential oil of rhizome by GCMSMS analysis and the main constituent was eucalyptol. With respect to biochemical analysis, the accessions IC373608, IC210421, IC210656 and IC582825 were found to be superior.

The efficacy of methanolic extract of rhizomes and leaves of *Alpinia calcarata* at 1000 ppm concentration was screened against five plant pathogens viz, *Rhizoctonia* spp., *Colletotrichum* spp., *Fusarium* spp., *Phytophthora* spp. and *Ralstonia solanacearum* under *in vitro* conditions. The anti-fungal activity of rhizome and leaf extract was comparable. Accessions IC550112, IC373608, IC210421 and IC210656 recorded the highest antifungal activity against the tested fungi. However, no accession showed any zone of inhibition for phyto pathological bacteria *R. solanacearum* with methanolic extract of leaf and rhizome.

The genotypic variability of *Alpinia calcarata* accessions was assessed using yield parameters, biochemical parameters of rhizome, and the antimicrobial activities of leaf and rhizome extract. Accessions IC373608, IC582825, IC210421, IC550112 and IC210656 were identified as the superior genotypes with desirable traits and can be utilized for further crop improvement programme.