SURVEY, COLLECTION AND CHARACTERIZATION OF '*Kizharnelli*' (*Phyllanthus* spp.) OF KERALA

By

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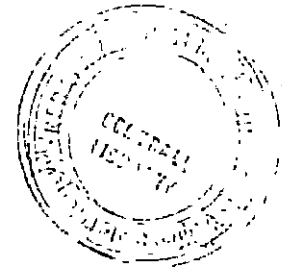
(2013-12-107)



THESIS

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DECLARATION

I hereby declare that the thesis entitled **"Survey, collection and characterization of '*Kizharnelli*' (*Phyllanthus* spp.)"** 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 of any degree, diploma, fellowship or other similar title, of any other University or Society.

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(2013-12-107)

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Date: 25-9-2015

CERTIFICATE

Certified that thesis entitled **"Survey, collection and characterization of '*Kizharnelli*' (*Phyllanthus* spp.)"** is a bonafide record of research work done independently by **Ms. Shafna Kalarikkal (2013-12-107)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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We, the undersigned members of the advisory committee of **Ms. Shafna Kalarikkal. (2013-12-107)**, a candidate for the degree of **Master of Science in Horticulture**, with major field in **Plantation Crops and Spices**, agree that the thesis entitled **"Survey, collection and characterization of '*Kizharnelli*' (*Phyllanthus* spp.)"** may be submitted by **Ms. Shafna Kalarikkal**, in partial fulfilment of the requirement for the degree.

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ABBREVIATIONS

NBPGR	- National Bureau of Plant Genetic Resources
TBGRI	- Tropical Botanical Garden and Research Institute
RAPD	- Random Amplified Polymorphic DNA
ISSR	- Inter Specific Sequence Repeats
AFLP	- Amplified Fragment Length Polymorphism
UPGMA	- Unweighted Pair Group Method of Analysis
PCR	- Polymeric Chain Reaction
SCAR	- Sequence Characterized Amplified Regions
MIC	- Minimum Inhibitory Concentration
EC_{50}	- Effective Concentration
IC_{50}	- Inhibitory Concentration
UV	- Ultra Violet
CTX	- Cyclophosmide induced toxicity
WBC	- White Blood Corpuscles
TPC	- Total Phenolic Content
DPPH	- 2, 2-diphenyl-1-picrylhydrazyl
FRAP	- Ferric Reducing Antioxidant Power
BHA	- Butylated Hydroxy Anisole
LCMS	- Liquid Chromtagraphy Mass Spectroscopy
aHSC	- Activated Hepatic Stellate Cells
HPLC	- High Performance Liquid Chromatography
MS	- Murashige and Skoog
RD	- Raw Drug

Introduction

1. INTRODUCTION

The genus *Phyllanthus* L. belonging to the family Euphorbiaceae, consists of about 833 species, (Govaerts *et al.,* 2000) and is chiefly distributed in moist humid tropics. Members of Euphorbiaceae are pantropical which include trees, shrubs, semi-succulents and annual herbs (Upadhyay *et al.,* 2010). In India, the genus is represented by 40 species (Santapau and Henry, 1972). In total, eight species of herbaceous *Phyllanthus* have been identified from Kerala. The herbs known as Bhumiamalaki, in sanskrit refer to a complex group of various *Phyllanthus* species (Chowdhary and Rao, 2002). Although these species closely resemble each other, they also show sufficient characters to maintain them as distinct species. Confusion exists in identification of these herbaceous *Phyllanthus* spp., mainly due to their similarity in gross morphology, close proximity in growth habitat as well as referring them with a common vernacular name, '*Kizharnelli*'.

The most wide spread species of the genus, *Phyllanthus amarus* Schum & Thonn., is widely distributed in all tropical regions of the world. The plant is a common arable weed of distributed ground in southern Florida, the Bahamas, the West Indies and tropical America and is naturalized in old world tropics. Other commonly occurring *Phyllanthus* spp. of Kerala are, *Phyllanthus airy-shawii* Brunnel & Roux *Phyllanthus maderaspatensi* L., *Phyllanthus rheedei* Wight. *Phyllanthus urinaria* L., *Phyllanthus virgatus* G. Frost. var. *virgatus, and Phyllanthus virgatus* G. Frost. var. *gardnerianus* Wight, Govaerts & Radcl. (Ganeshaiah *et al.*, 1998).

Phyllanthus spp. is reputed for their hepatoprotective activity and are used in traditional Indian medicine against jaundice. Phytochemical properties of *Phyllanthus* have recently become a focal point of several investigations due to their broad therapeutic uses, wide distribution as well as diverse secondary metabolite entities. *Phyllanthus amarus* is claimed to be an excellent remedy for infective hepatitis. Hypoglycaemic, carminative, antispasmodic and diuretic

properties are also associated with the species. The lignans, phyllanthin and hypophyllanthin, have been identified as the major therapeutically active constituents of the herb (Syamsundar *et al.*, 1985).

Misuse of herbal medicines or natural products, starts with wrong identification. Hence, confirmation of identity of the crude drug through characterization of distinct morphological features is of utmost importance to identify adulteration of raw drugs. Biochemical characterization gains importance because, once the plant is dried and made into powder form, it loses its morphological identity and is easily prone to adulteration. Hence, morphological and biochemical characterization ensures plant identity and lays down standardization parameters which will help to prevent adulteration. Such studies will help in authentication of the plants and ensures reproducible quality of herbal products which will lead to safety and efficacy of natural products.

User industries of Kerala obtain raw drug of *Phyllanthus* through collections from wild or from marginal lands. Trade in *Phyllanthus* as a bulk herb is rampant, which consists of a heterogeneous mixture of various species of the genus. Several branded or patented herbal products of our country increasingly make use of *Phyllanthus amarus* as a component drug, ever since modern research focused on antiviral properties of the plant, as a cure for the Hepatitis-B viral disease. But preponderance of other *Phyllanthus* spp. often leads to ignorant as well as deliberate adulteration or substitution. This has resulted in lowering the efficacy of the medication for its intended purpose.

Though *P.amarus* has been extensively studied phytochemically, and is known to contain phyllanthin and hypophyllanthin, other commonly occurring *Phyllanthus* species have not been subjected to in-depth phytochemical and clinical investigations. Hence, surveying agroclimatic zones of Kerala and characterizing natural population of *Phyllanthus* spp. morphologically and phytochemically, will ensure correct identity and prevent erroneous conclusions regarding their therapeutic efficacy. Also, the possibilities of genuine and

legitimate substitution of *Phyllanthus amarus* with related species, without comprising the efficacy of the drug, could be explored. Hence, the study 'Survey. collection and characterization of '*Kizharnelli*' (*Phyllanthus* spp.) of Kerala' was taken up with the following objectives.

- Survey and collection of *Phyllanthus* accessions from select locations of Kerala
- Morphological and phytochemical characterization of collected accessions of *Phyllanthus*
- Ascertain the zone wise and region wise distribution of *Phyllanthus* spp.
- Assessment of performance of *Phyllanthus* accessions in pot culture
- Ascertain the quality of traded crude drug of *Phyllanthus* by detecting species admixtures and estimating phytochemical constitution

Review of Literature

2. REVIEW OF LITERATURE

The genus *Phyllanthus*, constitutes one of the most important groups of plants with medicinal value (Ved and Goraya, 2008). The name '*Phyllanthus*' means "leaf and flower", which is so named, because of its appearance, where flower, fruit and leaf appear fused (Kumar *et al.*, 2010). In the last few decades, plants belonging to the genus *Phyllanthus*, of the family Euphorbiaceae, came into focus due to their wide distribution, diversity in genus, broad therapuetical potential and wide range of secondary metabolites present in them. Euphorbiaceae family includes upright or prostate herbs or shrubs, often with milky acrid juice (Lewis and Elvin-Lewis, 1977).

Webster (1994) reported that genus *Phyllanthus* consists of approximately 1000 species, spread all over American, African, Australian, and Asian continents. Fifty three species of *Phyllanthus* are distributed in India of which 23 are endemic (Balakrishnan and Chakrabarthy, 2007). *Phyllanthus amarus* is the most widespread and important species of this genus, which is distributed throughout the tropical and subtropical countries of the world including India.

P. amarus is a tropical and sub-tropical weed, mostly found in moist, shady and sunny places (Cabieses, 1993). It is commonly known as *Bhumiamalaki*, *Jamgli amli*, *Jaramla* and *Kizharnelli* in Malayalam. *P. amarus* was first identified in central and southern India in 18th century but is now found in many countries like Philippines, Cuba and Nigeria. It is commonly called 'carry me seed' 'stone breaker' or 'gulf leaf flower'(Igwe *et al.*, 2007). *Phyllanthus amarus* is a branching annual glabrous herb which is 30-60 cm high with slender, leaf-bearing branchlets and distichous leaves which are subsessile and elliptic-oblong with obtuse, rounded base. Flowers are yellowish, whitish or greenish and axillary. Male flowers occur in groups of 1-3, whereas female flowers are solitary. Fruits are depressed-globose like smooth capsules and are present underneath the branches. Seeds are trigonous and pale brown with longitudinal parallel ribs on the back (Itoro *et al.*, 2013). The species plays an important role in health care

management throughout the world. The herb is used in traditional medicine for more than 3,000 years. Every part of this plant has been investigated as a source of valuable compounds. Standardization of herbal drugs to ensure their quality and efficacy is most desirable at this time, when world wide interest on herbal medicine has gained momentum.

2.1. CHARACTERIZATION, SPECIES IDENTIFICATION AND PHYLOGENY OF *Phyllanthus* spp.

Webster and Airyshaw (1971) observed that, true *P. niruri* L., is native of New world and endemic to America and does not occur in India.

In 1985, Mitra and Jain, after critical examination of Indian materials of *Phyllanthus* revealed that the *Phyllanthus niruri* L. described in Flora of British India (Hooker, 1887) is a mixture of three closely related but distinct species namely, *P. amarus*, *P. debilis* and *P. fraternus.*

Kandavel *et al.* (2011) reported morphological characters of herbaceous *Phyllanthus* spp. seen in Tiruchirappalli district of Tamil Nadu as follows.

Table 1. Morphological characters of herbaceous *Phyllanthus* spp. seen in Tiruchirappalli district of Tamil Nadu.

Character	*P. amarus*	*P. airy-shawii*	*P. maderaspatensis*	*P. virgatus* var. *virgatus*
Stem shape	Terete	Angular	Angular	Angular
Stem surface	Hispiulous	Glabrous	Glabrous	Glabrous
Cataphyll	Three, triangular-lanceolate; acuminate and turn black at maturity	Three, normally lanceolate and acuminate	Absent	Absent
Branchlet	Present	Present	Absent	Absent
Leaf shape	Oblong	Narrowly elliptic in upper part and cuneate at base	Spathulate	Oblong-elliptic
Leaf tip	Rounded	Acute	Apiculate	Obtuse

Khatoon *et al.* (2006) reported comparative macroscopic characters of three *Phyllanthus* spp. as follows.

Table 2. Comparitive macroscopic characters of three *Phyllanthus* spp.

Sl no.	*P. amarus*	*P. airy-shawii*	*P. maderaspatensis*
1	Plant 10- 60 cm tall	Plant 7- 50 cm tall	Plant 30- 90 cm tall
2	Stem terete, younger part rough	Stem terete, mostly naked below and tetragonous above	Stem glabrous
3	Branchlets 2-6 cm long with 10-20 leaves	Branchlets 2-11 cm long with 10-30 leaves.	Branchlet absent
4	Leaf elliptic, oblong to ovate, obtuse or minutely apiculate at apex	Leaves elliptic, oblong, rounded at apex	Leaves, rounded truncate or somewhat obcordate at the apex, mucronate, much tapering into a very short petiole
5	Flowers axillary, proximal two to three male flowers and all succeeding axils with bisexual cymules	Flowers are axillary, unisexual cymules proximal three to four male flowers, succeeding solitary female flowers	Flowers axillary, male flowers minute in small clusters, subsessile female flower solitary and larger with shortly stalked
6	Sepals five	Sepals six	Sepals six

In 1982, Manilal and Sivarajan, published a new species of *Phyllanthus* from Kerala, i.e., *Phyllanthus kozhikodianus*. This species has been recently merged with *Phyllanthus rheedei*.

Udayan *et al.* (2005) reported that, *P. rheedei* is an erect annual herb, up to 60 cm tall with elliptic or obovate, glabrous leaves, 2.5 x 1.5 cm, obtuse to sub acute at apex, rounded at base and sepals six.

Genetic diversity analysis using RAPD (Random Amplified Polymorphic DNA) markers, with *P. amarus* collected from different geographical locations in

India, showed that the accessions from the southern part of India have high intra population variation (Jain *et al.*, 2003). This variation may be attributed to the cross pollination mechanisms in populations and also because they grow as weeds without much anthropogenic intervention (Mitra and Jain, 1985).

Isozymes also have been used to assess the genetic variability in south Indian populations of *P. amarus,* to indentify superior genotypes for improving drug quality and for formulating strategies for *in situ* conservation and sustainable utilization (Geetha *et al.*, 2003).

The extent of genetic diversity has been investigated in *P. amarus, P. debilis* and *P. virgatus* using RAPD and ISSR (Interspecific Sequence Repeats) markers, and an average polymorphism of 68.2 per cent and 69.7 per cent, respectively, was observed (Palaniappan and Marappa, 2006). AFLP (Amplified Fragment Length Polymorphism) profile along with morphological study could confirm the identification of *P. ajmerianus* (Vishwanatha *et al.*, 2006).

Use of molecular markers for the identification of *Phyllanthus* spp. has proved to be a reliable tool. Species specific SCAR (Sequence Characterized Amplified Regions) markers were developed for identification of *Phyllanthus* species (*P. amarus, P. fraternus, P. debilis* and *P. urinaria*) used in the dry leaf bulk trade (Theerakulpisut *et al.*, 2008).

Mundra and Paria (2009) first reported the heteroblastic development in *Phyllanthus urinaria* and in the genus *Phyllanthus*. The changes in the morphology of the leaves are undoubtedly the most conspicuous feature of heteroblastic development in vascular plants. In the simplest case (as is the case with most of the *Phyllanthus* spp.), there is merely an increase in size of the leaves, without any change in form. However, in *P. urinaria*, irrespective of any form changes, there is an increase in leaf size (both length and width) followed by a subsequent fall in the terminal portions (mature leaves) of the shoot. Leaf shape is the most obvious trait that changes during heteroblastic development.

In *P. urinaria*, apart from size changes, there are progressive changes in leaf shape from node to node. In addition to changes in leaf shape, the ontogeny of a plant frequently involves changes in the phyllotaxy. This heteroblastic development of leaves serves as a marker character and will help in identification of this taxon, from other closely related species of genus *Phyllanthus*, at juvenile stage i.e. much before flowering and fruiting stages, which in turn, can also be utilized in the conservation of this medicinally important plant. Further, the knowledge of heteroblastic development in *P. urinaria* can be of importance in morphological and physiological studies.

An investigation was undertaken by Rout *et al.* (2010) to describe the relationships among twelve species of *Phyllanthus* collected in India using molecular markers. In total, 259 marker loci were assessed, out of which 249 were polymorphic, revealing 96.13 per cent polymorphism. Nei's similarity index varied from 0.35 to 0.76 for RAPD and from 0.31 to 0.76 for ISSR marker systems. Cluster analysis by the unweighted pair group method (UPGMA) of Dice coefficient of similarity generated dendrograms with more or less similar topology for both the analyses, offered a better explanation for diversity and affinities between the species. The phylogenetic tree obtained from both RAPD and ISSR markers has divided the 12 species into two groups: group I consisting of only one species *Phyllanthus angustifolius* and group II with the rest of the 11 species. These results were in compliance with notable morphological characters. This study revealed high variation among the species of *Phyllanthus* and will help to identify different *Phyllanthus* spp.

PCR-RFLP approach of ITS (Internal Transcribed Spacers) region has been successful in discriminating *P. amarus, P. debilis and P. urinaria* (Manissorn *et al.*, 2010).

The ITS sequences generated phytograms aid in deducing affinities among *P. emblica*, *P. reticulatus*, *P. amarus*, *P. fraternus* and *P. urinaria*. Phylogenic relationship of 23 *Phyllanthus* spp. of Thailand have been analysed by sequencing

ITS regions (Manissorn *et al.*, 2010). RAPD and ISSR markers have been used to analyse phylogenic relationship between twelve species of *Phyllanthus* (Rout *et al.*, 2010).

Srirama *et al.* (2010) assessed species admixtures in raw drug trade of *Phyllanthus* using DNA barcoding tools. They analysed sequence variations of psbA-trnH region of the chloroplast to identify the *Phyllanthus* spp. admixtures.

Ethnomedicinal uses and pharmacological activities among *P. amarus, P. fraternus, P. debilis* and *P. urinaria* are varied but these plant species commonly grow together in the same open habitats and wastelands. In Bangladesh, China, India, Pakisthan, and Thailand, *P. amarus, P. fraternus, P. debilis* and *P. urinaria* grow together and lead to confusion in identification of these herbaceous species. Systematic studies on herbaceous *Phyllanthus* spp., using morphological and anatomical parameters, could identify these *Phyllanthus* herbs (Kandavel *et al.*, 2011). Ravikant *et al.* (2011) described southern India to be the genetic hot spot of *Phyllanthus* spp.

Senapati *et al.* (2011) identified species-specific diagnostic markers for ten *Phyllanthus* species, using Inter Simple Sequence Repeat- Polymerase Chain Reation (ISSR-PCR).

Bandyopadhyay and Raychaudhari (2013) compared RAPD, SCAR and RFLP markers for identification of five *Phyllanthus* spp. and concluded that AFLP is a better polymorphic marker.

Leaf epidermal studies were carried out by Uka *et al.* (2014) on six species of *Phyllanthus* occurring in South Eastern Nigeria. The species investigated include *P. amarus, P. urinaria, P. odontadenius, P. niruroides, P. mullerianus* and *P. discoideus*. The study was done to investigate their taxonomic relationship and to identify epidermal features that can be recognized and employed as useful taxonomic characters. Qualitative features of the epidermal morphology showed variations in shapes of the epidermal cells and types of stomata, which varied from wavy, polygonal to sinuous and anisocystic, tetracystic to paracystic

respectively, in different species. Differences were found in the distribution of the stomata as well as variation in the cell wall contours and thickness.

2.2. MEDICINAL SIGNIFICANCE OF *Phyllanthus amarus*

Phyllanthus amarus is widely used in the Indian Systems of Medicine. The extract of *P. amarus* is used as a blood purifier, for light malaria fevers and anaemia (Heyde, 1990). According to Unander *et al.* (1995) the aerial parts of the herb *P. amarus* have been widely used in folk medicines in India and other tropical countries for the treatment of various diseases and disorders such as jaundice, diarrhoea, constipation, kidney ailments, ulcers, ring worm, malaria, genitourinary infections, haemorrhoids and gonorrhoea. This plant is traditionally used around the world in the treatment of liver ailments and kidney stones. In Spanish, it is called as 'chanca piedra' meaning 'stone breaker or shatter stone'. In South America, it is used to eliminate gall bladder and kidney stones, and to treat gall bladder infections (Foo and Wong, 1992).

Presently, *Phyllanthus amarus* is gaining importance because of its novel antiviral activity against hepatitis B virus and for several other biological activities such as kidney and gall bladder stones, for cold, flu, tuberculosis and other viral infections; liver diseases and disorders including liver cancer, hepatitis and jaundice (Unander *et al.*, 1995). The inhibition of hepadnavirus DNA polymerase differed between cultivated *Phyllanthus amarus* and plants collected from the wild (Unander *et al.*, 1993). It acts against liver cell cancer toxicity and improves the immune system of patients and is found effective against hepatitis A (Jayaram *et al.*, 1997). Annamalai and Lakshmi (2009) reported that the pharmaceutical interest in *P. amarus* has increased in recent years because of the efficacy of the whole herb against Hepatitis B virus. 'Ayurviva' is a drug available in the market, containing *P. amarus* which is used for liver disorders, hepatitis, loss of appetite, general debility and convalescence and 'Lovanthin' against Hepatitis B (Kumar *et al.*, 2010).

P. amarus has been described in Ayurveda by the sanskrit name Bhumiamalaki. It is described to have properties of Rasa, Guna, Veerya and Vipaka. The ayurvedic literature has shown its uses as Kaasahara (antitussive), Shawaasahara (antispasmodic, antidyspnic), Kaphapittahara (relieving the kapha, pitta and dosha), Pipaasaaghna (which relieves polydipsia), Raktapittahara (haemorrhage diseasae), Paanduhara (antianaemic), Kaamalaahara (curing jaundice), Kushthaghna (against leprosy), Daahaghna (refrigerant, relieving burning sensation), Kshatakshayaghna (indicated in trauma) and Mootrarogahara (against urinary disorders) (Patel *et al.*, 2011).

In Ayurvedic system of medicine, *P. amarus* is considered as an acrid alexipharmic and is used in thirst, bronchitis, asthma, leprosy, anaemia, urinary discharge, anuria, hiccups and as a diuretic. In Unani system of medicine, the herb is used as a stomachic and against sores and chronic dysentery. The fresh root is believed to be a remedy for jaundice. The infusion of the roots and leaves is a good tonic and diuretic, when taken as repeated doses (Mohideen *et al.*, 2011).

In Brazilian folk medicine, the infusion of leaves stems and roots of *P. amarus* are used in various diseases and disorders. Leaves are used as expectorant and diaphoretic and as a tonic to liver (Kirtikar and Basu, 1987). The extract of *P. amarus* along with a small amount of turmeric is taken orally for 3-5 days to cure jaundice (Prasad *et al.*, 2008). It has bitter, astringent, stomachic, diuretic, febrifuge and antiseptic properties. The whole plant is used in the treatment of gonorrhea, menorrhagia and other genital affections. It is also administered against gastropathy, diarrhoea, dysentery, intermittent fevers, ophthalmopathy, scabies, ulcers and wounds (Patel *et al.*, 2011).

P. amarus shows antifertility activity, where alcoholic extract of the herb brought changes in 3-beta and 17-beta Hydroxyl Steroid Dehydrogenase (HSDs) levels, thereby effecting hormonal conversions in the female mice that was confirmed by observation of lack of pregnancy in cohabited normal females and male mice (Rao and Alice, 2001).

The treatment with the aqueous extract of *Phyllanthus amarus* exhibited potent anticarcinogenic activity against 20 - methylcholanthrene (20-MC) induced sarcoma development. The antitumour and anticancer activity of *P. amarus* may be attributed to its inhibition of metabolic activation of carcinogen as well as the inhibition of cell cycle regulators and DNA repair confirming the significant anti-mutagenicity of the plant extract (Rajeshkumar *et al.*, 2002).

Antiamnesic activity of aqueous extract of leaves and stems of *P. amarus* were evaluated for nootropic effects and brain cholinesterase activity in male Swiss albino mice and the result reveals a dose dependent attenuation of diazepam and scopolamine induced amnesic deficits and reduction in brain cholinesterase activity. Since the reduction in cholinesterase in linked with increase in acetylcholine concentration in brain, which further is responsible for improving memory, it provides a rationale to use this therapeutic potential in the management of patients with cognitive disorders (Joshi and Parle, 2007).

The diuretic, hypotensive and hypoglycemic effects of *P. amarus* were assessed and significant increase in urine volume, urine and serum Na levels was observed after treatment with the extract obtained from the herb. The significant reduction in systolic blood pressure in non-diabetic hypertensive subjects was noted that further confirmed the diuretic potential of the plant (Wright *et al.*, 2007).

Antidiabetic potential of *P. amarus* was investigated in an experimental model, where fasted rats were made diabetic, by single intraperitoneal injection of 120 mg per kg of alloxan monohydrates and then two doses of the aqueous and hydroalcoholic extract of *P. amarus* administered orally, which were then compared with the normal control group that received distilled water only. After 15 days of treatment, the result revealed that aqueous and hydroalcoholic extract of *P. amarus* decrease the blood glucose level significantly. Serum analysis of the treated experimental animals showed an increase in insulin and reduction in the malondialdehyde concentration, which demonstrated the potential antidiabetic

property of aqueous and hydroalcoholic extract of *P. amarus* (Evi and Degbeku, 2011).

2.3. MEDICINAL SIGNIFICANCE OF HERBACEOUS *Phyllanthus* spp., OTHER THAN *Phyllanthus amarus.*

Other herbaceous *Phyllanthus* spp. except *P. amarus*, were also reported to have medicinal properties. In china, the extract of *P.virgatus* is fed to children suffering from malnutrition due to worm infestation. Gond tribe of India use this as an antiseptic and anti-inflammatory agent (Tiwari and Padhye, 1993). The whole extract of *P. maderaspatensis* is reported to shown antihepatotoxic, hepatoprotective and cholerectic activities (Asha and Pushpangadan, 1998). *P. kozhikodianus* provides protection to liver against chemical induced liver damage (Thyagarajan *et al.,* 2002). The plant extracts of *P. rheedei* have been analysed for pharmacognostic properties and it was shown to be hepatoprotective (Thyagarajan *et al.*, 2002).

Traditionally, in India, the herb, *P. fraternus* was used as a mild laxative, to expel worms and intestinal gas. The plant extracts are used for treating many types of biliary and urinary conditions like gall bladder, kidney stones and bacterial infections such as cystitis, prostatitis, viral infections, hepatitis, flu, tuberculosis, liver diseases, anaemia, veneral diseases and urinary tract diseases (Nishiura *et al.*, 2004). *P. debilis* shows antihepatotoxic and anti-inflammatory activity properties (Chandrasekhar *et al.*, 2005).

Ethanolic extract of the herb, *P. maderaspatensis* demonstrated chemoprotective effect in modulating cisplatin - induced nephrotoxicity and genotoxicity, thus proving its antioxidative property (Chandrasekhar *et al.*, 2006). The lignin virgatusin is found in the plant parts of *P. virgatus* and it inhibits growth of gram-positive bacteria (Maruyama *et al.*, 2007).

The ethanolic extract of *P. maderaspatensis* is taken as a popular dietary supplement in the southern part of India (Bommu *et al.*, 2008). Muthuvan tribe of

Kerala use all parts of *P. rheedi* as a cure for liver diseases. It also shows hepatoprotective, antihyperglycemic, antihyperlipidemic and antioxidant effects The plant extracts of *P. rheedei* have antihyperglycemic, antihyperlipidemic and antioxidant effects (Suresh and Asha, 2008).

The plant parts of *P. urinaria* have been shown to have antibacterial activity against *Helicobacter pylori*, which cause peptic ulcers and gastric cancers (Lai *et al.*, 2008). The ethanolic extract of the herb, *P. urinaria* has anti-inflammatory and antioxidant activity (Fang *et al.*, 2008). The aqueous extract of *P. debilis* shows antihyperglycemic property (Wanniarachchi *et al.*, 2009).

Aqueous or methanolic extract of *P. urinaria* is used the treating cancer (Haung *et al.*, 2003). *P. fraternus* is reported to have antimicrobial property (Chanda *et al.*, 2011). The leaf juice of *P. debilis* is taken orally by the Kamar, Gond and Halda tribes of Chattisgarh in India, for relief of problems related to sickle cell anaemia (Acharya *et al.*, 2012). *P. amarus* along with *P. urinaria* demonstrate the strongest antiviral activity against Herpes Simplex Virus type-1 and Herpes Simplex Virus type-2 which is attributed to its action in the early stage of infection and replication (Tan *et al.*, 2013). The acetone extract of *P. urinaria* has been found to inhibit herpes simplex virus infection (Zhong *et al.*, 2013).

2.4. ANTIMICROBIAL ACTIVITY OF HERBACEOUS *Phyllanthus* spp.

Antimicrobial activity of ethanol and water extracts of *P. amarus* were evaluated against the test organisms *Salmonella typhi.* Ethanolic, cold water and hot water extract of *P. amarus* were employed for antimicrobial evaluation by agar cup diffusion methods which were compared against standard antibiotics that were evaluated by disc diffusion method. The result demonstrates ethanolic extract to be the most potent against the test bacteria, with diameter of 8.0 mm as growth inhibition zone. This study establishes one of the traditional uses of *P. amarus* against typhoid fever (Oluwafemi and Debiri, 2008).

Antimicrobial potential of *P. amarus* was investigated using agar well diffusion method, for activity against several drug resistant pathogens such as *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Klebsiella species*. The results revealed that minimum inhibitory concentration (MIC) of the ethanolic plant extracts on *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Klebsiella* species were at 10 mg ml^{-1}, 50 mg ml^{-1}, 150 mg ml^{-1} and 100 mg ml^{-1} while the minimum bactericidal concentration were at 50 mg ml^{-1}, 100 mg ml^{-1}, 150 mg ml^{-1} and 150 mg ml^{-1} respectively (Adegoke *et al.*, 2010).

The solvents hexane, petroleum ether, chloroform, acetone and methanol extract of *Phyllanthus* leaves were tested for antibacterial activity against *Pseudomonas aeruginosa, Klebsiella pneumonia, Proteus mirabilis, Streptococcus faecalis, Enterobacter* sp., *Serratia marcescens, Staphylococcus aureus* and *Escherichia coli* by agar well diffusion method. The results demonstrated the highest inhibitory activity of methanol extract of *P. amarus* against the above bacterial species (Saranraj and Sivasakthivelan, 2012).

Studies on hexane, chloroform, ethyl acetate, acetone and methanol extracts of stem bark of *P. amarus* demonstrated the antimicrobial activity for all these extracts with a diameter that ranges between 11 mm 24 mm against *Streptococcus pyogenes, Staphylococcus aureus, Salmonella typhi, Escherichia coli, Candida albican, Aspergillus flavus* (Ushie *et al.*, 2013).

Antimicrobial activity of the methanolic extract of *P. amarus* as studied by agar dilution method and disc diffusion showed significant concentration-dependent antibacterial activity specifically for gram-negative microbes. It was also observed that antibacterial action was mainly due to isolated phyllanthin (Mazumder *et al.*, 2006).

The antiviral property of *P. amarus* has been attributed to the compounds niranthin, nirtetralin, hinokinin, geraniin and corilagin (Notka *et al.*, 2004). Antibacterial activity has been shown by phyllanthin and virgatusin in *P. amarus*

(Leite *et al.*, 2006). The lignin virgatusin from *P. virgatus* also shows antibacterial activity (Maruyama *et al.*, 2007). Rutin and Quercetin found in *P. urinaria* exhibit antiviral property (Fang *et al.*, 2008)

2.5. PHYTOCHEMISTRY OF *Phyllanthus* spp.

Phytochemistry is regarded as the heart of herbal therapy and phytochemical research plays an important role in the development of herbal medicines. It constantly addresses a challenge because of the large number of compounds present as mixtures in the extract in trace amounts. However, screening of prefractionated extracts allows quick identification and dereplication of extract that depicts compound whose activity is masked in crude extracts. Though phytochemical research is comparatively slow as compared to synthetic chemistry by all advanced methods including dereplication, mechanism based cleaning and drug design using natural molecules, it has the potential to discover and develop active new chemical entities of rich medicinal values (Newman and Cragg, 2007). Nahar *et al.* (2010) listed the various classes of phytochemicals found in *Phyllanthus* spp.

Most of the herbaceous *Phyllanthus* spp. have been shown to contain different combination of secondary metabolites such as alkaloids, flavanoids, lignans, phenols, tannins and terpenes, which render them with medicinal properties (Calixto *et al.*, 1998). In *P. amarus,* the whole plant is medicinal, possesessing a wide number of classes of biologically active compounds, which include, alkaloids, flavanoids, steroids, terpenoids, lignans, lipids and coumarins. Phyllanthin and hypophyllanthin are the important lignans isolated from *P. amarus* which are considered as the major active ingredients of the species (Row *et al.*, 1967). Khatoon *et al.* (2006) conducted pharmacognistic studies on *P. amarus* and reported that the plant contains 6.23 per cent total ash, 10 per cent alcohol extract, 22.25 per cent water soluble extract, 0.2 per cent phyllanthin, 0.3 per cent hypophyllanthin, 0.7 per cent phenols, 1.9 per cent sugar and 0.7 per cent

tannins. Among other *Phyllanthus* herbs, the phytochemistry of *P. amarus* is well studied (Patel *et al.*, 2011).

Niranthin, nirtetralin, phyltetralin and lintetralin are the four flavanone glycoside reported to be obtained from the leaves of *P. amarus* (Ganeshpure *et al.*, 1981). An unusual ellagitannin, Phyllanthusiin D (I), was isolated from the biological active polar fraction of aerial parts of *P. amarus* whose structure was established as 1-galloyl-2, 4-(acetonyl-dehydrohexahydroxydiphenoyl)-3, 6-hexahydroxy di phenoyl-glucopyranoside by chemical and spectroscopic methods (Foo and Wong, 1992) .

A novel cyclic hydrolysable tannin namely amarulone was obtained from the whole plant of *P. amarus* (Foo, 1993). *P. amarus* has been reported to possess didehydrohexahydroxyldiphenoyl hydrolysable tannin named amariin. In addition, geranin, corilagin, 1, 6-digalloylglucopyranoside, rutin, quercetin-3-o-glucopyranoside were isolated from the polar fraction of aerial parts of this herb (Foo and Lower, 1993).

Two new Securinega-type alkaloids isobubbialine and epibubbialine were isolated from the leaves of *P. amarus*. Other known alkaloids are securinine, norsecurinine, and phyllanthine, the structures of which have been detected by means of UV, IR, mass and NMR spectroscopy (Houghton *et al.*, 1996).

The whole plant of *P. amarus* contains new secosterols named as amarosterol-A characterized as 13, 14-seco-stigma-5(6), 14(15)-diene-3-a-ol (I) and amarosterol-B characterized as 13, 14- seco-stigma-9(11), 14(15)-diene-3-a-ol (II), whose structures have been elucidated on the basis of spectral and chemical studies (Ahmad and Alam, 2003). In addition, 2, 3, 5, 6-tetrahydroybenzyl acetate and phyllangin are the two new compounds isolated from the whole plant of *P. amarus* (Wei *et al.*, 2004).

P. amarus has the maximum reports of pharmaceutically important compounds isolated from aqueous or organic solvent extracts. The lignans such as phyllanthin, hypophyllanthin, niranthin, nirtetralin, virgatusin and

heliobupthalmin lactone are common to *P. amarus, P. maderaspatensis and P. virgatus* (Shanker *et al.*, 2011).

Flavanoids such as rutin, quercetin, quercitrin, kaempferol and astragalin are present in both *P. amarus* and *P. urinaria* (Foo and Wong, 1992). The lignin hinokinin has been isolated from *P. amarus, P. tenellus and P. virgatus* (Huang *et al.*, 2003). According to Londhe *et al.* (2008) hepatoprotective property of *P. amarus* is attributed to amariin and geraniin, whereas phyllanthin and hypophyllanthin have been suggested to be anti-inflammatory and antiapoptotic.

Decalactone isolated from *P. debilis* is shown to possess antihepatotoxic ability (Ahmed *et al.*, 2009). The lignin phyllanthin renders hepatoprotective property to *P. amarus* (Chirdchupunseree and Pramyothin, 2010). Anticancer or antitumour properties have been related to the presence of phyllanthin, hypophyllanthin, niranthin and polyphenols. Among these, niranthin is present *in P. fraternus, P. maderaspatensis, P. urinaria* and *P. virgatus* (Shanker *et al.*, 2011). Srirama *et al.* (2010) pointed out that phyllanthin and hypophyllanthin may not be the only compounds responsible for the hepatoprotective activity.

The antiviral activity in *P. tenellus* and *P. virgatus* is also attributed to niranthin, nirtetralin, hinokinin (Huang *et al.*, 2003).

The anti-inflammatory activity in *P.urinaria* is attributed to the phytochemicals, phyltetralin, phyllanthin, quercetin, rutin, rhamnocitrin and *β*-sitosterol (Santos *et al.*, 1995). The alkaloid norsecurinine is associated with antifungal property of *P. amarus* (Sahni *et al.*, 2005) and the compounds, *β*-sitosterol and *β*-amyrin are associated with analgesic property of *P. urinaria* (Recio *et al.*, 1995).

Two alkamides (E, E-2,4- octadienamide and E,Z-2,4- decadienamide) have been isolated from *P. fraternus* which contributes to the antiplasmodial property of the herb (Sittie *et al.*, 1998). Geraniin is the compound which is found common in *P. amarus, P. urinaria* and *P. virgatus*, which shows antiviral property (Yang *et al.*, 2007). The flavanoids (rutin and quercetin-3-0-glucoside) and ellagitannins

(geraniin, amariin, repandusinic acid,corilagin and Phyllanthusiin) in *P. amarus* and *P. urinaria* have a role in radio protective property (Londhe *et al.*, 2009).

2.5.1. Phyllanthin and hypophyllanthin content in herbaceous *Phyllanthus* spp.

Scientific community are largely attracted to phyllanthin and hypophyllanthin as a potent phytomolecule, than any other reported potent sources (Negi *et al.*, 2007). *P. amarus* has been reported to possess two lignans namely phyllanthin and hypophyllanthin obtained from the leaves of the plant that has been noted to enhance the cytotoxic responses with cultured multidrug-resistant cells (Somanabandhu, 1993).

According to Khatoon *et al.* (2006) phyllanthin is absent in *P. maderaspatensis*. Tripathi *et al.*, (2006) have reported that both phyllanthin and hypophyllanthin are present in *P. amarus* and *P. fraternus* but the concentration of these two lignans varies substantially in the two species. Sharma *et al.*, (2011) have reported the absence of phyllanthin and hypophyllanthin from *P. maderaspatensis* and *P.urinaria.*

2.6. DETECTION AND ESTIMATION OF MAJOR ACTIVE INGREDIENTS IN HERBACEOUS *Phyllanthus* spp.

A simple, precise and rapid high-performance thin-layer chromatographic method has been developed for the estimation of phyllanthin, an important lignan of *P. amarus*. Separation of phyllanthin was carried out on silica gel 60 F254 layers eluted with hexane: ethyl acetate (2:1), and the analytes were visualized through colour development with 10 per cent concentrated sulphuric acid in ethanol. Scanning and quantification of spots was performed at 200 nm. The proposed method being precise and sensitive, can be used for the detection, monitoring and quantification of phyllanthin from *P. amarus* (Nayak *et al.*, 2010)

Soxhlet assisted extraction of *P. amarus* leaves was carried out to recover the maximum extractable amount of phyllanthin. After the extraction, 10.62 ± 0.35 mg phyllanthin per gram of *P. amarus* leaves was obtained. Optimum chromatographic separation of phyllanthin was achieved with acetonitrile : water (45:55, *V/V*) with a flow rate of 1 ml min^{-1}. UV detection of analysis was carried out at 230 nm. The resulting chromatograms showed a retention time of 13.12 minutes for phyllanthin (Patel *et al.*, 2011).

2.7. ANTIOXIDANT ACTIVITY OF *Phyllanthus* spp.

Chemoprotective activity of 75 per cent methanolic extract of *P. amarus* plant was studied against cyclophosphamide (CTX) induced toxicity in mice. Administration of CTX produced significant myelosuppression as seen from the decreased WBC count and bone marrow cellularity. Administration of *P. amarus* extract at doses 250 and 750 mg per kg body weight significantly reduced the myelosuppression and improved the WBC count, bone marrow cellularity as well as the number of maturing monocytes that accounted for its chemoprotective activity (Kumar and Kuttan, 2005).

Kumaran and Karunakaran, (2007) conducted a study, to evaluate the antioxidant activity of methanol extracts of five plants from the genus *Phyllanthus*, by various antioxidant assays, including total antioxidant, free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, nitric oxide scavenging, reducing power and metal ion chelating activities. The various antioxidant activities were compared to standard antioxidants such as butylated hydroxytoluene and ascorbic acid. All the extracts showed strong antioxidant activity in all the tested methods. Among the five plants, *P. debilis* has been found to possess the highest activity in all tested models, the activity decreasing in the order *P. debilis* > *P. urinaria* > *P. virgatus* > *P. maderaspatensis* > *P. amarus*. Gheldof and Engeseth (2002) reported that, there is a linear correlation between the total phenol content and antioxidant activity.

Lim and Murtijaya (2006) evaluated the total phenolic content (TPC) and antioxidant activity of fresh and dried *P. amarus* plant materials using the Folin-Ciocalteau method, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity and ferric reducing antioxidant power (FRAP) assays. Different drying treatments led to a significant reduction in antioxidant properties of *P. amarus* methanolic extracts, with microwave drying causing the highest decrease in TPC and antioxidant activity exhibited by the reduction in both radical scavenging activity and FRAP. On the other hand, boiling water extracts appeared to exhibit significantly stronger antioxidant potentials even in dried plant materials due to greater solubility of compounds, breakdown of cellular constituents as well as hydrolysis of tannins.

The aqueous extract of the herb *P. fraternus* shows antioxidant property (Sailaja and Setty, 2006). Kumaran and Karunkaran (2007) reported that methanol extract of powdered air dried *P. amarus* showed high antioxidant activities.

In *P. urinaria*, the phytoconstituent, geraniin (ellagitannin) (Yang *et al.*, 2007), methyl gallate and trimethyl 1-3,4 dehydrochebulate (triterpene) shows antioxidant activity (Fang *et al.*, 2008). Antioxidant activity is shown by rutin, quercetin (flavanoids), amariin, repandusinic acid A, corilagin, Phyllanthusin, A, B, C, geraniin (ellagitannins) (Londhe *et al.*, 2008) and phyllanthin (lignin) (Krithika *et al.*, 2009) in *P. amarus.*

Lim and Murtijaya (2006) found that boiled water extract of the fresh and dried *P. amarus* plant had comparatively greater antioxidant activity than microwave assisted extraction method employed for the extraction.

Methanolic extract of *P. amarus* was found to inhibit lipid peroxidation and scavenge hydroxyl and superoxide radicals (Kiran *et al.*, 2011). Sen and Batra (2013) observed that the DPPH free radical scavenging activity was concentration dependent and reaches maximum at a concentration of 20 mol ml^{-1} for phyllanthin and 300 g ml^{-1} for *P. amarus* extract. Further, since phyllanthin possess very high

antioxidative property as evident by its low IC_{50} value of 7.4 mol ml^{-1} as compared to *P. amarus* extracts, its contribution in antioxidative effects is evident.

Eighty per cent methanol extracts obtained from seven *Phyllanthus* spp. were evaluated by Eldeen *et al.* (2011) for antiradical scavenging effects and phenolic contents using the DPPH assay and Folin–Ciocalteau colorimetric method, respectively. A remarkable DPPH scavenging effect was observed with *P. myrtifolius, P. reticulatus* and *P. urinaria* (IC_{50} of 10.2, 10.8 and 17.4 µg/ml, respectively). Highest total phenolic contents were recorded for *P. myrtifolius and P. urinaria* (207 and 205 mg/GAE/g respectively). With the exception of *P. amarus*, *P. debilis* and *P. pulcher*, total phenolic contents were correlated with DPPH radical scavenging activity.

Nimmi *et al.* (2012) conducted a comparative study of antioxidant properties on two varieties of *Phyllanthus* (*P. niruiri* and *P. urinaria*) leaves growing in Bangladesh. Five complimentary test methods namely DPPH free radical scavenging activity, reducing power assay, total antioxidant capacity, total phenolic and flavonoid contents determination were used for this. Based on the concentrations expressed by the ratio of crude sample per solvent volume, the investigated *P. niruri* and *P. urinaria* leaf extracts exhibited significant results. At two mg ml^{-1} concentration, DPPH radical scavenging capacity of the methanol extract of both the plants was found to show significant (>90%) activity which is comparable to ascorbic acid and butylated hydroxyl anisole. In case of reducing power tests for both the extract, the activity of *P. urinaria* is comparable to that of ascorbic acid and butylated hydroxyanisole (BHA) and that of *P. niruri* is comparable to BHA. *P. niruri* showed better antioxidant potential than *P. urinaria,* based on the observed results of their corresponding methanol extracts.

Sen and Batra (2013) studied the free radical scavenging activity of *in vitro* callus, induced using internodal explant on MS (Murashige and Skoog) medium fortified with 2,4-D (0.6 mg/l) using 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. The analysis was carried out for *in vivo* plant and *in vitro* callus

to determine the quantitative phenolic content along with their antioxidant activity of different plant extracts. The result of the present study showed that the methanol extract of *P. amarus* contains highest amount of phenolic compounds and exhibits the greatest anti-oxidant activity in comparison to other extracts. It was observed that, the *in vitro* plant extract shows more phenolic contents and revealed better antioxidant activity as compared to *in vivo* plant extraction.

The antioxidant property of the 70 per cent aqueous ethanol extract of *P. amarus* roots and its ether-soluble, ethyl acetate-soluble, and aqueous fractions was investigated by various *in vitro* assays. The root extracts showed higher DPPH, hydroxyl, superoxide, and nitric oxide radical scavenging and reducing power activity. Among all the samples, the ethyl acetate-soluble fraction demonstrated highest radical scavenging activity and total phenolic content. Twenty-eight different phenolic compounds were identified by LCMS analysis of the ethyl acetate-soluble fraction. Majority of the compounds were found to exist as their glycosides, and many of these were gallic acid derivatives. Free epicatechin and gallic acid were also identified in the ethyl acetate-soluble fraction. The present investigation suggested that *P. amarus* root is a potent antioxidant and can be used for the prevention of diseases related to oxidative stress (Maity *et al.*, 2013).

2.8. POST HARVEST HANDLING OF RAW DRUGS OF *Phyllanthus* spp.

Extraction studies conducted by Manjusha (2010) revealed that ethanolic extraction was the best extraction method, recording 0.07 per cent phyllanthin and 0.89 per cent hypophyllanthin, with a recovery of 18.66 per cent. The extract obtained through vapour heat treatment followed by mechanical pressing, recorded zero phyllanthin and negligible amount of hypophyllanthin. Shade dried whole plant recorded highest amount of alkaloids, viz., phyllanthin (0.125 per cent) and hypophyllanthin (0.186 per cent), where as sun dried chopped material recorded least amount of phyllanthin (0.013 per cent) and hypophyllanthin (0.121

per cent). Phyllanthin content was reduced gradually with the advancement of storage period.

2.9. SPECIES ADMIXTURES IN RAW DRUG SAMPLES OF *Phyllanthus* spp.

Adulteration and substitution are frequent in raw material trade of medicinal plants. Herbal adulteration is one of the common malpractices in herbal raw material trade. The deforestation and extinction of many species and incorrect identification of plants have resulted in adulteration and substitution of raw drugs (Mukharjee, 2002). Adulteration in market samples is one of the greatest drawbacks in promotion of herbal products. In adulterated drugs, it is invariably found that the Adverse Event Reports (AER) is not due to the intended herb, but rather due to the presence of an unintended herb (Uniyal and Joshi, 1993).

Adulteration is a practice of adding foreign substance in place of original crude drug partially or fully, which is inferior or substandard in therapeutic and chemical properties or addition of low grade or spoiled drugs or entirely different drugs similar to that of original drug adding with an intention of enhancement of profits (Nair *et al.*, 1983).

Many substitute drugs are mentioned in Ayurvedic texts. The principles to select substitute drugs are based on similarity of properties (Rasa, Guna Virya and Vipaka), but most important factor is therapeutic action (Karma). In terms of pharmacology, a substitute is generally used when original drugs are not available or may be available in small quantity. In ancient times, the vaidyas had to collect the drug on their own. The drugs which were less available in local area were replaced by other drugs known as substitute drugs (Pratinidhi Dravyas). The ancient acharyas e.g. Charaka and Sushruta have not given direct references or listed substitute drugs but, Acharya Vagbhata has stated that in case of non availability of any particular drug in the preparation of compound formulations, one should try to get another, which is similarly potent and has similar Rasa (Taste), Guna (Property), Virya (Potency) and Vipaka. Detailed description

regarding substitute drugs can be traced from the text books like Bhavaprakasha (Author Bhavamishra, 16th century), Yogaratnakara (Author Unknown, 17th century) and Bhaishajya ratnavali (Author Govind Das,14th century) (Mitra and Kannan, 2007).

2.9.1. Similarity in Morphology

Mucuna pruriens is adulterated with other similar Papilionaceae seeds having similarity in morphology. *M. utilis* (sold as white variety) and *M. deeringiana* (sold as bigger variety) are popular adulterants. Apart from this *M. cochinchinensis*, *Canavalia virosa* and *Canavalia ensiformis* are also sold in Indian markets. Authentic seeds are up to one cm in length with shining mosaic pattern of black and brown colour on their surface. *M. deeringiana* and *M. utilis* are bigger (1.5-2 cm) in size, while *M. deeringiana* is dull black and *M. utilis* is white or buff coloured (Neelam *et al.,* 2014).

2.9.2 Substitution of the species belonging to same family

The *Datura metal* and *Datura stramonium* which contain chemical constituents like alkaloids - scopalamine, atropine, hyocyamine, Hyoscine etc. The alkaloids are proved as bronchodialator, relaxant and inhibitor of secretion of mucous membrane. The alcoholic extract of *D. metal* shows anti helmentic activity. The alkaloid present in both the species are well proven bronchodilators and also they inhibit the secretion of mucous membrane of the respiratory tract. Thus, as far as the diseases of the respiratory tract are concerned both *D.metal* and *D. stramonium* are beneficial, while as Krimihara *D.metal* would be a better choice as it is a proven antihelmentic (Neelam *et al.,* 2014).

2.9.3. Confusion in vernacular names

Neelam *et al.* 2014 has also reported that, in Ayurveda, Parpata refers to *Fumaria parviflora* and in Siddha, 'Parpadagam' refers to *Mollugo pentaphylla.* Owing to the similarity in the names in traditional systems of medicine, these two herbs are often interchanged or adulterated. Because of the popularity of Siddha

medicine in some parts of South India, traders in these regions supply *Mollugo pentaphylla* as Parpata/Parpadagam and the North Indian suppliers supply *F. parviflora*. These two can be easily identified by the presence of pale yellow to mild brown coloured, thin wiry stems and small simple leaves of *Mollugo pentaphylla* and black to dark brown coloured, digitate leaves with narrow segments of *F. parviflora*. *Casuarina equisetifolia* for *Tamarix indica* and *Aerva lanata* for *Berginia ciliata* are some other examples for adulterations due to confusion in names.

P. amarus is sold in both fresh and dry forms by local traders in markets. Morphological, biochemical and anatomical characterization play a vital role in crude drug standardization (Agarwal *et al*., 2013). Of the *Phyllanthus* market samples, about 76 per cent contains *P. amarus* Schum. & Thonn. and the remaining percentage is shared by several other species (Srirama *et al*., 2010).

Materials and Methods

3. MATERIALS AND METHODS

The present study on "Survey, collection and characterization of *'Kizharnelli'* (*Phyllanthus* spp.) of Kerala" was carried out at Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara, Thrissur and Analytical Laboratory, CARe KERALAM, Thrissur during 2014-2015. The details regarding the experimental materials used and methodology adopted for conducting various aspects of the study are presented in this chapter.

The whole programme was carried out in two experiments, as detailed below

3.1. EXPERIMENT 1

3.1.1. Survey collection and characterization of accessions of *Phyllanthus* spp.

Exploratory surveys were conducted and accessions of *Phyllanthus* spp. collected from different agro-ecological zones of Kerala, (coastal areas. plains. midlands and high altitude regions) representing northern. central and southern Kerala. The collected accessions were raised in pots as well in the experimental field of Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara.

3.1.2 Location

The experimental site is situated at 12^0 32' N latitude and 74^0 20' E longitude at an altitude of 22.5 m above mean sea level. The area enjoys a typical warm humid tropical climate.

3.1.3 Methodology

The survey was carried out in high ranges, midlands, coastal regions and plains of southern, central and northern zones of Kerala, during July–August 2014, to explore the agro ecological diversity in *Phyllanthus* spp (Plate 1). Purposive sampling procedure was adopted during the survey, as the diversity and density of *Phyllanthus* was confined only to certain pockets, all over Kerala.

Plate 1. Survey and collection of *Phyllanthus* accessions

As per the purposive sampling procedure, preliminary survey of the area to be explored was done before the actual collection. The location of sampling sites and collection areas were planned in advance.

Thrissur district in central zone, Wayanad, Kasargod and Kozhikode districts in northern zone and Thiruvananthapuram district in southern zone were selected as the study areas for the conduct of survey.

In Wayanad, Kasargod and Kozhikode districts representing northern zone, survey was conducted at Bekal in Kasargod representing coastal areas in Pallikkara panchayat, Thachangad, Periyattadukkam and Thekkil in Kasargod representing plains, Adivaaram in Kozhikode representing midlands and Kallur, Kuruva, Puthur vayal in Wayanad representing high altitude regions during July 2014 and a total of 16 accessions were collected.

Thrissur district represented central zone in the survey. Survey was conducted during July 2014, and accessions were collected from Athirapally, Chavakkad, Guruvayur and Mannuthy representing high altitude region, coastal regions, plains and midlands respectively. A total of 14 accessions were collected from central zone.

Thiruvananthapuram district was selected for survey, representing southern zone. Survey was conducted during August 2014 and accessions were collected from Palode, representing high altitude regions, Sangumukham and Valiyathura representing coastal regions, Chippinchira, Irumbupalam representing midlands, and Thambanur representing plains. A total of 17 accessions were collected from southern zone.

The species being cross pollinated, accessions were collected as seedlings to ensure true representation of the species. The accessions were collected from waste lands, road sides, sea shores and uncultivated lands. In each accession, ten plants per accession were collected. The collected accessions were raised in pots for the assessment of distinct morphological characters. Voucher specimens and passport data of collected accessions were prepared. The passport data of the

collected accessions contained details on collection number, date of collection, collection source, village, district, latitude and longitude. The accessions raised in pots were evaluated for growth and yield parameters.

3.1.4. Preparation of herbarium sheets

The voucher herbarium specimens were prepared by pressing the specimens in a plant press, which consists of a wooden frame (for rigidity), corrugated cardboard ventilators (to allow air to flow through the press), and a blotter paper, typically a newspaper (to contain the plant material). Fresh and good specimens were selected and carefully arranged in the press to maximize preservation of diagnostic features. To prevent shrinkage and wrinkling of the plant material, the plant press was tightened using straps with buckles or bolts with wing nuts which ensure the extraction of moisture in the shortest period of time. To fix on a standard herbarium sheet, the plant specimen was pressed flat. As the specimens dried, it was necessary to further tighten the straps on the press to minimize shrinkage and wrinkling. The press dried specimens were mounted on the herbarium sheets using glue (Plate 2). The specimens were labeled which include informations like collector's name, scientific name, location details, habitat, plant habit, distinct plant features, date of collection and collection number, which can be preserved long term (Jain and Rao, 1977).

3.1.5. Morphological characterization

Morphological key characters observed for collected accessions included both quantitative and qualitative parameters. The quantitative parameters and qualitative character observed are listed in Table 3. *Phyllanthus* accessions were characterized employing NBPGR, descriptor (Singh *et al.*, 2003). The collected accessions were decoded to respective species using the key characters, reported in Flora of Madras Presidency (Gamble and Fischer, 1915-1936).

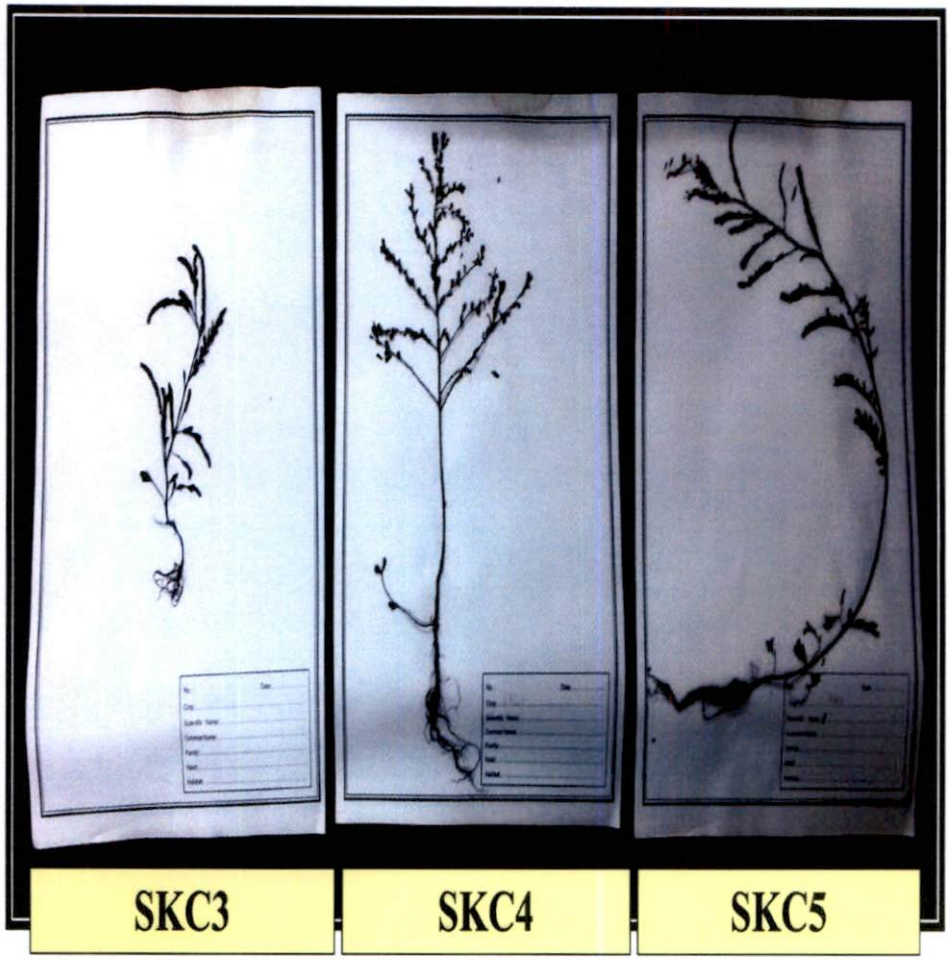


Plate 2. Voucher specimens for collected *Phyllanthus* accessions

Table 3. Morphological key characters observed for collected *Phyllanthus* accessions

Qualitative characters		
Growth characters	Growth habit Branching pattern	Recorded at flower initiation stage Recorded at flowering initiation stage
Stem characters	Stem colour Stem shape	Recorded at flower initiation stage Recorded at flower initiation stage
Leaf characters	Leaf shape Leaf apex Leaf margin Leaflet colour Leaf base Rachis colour	Recorded at flower initiation stage Recorded at flower initiation stage Recorded at flower initiation stage Recorded full foliage stage Recorded at flower initiation stage Recorded at flower initiation stage
Fruit characters	Capsule colour Capsule texture Capsule shape Peduncle colour	Recorded at near maturity stage Recorded at near maturity stage Recorded at near maturity stage Recorded at near maturity stage
Flower characters	Flower colour	Recorded at full bloom stage
Quantitative characters (Morphological)		
Stem characters	Stem height No. of branches per plant	Measured from ground level to the extended foliage of the plant at capsule maturity stage Recorded from the base of the stem at flowering stage
Leaf characters	Leaf length Leaf width Number of leaflets per compound leaf	Recorded at flower initiation stage Recorded at flower initiation stage Recorded at flower initiation stage
Fruit characters	Number of capsules per branch	Recorded at near maturity stage
Flower characters	No. of sepals	Recorded at full bloom stage
Yield parameters	Fresh weight per plant (g) Dry weight (g) per plant	Recorded on fresh weight basis of whole plant at complete flowering stage
Quantitative characters (Biochemical)		
Biochemical attributes	Content of total extractives (g) Content of total phenol (mg/g) Phyllanthin content (%) Antioxidant capacity (µg/ml)	Collected whole plants at complete flowering stage

3.1.6 Cluster analysis of *Phyllanthus* accession

Data based on qualitative and quantitative characters of the *Phyllanthus* accessions were compared with Euclidean coefficient and was clustered by the Unweighed Pair Group Average Method (UPGAM) developed by Sneath and Snokel (1973) using NTSYS pc 2.02 software. Similarly, matrices were computed and dendrograms were constructed accordingly.

3.1.7 Pot culture of *Phyllanthus* accessions

The collected *Phyllanthus* accessions were grown in plastic pots of 60 cm diameter containing sand, soil and farm yard manure, in the ratio 1:1:1. The plants were regularly irrigated and weeded and manured with dry cowdung powder (Plate 3). The planted accessions were evaluated based on the field establishment, production of new shoots and overall performance in the prevailing conditions. The species wise, region wise and zone wise performance of the accessions were assessed by observing biometric characters like plant length (cm), number of branchlets per plant, number of leaflets per compound leaf and east-west spread (cm) and yield parameters like fresh and dry weights per plant (g).

Design: Completely Randomized Design (CRD)

Number of replications: 3

Number of plants per replication: 10

3.1.8. Biochemical attributes

Biochemical analyses of the collected accessions were done at CARe KERALAM, Koratty, Thrissur.

3.1.8.1. *Estimation of content of total extractives of collected accessions*

Solvent extraction was carried out in a batch process using analytical grade solvents for the estimation of content of total extractives. The extraction was performed in a soxhlet apparatus consisting of a 500 ml round bottom flask, 100 ml extractor and a condenser. The extractor was loaded

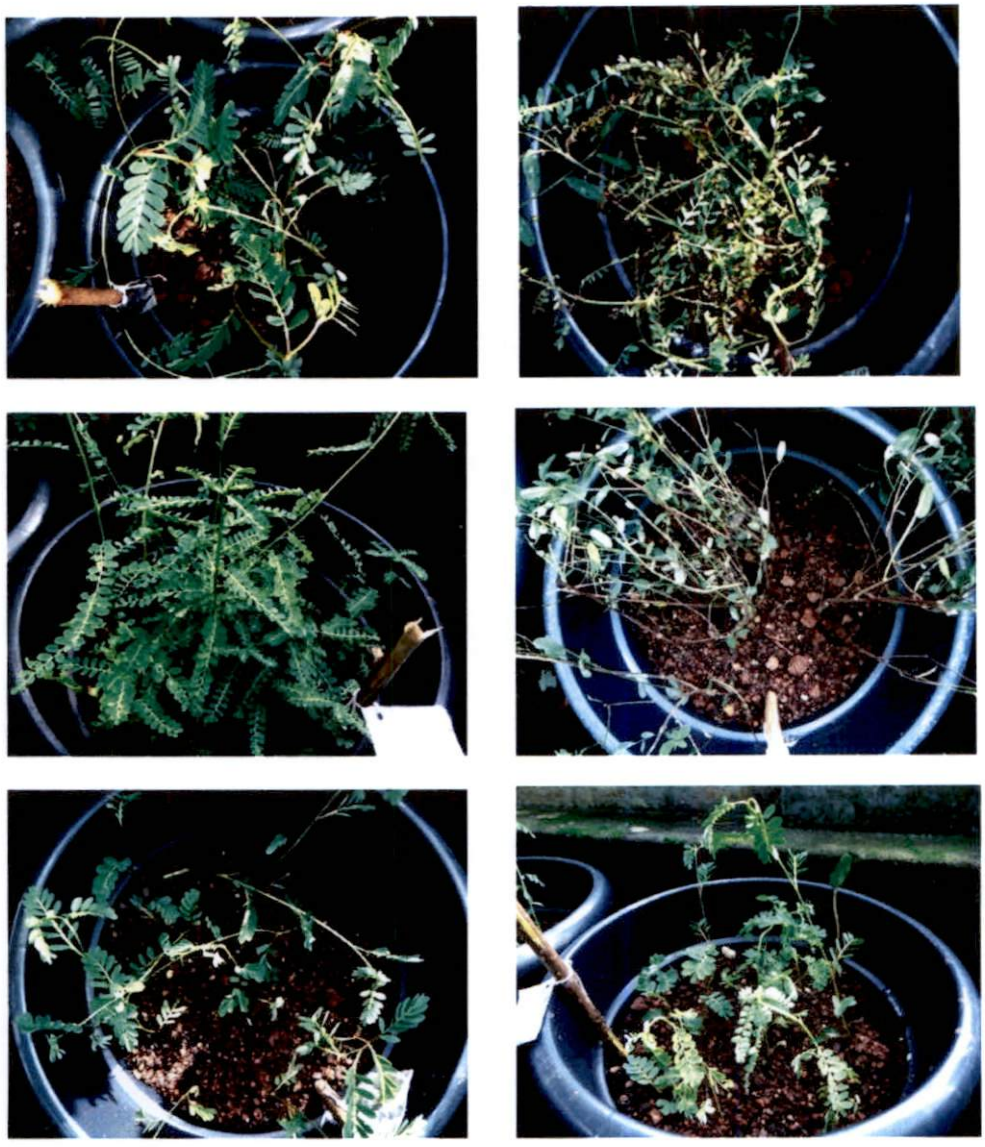


Plate 3. Pot culture of collected *Phyllanthus* accessions

with 5 g sample in a filter paper packet and made into a thimble. The solvent used was chloroform, which was taken in a round bottom flask. The whole apparatus was heated to a temperature of 100° C in a water bath. When the liquid reached the overflow level a siphon aspirated the solution of extractor and unloaded it back to the flask. During each cycle, a portion of the non volatile compounds dissolved in the solvent. After many cycles, the desired compound was concentrated in the distillation flask. The operation was repeated until the complete extraction was achieved. After the extraction, the solvent was removed by evaporating, yielding the extracted compound. The non-soluble portion of the extracted solid remained in the thimble, and was discarded. The extract was transferred to a 50 ml beaker and the solvent left out was evaporated. The difference between the initial weight of the beaker and final weight after the addition of the extract gave the total content of extract in the plant sample, which was expressed in grams.

3.1.8.2. ***Estimation of content of phyllanthin in collected Phyllanthus accessions***

Estimation of phyllanthin was done based on the procedure outlined by Sharma *et al.* (1993). The phyllanthin standard was procured from Sigma Aldrich. Bangaluru, during March, 2015. The methanol extracts (100 mg) and aqueous extracts (300 mg) of *Phyllanthus* spp. were dissolved in 10 ml of HPLC grade methanol. They were sonicated again for 6 minutes and filtered with 0.22 µ filters. The filtrate was used for estimation of phyllanthin using HPLC. The procured phyllanthin dissolved separately at a concentration of 0.16 mg ml^{-1} in methanol was used as the standard. Phyllanthin was detected using a C18 column at 230 nm in HPLC. The mobile phase consisted of acetonitrile and phosphate buffer in the ratio of 83:17. The phyllanthin standard was used for obtaining the standard curves. The flow rate was adjusted to 1.9 ml min^{-1}. The presence of phyllanthin in the extracts was determined depending on the retention time, and the concentrations corresponding to their peak area was estimated using the standard curves obtained from reference phyllanthin.

3.1.8.3. *Estimation of total content of phenols*

The total phenolic content of plant extracts was determined using Folin-ciocalteu reagent (Yu *et al.*, 2002). The plant extracts (100 µl) was mixed with 500 µl of the Folin-ciocalteu reagent and 1.5 ml of 20 per cent sodium carbonate. The mixture was shaken thoroughly and made up to 10 ml using distilled water. The mixture was allowed to stand for 2 hrs. Then the absorbance at 765nm was determined. These data were used to estimate the phenolic contents using a standard curve obtained from various concentration of gallic acid.

Content of total phenol (mg/g) =

$$= \frac{\text{Optical density of the test}}{\text{Optical density of the standard}} \times \frac{\text{Concentration of the standard}}{\text{Volume of the sample taken}} \times 100$$

3.1.8.4. *In vitro antioxidant capacity*

The free radical scavenging activity of the extracts, based on the scavenging activity of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical, was determined by the method described by Braca *et al.* (2001). Plant extract (0.1 ml) was added to 3 ml of a 0.004 per cent methanol solution of DPPH. Absorbance at 517 nm was determined after 30 min, and the percentage inhibition activity was calculated from $[(A_0-A_1)/A_0] \times 100$, where A_0 is the absorbance of the control, and A_1 is the absorbance of the extract per standard.

3.2. EXPERIMENT - II

3.2.1. Assessment of quality of traded crude drug of *Phyllanthus* in Kerala

Samples of traded crude drug of *Phyllanthus* was procured from user industries like Oushadhi, Thrissur and Kottakkal Aryavaidyasala, Kottakkal, Malappuram.

Species admixtures in the collected samples were analysed using morphotaxonomic keys which are used to distinguish *Phyllanthus* spp. and by organoleptic evaluation of characters like apperance, colour, flavour, texture.

odour, taste, after taste, species admixtures and over acceptability using nine point hedonic scale (Table 4).

Table 4. Nine point Hedonic scale

Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

The scores of organoleptic evaluation were statistically analysed by Kendall's Concordance Test to test the level of significance. The traded crude drug of *Phyllanthus* were analysed for content of total extractives, content of total phenol, content of phyllanthin and for antioxidant capacity. The biochemical parameters of raw drug samples of *Phyllanthus* were compared with genuine reference samples that were collected by surveying different agro ecological zones to determine the extent of adulteration or substitution.

Results

4. RESULTS

The present study was conducted at Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara, Thrissur and CARe KERALAM. Koratty, Thrissur, during 2013-2015 with the objective of morphological and biochemical characterization of herbaceous *Phyllanthus* spp. of Kerala and to assess species admixtures of crude drug of *Phyllanthus* in traded samples. The results of the study are presented in this chapter.

4.1. EXPERIMENT I – SURVEY, COLLECTION AND CHARACTERIZATION OF *Phyllanthus* ACCESSIONS FROM VARIOUS ZONES OF KERALA.

4.1.1 Survey and collection of *Phyllanthus* accessions from various zones of Kerala.

The survey was conducted to elucidate information on the variance of *Phyllanthus* spp., throughout Kerala. The zone wise classification of Kerala as outlined by Kerala Agricultural University was adopted to facilitate the study on topographical and temporal variations of the species. The survey was conducted in all the three zones, namely, south, central and north. Among the zones itself, further classification was done based on altitude to study the influence of micro climate favouring a habitat for *Phyllanthus* as well. Thus, the survey was intensively carried out in the high ranges, plains, coastal and midlands regions of all the zones. The number of accessions collected altitude wise and zone wise, are listed in the Table 5a. A total of fourty seven *Phyllanthus* accessions were collected from coastal, plains, midlands and high ranges of southern (Plates 4(A), 4(B), 4(C) and 4(D), central (Plates 5(A), 5(B), 5(C) and 5(D)) and northern zones (Plates 6(A), 6(B), 6(C) and 6(D)) of Kerala.

Table 5a. *Phyllanthus* accessions collected from southern, central and northern zones of Kerala

Sl. No	Zones	Temporal sites	No. of collected accessions	Code of the collected accessions
1	Southern Kerala (SK)	Coastal (C)	4	SKC1, SKC2, SKC3, SKC4
		Plain (P)	3	SKP1, SKP2,SKP3
		Midland (M)	4	SKM1, SKM2, SKM3,SKM4
		High range (H)	6	SKH1, SKH2, SKH3, SKH4, SKH5, SKH6
2	Central Kerala (CK)	Coastal (C)	3	CKM1, CKM2, CKM3
		Plain (P)	3	CKM1, CKM2, CKM3
		Midland (M)	5	CKM1, CKM2, CKM3,CKM4,CKM5
		High range (H)	3	CKM1, CKM2, CKM3
3	Northern Kerala (NK)	Coastal (C)	3	NKM1, NKM2, NKM3
		Plain (P)	6	NKP1, NKP2, NKP3, NKP4, NKP5, NKP6
		Midland (M)	3	NKM1, NKM2, NKM3
		High range (H)	4	NKM1, NKM2, NKM3, NKM4

Plate 4 . *Phyllanthus* accessions collected from southern zone

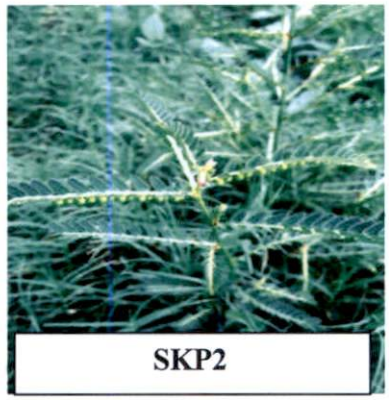
SKC1

SKC2

SKC3

(A). Coastal region

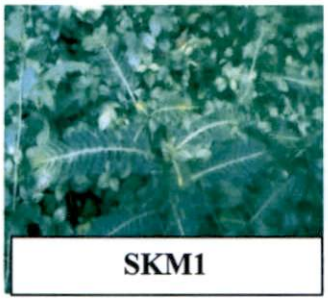
SKP1



SKP2

SKP3

(B). Plains



SKM1

SKM2

SKM3

SKM4

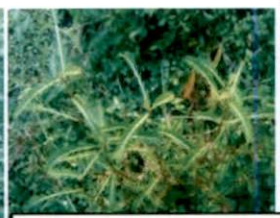
(C). Midlands

SKH1

SKH2

SKH3

SKH4



SKH5

SKH6

(D). High ranges

CKM1

CKM2

CKM3

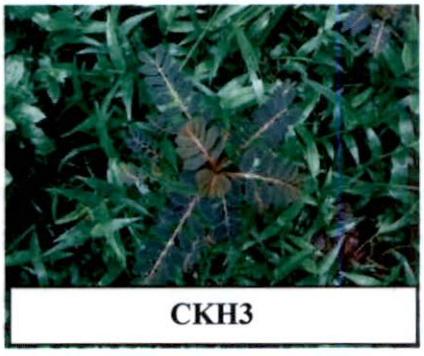
CKM4

CKM5

(C). Midlands

CKH1

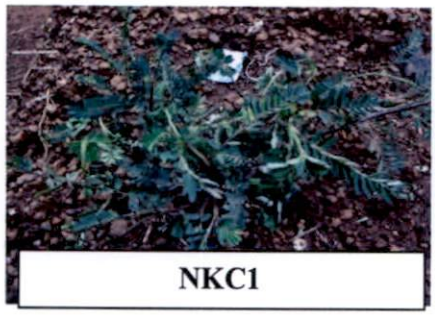
CKH2



CKH3

(D). High ranges

Plate 6. *Phyllanthus* accessions collected from northern zone

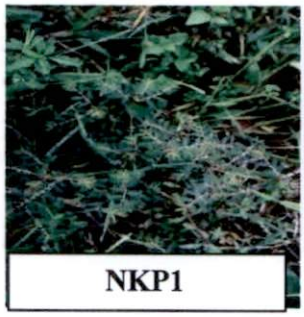


NKC1

NKC2

NKC3

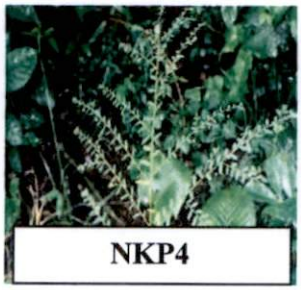
A). Coastal regions



NKP1

NKP2

NKP3



NKP4

NKP5

NKP6

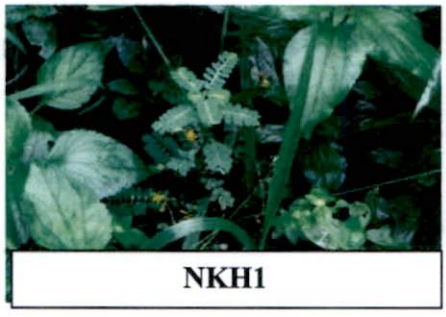
(B). Plains

NKM1

NKM2

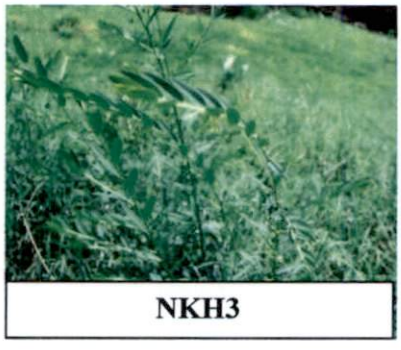
NKM3

(C). Midlands



NKH1

NKH2



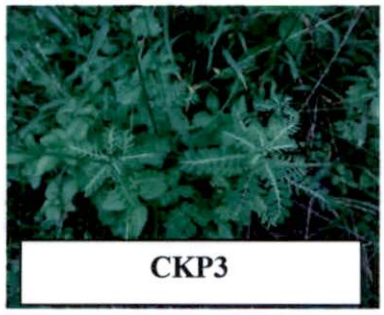
NKH3

NKH4

(D). High ranges

Plate 5. *Phyllanthus* accessions collected from central zone

(A). Coastal regions



(B). Plains

The passport data of collected accessions are presented in Tables 5b, 5c and 5d which include details on collector's number, date of collection, village, district, latitude and longitude. The accessions were collected from roadsides, seashores, garden lands and uncultivated lands. Maximum number of accessions was collected from road sides followed by uncultivated lands. Garden lands harboured comparatively lesser population of herbaceous *Phyllanthus* spp. under study.

4.1.2. Morphological characterization of collected *Phyllanthus* accessions

The fourty seven *Phyllanthus* accessions were characterized based on morphological traits. Both qualitative and quantitative characters were considered for morphological characterization of the accessions. NBPGR descriptor was used for characterization of *Phyllanthus* accessions. The results based on the characterization of the collected accessions are presented below.

4.1.2.1. *Characterization of Phyllanthus accessions based on morphological qualitative characters*

Out of the fifteen qualitative characters observed, no notable variability was observed for eight qualitative characters viz., growth habit, branching pattern, stem shape, leaf margin, flower colour, capsule colour, capsule texture and capsule shape (Hence, these eight characters were not considered in the further characterization (Tables 6a, 6b and 6c). The characters with less variability are presented in the Table 6d, along with their general observations on them.

Table 5b. Passport data of collected accessions of *Phyllanthus* species from southern zone

Collector's No.	Date of collection	Collection source	Village	District	Latitude	Longitude
SKM1	2-8-2014	Roadside	Nedumangad	Thiruvanathapuram	8.4875^0 N	76.9525^0 E
SKM2	2-8-2014	Roadside	Nedumangad	Thiruvanathapuram	8.4875^0 N	76.9525^0 E
SKM3	2-8-2014	Uncultivated land	Nedumangad	Thiruvanathapuram	8.4875^0 N	76.9525^0 E
SKM4	2-8-2014	Uncultivated land	Nedumangad	Thiruvanathapuram	8.4875^0 N	76.9525^0 E
SKC1	2-8-2014	Seashore	Valiyathura	Thiruvanathapuram	8.4784^0 N	76.9119^0 E
SKC2	2-8-2014	Seashore	Shangumugham	Thiruvanathapuram	8.4811^0 N	76.9124^0 E
SKC3	2-8-2014	Seashore	Shangumugham	Thiruvanathapuram	8.4811^0 N	76.9124^0 E
SKC4	2-8-2014	Seashore	Shangumugham	Thiruvanathapuram	8.4811^0 N	76.9124^0 E
SKP1	2-8-2014	Roadside	Thampanoor	Thiruvanathapuram	8.48633^0 N	76.95170^0 E
SKP2	2-8-2014	Roadside	Thampanoor	Thiruvanathapuram	8.48633^0 N	76.95170^0 E
SKP3	2-8-2014	Roadside	Thampanoor	Thiruvanathapuram	8.48633^0 N	76.95170^0 E
SKH1	1-8-2014	TBGRI garden	Palode	Thiruvanathapuram	8.7033^0 N	77.0264^0 E
SKH2	1-8-2014	TBGRI garden	Palode	Thiruvanathapuram	8.7033^0 N	77.0264^0 E
SKH3	1-8-2014	TBGRI garden	Palode	Thiruvanathapuram	8.7033^0 N	77.0264^0 E
SKH4	1-8-2014	TBGRI garden	Palode	Thiruvanathapuram	8.7033^0 N	77.0264^0 E
SKH5	1-8-2014	TBGRI garden	Palode	Thiruvanathapuram	8.7033^0 N	77.0264^0 E
SKH6	1-8-2014	TBGRI garden	Palode	Thiruvanathapuram	8.7033^0 N	77.0264^0 E

Table 5c. Passport data of collected accessions of *Phyllanthus* species from central zone

Collector's No.	Date of collection	Collection source	Village	District	Latitude	Longitude
CKM1	20.7.2014	Roadside	Mannuthy	Thrissur	10.5289 ^{0}N	76.2624 ^{0}E
CKM2	20.7.2014	Roadside	Mannuthy	Thrissur	10.5289 ^{0}N	76.2624 ^{0}E
CKM3	20.7.2014	Uncultivated land	Mannuthy	Thrissur	10.5289 ^{0}N	76.2624 ^{0}E
CKM4	20.7.2014	Uncultivated land	Mannuthy	Thrissur	10.5289 ^{0}N	76.2624 ^{0}E
CKM5	20.7.2014	Uncultivated land	Mannuthy	Thrissur	10.5289 ^{0}N	76.2624 ^{0}E
CKC1	20.7.2014	Seashore	Chavakkad	Thrissur	10.5833 ^{0}N	76.0188 ^{0}E
CKC2	20.7.2014	Seashore	Chavakkad	Thrissur	10.5833 ^{0}N	76.0188 ^{0}E
CKC3	20.7.2014	Seashore	Chavakkad	Thrissur	10.5833 ^{0}N	76.0188 ^{0}E
CKP1	20.7.2014	Uncultivated land	Ariyannur	Thrissur	10.6039 ^{0}N	76.0812 ^{0}E
CKP2	20.7.2014	Uncultivated land	Ariyannur	Thrissur	10.6039 ^{0}N	76.0812 ^{0}E
CKP3	20.7.2014	Uncultivated land	Ariyannur	Thrissur	10.6039 ^{0}N	76.0812 ^{0}E
CKH1	21.7.2014	Uncultivated land	Athirappilly	Thrissur	10.2886 ^{0}N	76.5483 ^{0}E
CKH2	21.7.2014	Uncultivated land	Athirappilly	Thrissur	10.2886 ^{0}N	76.5483 ^{0}E
CKH3	21.7.2014	Uncultivated land	Athirappilly	Thrissur	10.2886 ^{0}N	76.5483 ^{0}E

Table 5d. Passport data of collected accessions of *Phyllanthus* species from northern zone

Collector's No.	Date of collection	Collection source	Village	District	Latitude	Longitude
NKM1	13-7-2014	Road side	Adivaaram	Kozhikode	11.48805^0 N	76.0130^0 N
NKM2	13-7-2014	Road side	Adivaaram	Kozhikode	11.48805^0 N	76.0130^0 N
NKM3	13-7-2014	Road side	Adivaaram	Kozhikode	11.48805^0 N	76.0130^0 N
NKC1	12-7-2014	Seashore	Bekal	Kasargod	12.39220 N	75.03250 E
NKC2	12-7-2014	Seashore	Bekal	Kasargod	12.39220 N	75.03250 E
NKC3	12-7-2014	Seashore	Bekal	Kasargod	12.39220 N	75.03250 E
NKP1	12-7-2014	Road side	Periyattadukkam	Kasargod	12.50330N	74.98960N
NKP2	12-7-2014	Road side	Thachangad	Kasargod	12.50330N	74.98960N
NKP3	12-7-2014	Road side	Thachangad	Kasargod	12.50330N	74.98960N
NKP4	12-7-2014	Road side	Thekkil	Kasargod	12.50330N	74.98960N
NKP5	12-7-2014	Road side	Periyattadukkam	Kasargod	12.5033^{0}N	74.9896^{0}N
NKP6	12-7-2014	Road side	Periyattadukkam	Kasargod	12.5033^{0}N	74.9896^{0}N
NKH1	14-7-2014	Uncultivated lands	Puthur vayal	Wayanad	11.6304^{0}N	76.0867^{0}N
NKH2	14-7-2014	Uncultivated lands	Puthur vayal	Wayanad	11.6304^{0}N	76.0867^{0}N
NKH3	14-7-2014	Uncultivated lands	Puthur vayal	Wayanad	11.6304^{0}N	76.0867^{0}N
NKH4	14-7-2014	Uncultivated lands	Kallur	Wayanad	11.7506^{0}N	76.0031^{0}N

Table 6a. Non-variable qualitative characters of *Phyllanthus* accessions in southern zone

Zone	Temporal sites	Acc. No.	Growth habit	Branching pattern	Stem shape	Leaflet margin	Flower colour	Capsule colour	Capsule texture	Capsule shape
Southern Kerala (SK)	Midlands (M)	SKM1	Erect	Non-spreading	Terete	Entire	Pale green	Yellowish green	Rough	Depressed globose
		SKM2	Erect	Non-spreading	Terete	Entire	Pale green	Yellowish green	Smooth	Depressed globose
		SKM3	Erect	Non-spreading	Angular	Entire	Pale green	Yellowish green	Smooth	Depressed globose
		SKM4	Erect	Non-spreading	Angular	Entire	Pale green	Yellowish green	Smooth	Depressed globose
	Coastal (C)	SKC1	Erect	Non-spreading	Terete	Entire	Pale green	Yellowish green	Smooth	Depressed globose
		SKC2	Erect	Non-spreading	Angular	Entire	Pale green	Yellowish green	Smooth	Depressed globose
		SKC3	Erect	Non-spreading	Angular	Entire	Pale green	Yellowish green	Smooth	Depressed globose
		SKC4	Erect	Non-spreading	Terete	Entire	Pale green	Yellowish green	Rough	Depressed globose
	Plains (P)	SKP1	Erect	Non-spreading	Terete	Entire	Pale green	Yellowish green	Smooth	Depressed globose
		SKP2	Erect	Non-spreading	Terete	Entire	Pale green	Yellowish green	Rough	Depressed globose
		SKP3	Erect	Non-spreading	Terete	Entire	Pale green	Yellowish green	Rough	Depressed globose
	High ranges (H)	SKH1	Erect	Non-spreading	Terete	Entire	Pale green	Yellowish green	Smooth	Depressed globose
		SKH2	Erect	Non-spreading	Angular	Entire	Pale green	Yellowish green	Smooth	Depressed globose
		SKH3	Erect	Non-spreading	Angular	Entire	Pale green	Yellowish green	Smooth	Depressed globose
		SKH4	Erect	Non-spreading	Terete	Entire	Pale green	Yellowish green	Rough	Depressed globose
		SKH5	Erect	Non-spreading	Angular	Entire	Pale green	Yellowish green	Smooth	Depressed globose
		SKH6	Erect	Non-spreading	Terete	Entire	Pale green	Yellowish green	Rough	Depressed globose

Table 6b. Non-variable qualitative characters of *Phyllanthus* accessions in central zone

Zone	Temporal sites	Acc. No.	Growth habit	Branching pattern	Stem shape	Leaflet margin	Flower colour	Capsule colour	Capsule texture	Capsule shape
Central Kerala (CK)	Midlands (M)	CKM1	Erect	Non-spreading	Terete	Entire	Pale green	Yellowish green	Smooth	Depressed globose
		CKM2	Erect	Non-spreading	Terete	Entire	Pale green	Yellowish green	Smooth	Depressed globose
		CKM3	Erect	Non-spreading	Angular	Entire	Pale green	Yellowish green	Rough	Depressed globose
		CKM4	Erect	Non-spreading	Terete	Entire	Pale green	Yellowish green	Smooth	Depressed globose
		CKM5	Erect	Non-spreading	Angular	Entire	Pale green	Yellowish green	Smooth	Depressed globose
	Coastal (C)	CKC1	Erect	Non-spreading	Terete	Entire	Pale green	Yellowish green	Smooth	Depressed globose
		CKC2	Erect	Non-spreading	Terete	Entire	Pale green	Yellowish green	Rough	Depressed globose
		CKC3	Erect	Non-spreading	Terete	Entire	Pale green	Yellowish green	Rough	Depressed globose
	Plains (P)	CKP1	Erect	Non-spreading	Terete	Entire	Pale green	Yellowish green	Rough	Depressed globose
		CKP2	Erect	Non-spreading	Terete	Entire	Pale green	Yellowish green	Smooth	Depressed globose
		CKP3	Erect	Non-spreading	Terete	Entire	Pale green	Yellowish green	Smooth	Depressed globose
	High ranges (H)	CKH1	Erect	Non-spreading	Terete	Entire	Pale green	Yellowish green	Smooth	Depressed globose
		CKH2	Erect	Non-spreading	Terete	Entire	Pale green	Yellowish green	Rough	Depressed globose
		CKH3	Erect	Non-spreading	Terete	Entire	Pale green	Yellowish green	Rough	Depressed globose

Table 6c. Non-variable qualitative characters of *Phyllanthus* accessions in northern zone

Zone	Temporal sites	Acc. No.	Growth habit	Branching pattern	Stem shape	Leaflet margin	Flower colour	Capsule colour	Capsule texture	Capsule shape
Northern Kerala (NK)	Midlands (M)	NKM1	Erect	Non-spreading	Terete	Entire	Pale green	Yellowish green	Rough	Depressed globose
		NKM2	Erect	Non-spreading	Angular	Entire	Pale green	Yellowish green	Smooth	Depressed globose
		NKM3	Erect	Non-spreading	Terete	Entire	Pale green	Yellowish green	Smooth	Depressed globose
	Coastal (C)	NKC1	Erect	Non-spreading	Terete	Entire	Pale green	Yellowish green	Rough	Depressed globose
		NKC2	Erect	Non-spreading	Angular	Entire	Pale green	Yellowish green	Smooth	Depressed globose
		NKC3	Erect	Non-spreading	Terete	Entire	Pale green	Yellowish green	Smooth	Depressed globose
	Plains (P)	NKP1	Erect	Non-spreading	Terete	Entire	Pale green	Yellowish green	Smooth	Depressed globose
		NKP2	Erect	Non-spreading	Terete	Entire	Pale green	Yellowish green	Rough	Depressed globose
		NKP3	Erect	Non-spreading	Angular	Entire	Pale green	Yellowish green	Rough	Depressed globose
		NKP4	Erect	Non-spreading	Angular	Entire	Pale green	Yellowish green	Rough	Depressed globose
		NKP5	Erect	Non-spreading	Terete	Entire	Pale green	Yellowish green	Rough	Depressed globose
		NKP6	Erect	Non-spreading	Angular	Entire	Pale green	Yellowish green	Rough	Depressed globose
	High ranges (H)	NKH1	Erect	Non-spreading	Terete	Entire	Pale green	Yellowish green	Rough	Depressed globose
		NKH2	Erect	Non-spreading	Angular	Entire	Pale green	Yellowish green	Smooth	Depressed globose
		NKH3	Erect	Non-spreading	Angular	Entire	Pale green	Yellowish green	Smooth	Depressed globose
		NKM4	Erect	Non-spreading	Angular	Entire	Pale green	Yellowish green	Smooth	Depressed globose

Table 6d. Non-variable qualitative characters of *Phyllanthus* accessions

Sl No.	Characters	Noticeable phenomenon
1	Growth habit	Erect
2	Branching pattern	Non - spreading
3	Stem shape	Terete, angular
4	Leaf margin	Entire
5	Flower colour	Pale green
6	Capsule colour	Yellowish green
7	Capsule texture	Rough, smooth
8	Capsule shape	Depressed globose

Phyllanthus accessions collected were found to have erect growth habit with non- spreading branching pattern (Tables 6b, 6c and 6d). Entire leaf margin was noticed in all the accessions. Flower colour was pale green (Plate 7A) and capsule colour was yellowish green in all the accessions. Two types of stem shapes viz., terete and angular and capsule texture viz., rough and smooth types were found (Plates 7(B) and 7(C)). Observed shape of capsule was depressed globose (Plate 7(D)).

Notable variation was noted in qualitative characters like stem colour (Plates 8(A)), leaflet colour (Plates 8(B)), rachis colour, leaflet shape (Plates 8(C)), leaflet apex, leaflet base and peduncle colour (Table 6e, 6f and 6g).

In southern zone, the accessions SKM1, SKP2, SKH6 were observed to have dark green stem and dark green leaflets. All other accessions like SKM2, SKM3, SKM4, SKC1, SKC2, SKC3, SKC4, SKP1, SKH1, SKH2, SKH3 and SKH5 had light green stem (Table 6e). Purple coloured stem was observed for the accessions SKP3 and SKH4 of southern zone. Leaflet colour and rachis colour (light green) were similar for almost all accessions from southern zone, except SKM1, SKC3, SKC4, SKP3 and SKH4. Dark green leaflet colour was observed for the accessions SKM1 and SKC3 and purple rachis colour for the accessions

Plate 7. Non- variable qualitative characters of *Phyllanthus* accessions (Magnification- 35x)

(A).Yellowish green flower colour

(B).Rough textured capsules

(C). Smooth textured capsules

(D). Depressed globose capsules

Plate 8.Variable qualitative characters of *Phyllanthus* accessions

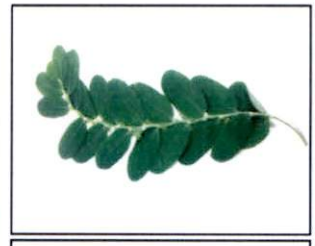
Dark green

Purple

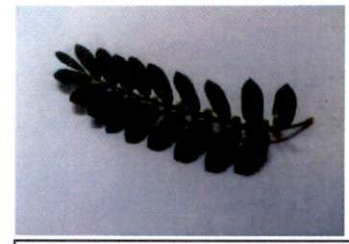
Light green

(A). Stem colour

Dark green



Dark green



Dark green

(B). Leaflet colour

Oblong with round apex

Obovate with obtuse apex

Round with obcordate apex

Elliptic with acute apex

Oblong with mucronate apex

(C). Leaflet shape

Table 6e. Variable qualitative characters of *Phyllanthus* accessions in southern zone

Zone	Temporal site	Acc. No.	Stem colour	Leaflet colour	Rachis colour	leaflet shape	Leaflet apex	Leaf base	Peduncle colour
Southern Kerala (K)	Midlands (M)	SKM1	Dark green	Dark green	Dark green	Obovate	Faintly mucronate	Round	Light green
		SKM2	Light green	Light green	Light green	Oblong	Obtuse	Round	Light green
		SKM3	Light green	Light green	Light green	Obovate	Obtuse	Round	Light green
		SKM4	Light green	Light green	Light green	Elliptic	Acute	Round	Light green
	Coastal regions (C)	SKC1	Light green	Light green	Light green	Oblong	Obtuse	Round	Light green
		SKC2	Light green	Light green	Light green	Elliptic	Acute	Round	Dark green
		SKC3	Light green	Dark green	Light green	Round	Obcordate	Acute	Light green
		SKC4	Light green	Dark green	Purple	Obovate	Faintly mucronate	Round	Purple
	Plains (p)	SKP1	Light green	Light green	Light green	Oblong	Obtuse	Round	Light green
		SKP2	Dark green	Dark green	Dark green	Obovate	Faintly mucronate	Round	Light green
		SKP3	Purple	Dark green	Purple	Oblong	Mucronate	Round	Purple
	High ranges (H)	SKH1	Light green	Light green	Light green	Oblong	Round	Round	Light green
		SKH2	Light green	Light green	Light green	Obovate	Acute	Obtuse	Light green
		SKH3	Light green	Light green	Light green	Obovate	Acute	Round	Light green
		SKH4	Purple	Dark green	Purple	Oblong	Mucronate	Round	Purple
		SKH5	Light green	Light green	Light green	Elliptic	Acute	Round	Light green
		SKH6	Dark green	Dark green	Dark green	Oblong	Mucronate	Round	Light green

Table 6f. Variable qualitative characters of *Phyllanthus* accessions in central zone

Zone	Temporal site	Acc. No.	Stem colour	Leaflet colour	Rachis colour	leaflet shape	Leaflet apex	Leaf base	Peduncle colour
Central Kerala (K)	Midlands (M)	CKM1	Light green	Light green	Light green	Oblong	Obtuse	Round	Light green
		CKM2	Light green	Light green	Light green	Obovate	Obtuse	Round	Light green
		CKM3	Dark green	Dark green	Dark green	Narrowly obovate	Acute	Round	Brownish green
		CKM4	Purplish green	Purple	Purple	Obovate	Faintly mucronate	Round	Light green
		CKM5	Light green	Light green	Light green	Elliptic	Round	Acute	Light green
	Coastal regions (C)	CKC1	Light green	Light green	Light green	Oblong	Obtuse	Round	Light green
		CKC2	Purplish green	Dark green	Light green	Obovate	Faintly mucronate	Oblique	Light green
		CKC3	Purplish green	Purple	Purple	Obovate	Faintlymucronate	Oblique	Purplish green
	Plains (P)	CKP1	Light green	Light green	Light green	Obovate	Faintly mucronate	Obtuse	Light green
		CKP2	Light green	Light green	Light green	Obovate	Obtuse	Round	Light green
		CKP3	Light green	Light green	Light green	Oblong	Obtuse	Round	Light green
	High ranges (H)	CKH1	Light green	Light green	Light green	Oblong	Obtuse	Round	Light green
		CKH2	Purplish green	Dark green	Purple	Narrowly obovate	Faintly mucronate	Obtuse	Purplish green
		CKH3	Purplish green	Purple	Purple	Narrowly obovate	Faintly mucronate	Obtuse	Purplish green

Table 6g. Variable qualitative characters of *Phyllanthus* accessions in northern zone

Zone	Temporal zone	Acc. No.	Stem colour	Leaflet colour	Rachis colour	leaflet shape	Leaflet apex	Leaflet base	Peduncle colour
Northern Kerala (NK)	Midlands (M)	NKM1	Light green	Dark green	Light green	Oblong	Obtuse	Round	Light green
		NKM2	Dark green	Dark green	Light green	Elliptic	Round	Acute	Light green
		NKM3	Dark green	Dark green	Greenish purple	Obovate	Obtuse	Round	Light green
	Coastal (C)	NKC1	Light green	Light green	Light purple	Oblong	Faintly mucronate	Obtuse	Light green
		NKC2	Brownish green	Dark green	Dark green	Elliptic	Acute	Acute	Light green
		NKC3	Light green	Light green	Light green	Oblong	Obtuse	Round	Light green
	Plains (P)	NKP1	Light green	Light green	Light green	Oblong	Obtuse	Round	Light green
		NKP2	Dark green	Dark green	Light purple	Oblong	Faintly mucronate	Obtuse	Light green
		NKP3	Dark green	Dark green	Light green	Obovate	Acute	Obtuse	Light green
		NKP4	Dark Green	Dark green	Brownish green	Linear ovate	Acute	Obtuse	Light green
		NKP5	Light green	Pinkish green	Light green	Oblong	Faintly mucronate	Oblique	Light green
		NKP6	Light green	Light green	Light green	Elliptic	Round	Acute	Light green
	High ranges (R)	NKH1	Light green	Dark green	Light green	Oblong	Round	Round	Light purple
		NKH2	Light green	Dark green	Green	Oblong	Mucronate	Round	Light green
		NKH3	Light green	Light green	Light green	Elliptic	Acute	Round	Light green
		NKH4	Brownish green	Dark green	Brownish green	Linear ovate	Acute	Obtuse	Brownish green

SKC4 SKP3 and SKH4. Characters like leaf shape and leaf apex were highly varying among the accessions collected from southern zone (Table 6e). Among the accessions, only SKC3 registered round leaf shape while obovate, oblong and elliptic shapes were observed for all other accessions. Leaf base was observed to be round in all accessions except SKC3 and SKH2. SKC3 had acute leaf base while SKH2 possessed obtuse leaf base. The peduncle colour was observed to be light green in all the accessions except SKC2 (dark green), SKP3, SKC4 and SKH4 (purple).

In central zone, other than dark green and light green stem colour, purplish green coloured stems were also observed for more than one accession (Table 6f). The accessions CKM4, CKC2, CKC3, CKH2 and CKH3 had purplish green stems. Majority of the accessions possessed light green rachis. Purple leaflet with purple rachis was observed for CKM4, CKC3 and CKH3. Varying leaflet shapes, obovate, oblong, narrowly obovate, and elliptic were observed among accessions of central zone. Varying leaf apices were also noted in collected accessions from central zone (Table 6f). Obovate leaf shape and obtuse as well as faintly mucronate leaf apices predominated. Obovate leaflet shape was observed for six accessions, CKM2, CKM4, CKC2, CKC3, CKP1 and CKP2. Leaflet base was highly varying, with accessions showing round, acute, oblique and obtuse leaf bases. Light green was the most common peduncle colour (Table 6f). Purplish green coloured peduncles were observed in accessions with purplish green coloured stem, viz., CKC3, CKH2 and CKH3. Most of the accessions with purplish green stem were found in midlands, coastal regions and high ranges of central zone. Stem colour, leaflet colour and rachis colour were light green for all the accessions collected from plains.

Accessions with dark and light green stems were observed from all the temporal sites viz., midlands, coastal regions, plains and high ranges in northern zone (Table 6g). All the accessions had stem and leaflet colour as light green or dark green except NKC2, NKP5 and NKH4. Both NKC2 and NKH4 had brownish green stem colour while, NKP5 had pinkish green leaflets. Leaflet

shapes, leaflet apices and leaflet bases were highly varying among the accessions (Table 6g). The accessions NKM1, NKM3, NKC3, and NKP1 had obtuse leaf apices with round leaf base. Light green peduncle colour was observed for almost all the accessions in northern zone, except NKH1 and NKH4, which had light purple and brownish green coloured peduncle respectively.

4.1.2.2. *Characterization of Phyllanthus accessions based on morphological quantitative characters*

Out of the eleven quantitative morphological characters observed, eight quantitative characters viz., stem length (cm) (Plate 9A), number of branchlets per plant, number of leaflets per compound leaf, leaf length (cm) (Plate 9B), leaf width (cm), number of capsules per branch, fresh weight (g) and dry weight (g) showed considerable variations among the accessions. Hence, remaining two quantitative characters including, pedicel length and number of sepals were not considered for further analysis. These two characters with their mean values are presented in the Table 7.

Table 7. Non- variable quantitative morphological characters of *Phyllanthus* accessions

Sl No.	Characters	Description (mean values)
1	Pedicel length (cm)	0.1, 0.6
2	Number of sepals	5, 6

Generally, pedicel lengths among the accessions were 0.1 cm and 0.6cm (Plate 10(A)). The accessions, CKM3, NKM4 , NKP4, NKH2 had pedicel lengths of 0.6cm each (Table 7a).The accessions, SKM2, SKC1, SKP1, SKH1, CKM1, CKC1, CKP3, CKH1, NKM2, NKC2, NKP3, NKP5 were found to have

Plate 9. Variable quantitative morphological characters of *Phyllanthus* accessions

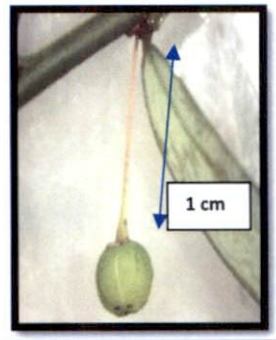
(A).Variation in stem length

(B). Variation in leaflet length

Plate 10. Non-variable quantitative morphological characters *Phyllanthus* accessions

(A). Pedicel length

Pedicel length- 0.1 cm



Pedicel length- 1.0 cm

(B). Number of sepals

5 numbers

6 sepals

Table 7a. Non-variable quantitative morphological characters of *Phyllanthus* accessions in southern, central and northern zone

Accessions from southern zone.	Pedicel length (cm)	No. of sepals
SKM1	0.1	6
SKM2	0.1	5
SKM3	0.1	6
SKM4	0.1	6
SKC1	0.1	5
SKC2	0.1	6
SKC3	0.1	6
SKC4	0.1	6
SKP1	0.1	5
SKP2	0.1	6
SKP3	0.1	6
SKH1	0.1	5
SKH2	0.1	6
SKH3	0.1	6
SKH4	0.1	6
SKH5	0.1	6
SKH6	0.1	6

Accessions from central zone	Pedicel length (cm)	No. of sepals
CKM1	0.1	5
CKM2	0.1	6
CKM3	0.5	6
CKM4	0.1	6
CKM5	0.1	6
CKC1	0.1	5
CKC2	0.1	6
CKC3	0.1	6
CKP1	0.1	6
CKP2	0.1	6
CKP3	0.1	5
CKH1	0.1	5
CKH2	0.1	6
CKH3	0.1	6

Accessions from northern zone	Pedicel length (cm)	No. of sepals
NKM1	0.1	5
NKM2	0.1	6
NKM3	0.1	6
NKC1	0.1	6
NKC2	0.1	6
NKC3	0.1	5
NKP1	0.1	5
NKP2	0.1	6
NKP3	0.1	6
NKP4	0.6	6
NKP5	0.1	6
NKP6	0.1	6
NKH1	0.1	5
NKH2	0.6	6
NKH3	0.1	6
NKM4	0.5	6

five number of sepals each and the rest of accessions had six number of sepals (Plates 10(B)).

Among the eight quantitative characters showing considerable variation, in southern zone, maximum mean stem length (60.1 cm), highest mean number of branchlets per plant (25.2), maximum mean leaf length (1.51 cm), highest mean number of capsules per branch (52.7), highest mean fresh weight (10.82 g) and highest mean dry weight (8.62 g) were registered by the accession SKC3. The accession SKM3 recorded a mean stem length of 52.6 cm. The same accession SKM3, recorded the highest mean number of leaflets per compound leaf (38.6) as well, followed by the accession SKH3 (38.1). Maximum mean leaf width (1.32 cm) was recorded by the accession SKM3 followed by SKH3 (1.31 cm). The accession SKH4 from high ranges recorded lowest values for mean stem length, mean number of branchlets per plant, mean number of leaflets per compound leaf, mean fresh weight and mean dry weight (Table 7b).

In central zone, the accession from midland, CKM3, recorded maximum values for mean stem length (87.1 cm), mean number of branchlets per plant (30.3), mean number of leaflets per compound leaf (40.9), mean leaf length (1.21 cm) mean number of capsules per branch (34.3), mean fresh weight (15.67 g) and mean dry weight (13.27 g). The accession CKM2, also from midlands, recorded maximum mean leaf width, 1.19 cm. The accessions CKH2, CKP1 and CKC2 recorded lowest values for mean stem length, mean number of branchlets per plant and mean number of leaflets per compound leaf respectively and CKM1 and CKP1 for mean leaf length. The accession CKH2 recorded lowest mean number of capsules per branch (10.6), lowest mean fresh weight (3.89 g) and lowest mean dry weight (1.98 g) (Table 7c).

In northern zone, the accession NKH4 recorded highest values for mean stem length (90.1 cm), mean number of branchlets per plant (31.50), mean leaf length (2.08 cm), mean fresh weight (16.21 g) and mean dry weight (13.81 g).

Table 7b. Variable quantitative morphological characters of *Phyllanthus* accessions in southern zone

Zone	Temporal site	Acc. No.	Stem length (cm)	No. of branchlets per plant	No. of leaflets per compound leaf	Leaflet length (cm)	Leaflet width (cm)	No. of capsules per branch	Fresh weight (g)	Dry weight (g)
Southern Kerala (SK)	Midlands (M)	SKM1	24.9	10.1	20.6	1.11	0.32	15.9	4.23	2.13
		SKM2	37.3	16.3	31.8	0.82	0.51	28.1	6.71	4.51
		SKM3	52.6	22.9	38.6	0.93	1.32	. 43.64	9.98	7.88
		SKM4	41.1	20.1	32.3	0.61	0.22	34.5	6.98	4.78
	Coastal regions (C)	SKC1	36.1	16.1	31.9	0.72	0.53	29.2	6.13	4.03
		SKC2	42.6	20.3	32.4	0.62	0.22	37.7	7.66	5.76
		SKC3	60.1	25.2	37.5	1.51	0.63	52.7	10.82	8.62
		SKC4	27.5	10.3	21.1	1.01	0.33	19.8	4.94	2.84
	Plains (P)	SKP1	34.7	15.6	31.3	0.82	0.54	27.2	6.24	4.04
		SKP2	21.3	09.7	20.1	1.01	0.33	16.7	3.62	1.42
		SKP3	26.9	10.2	21.2	1.03	0.35	19.6	4.84	2.74
	High ranges (H)	SKH1	33.1	15.4	31.1	0.71	0.51	27.4	5.62	3.34
		SKH2	45.6	20.9	32.9	0.63	0.21	38.3	7.75	5.65
		SKH3	51.2	22.7	38.1	0.93	1.31	44.9	8.70	6.60
		SKH4	22.1	09.9	20.3	1.11	0.32	17.5	3.75	1.55
		SKH5	43.6	20.5	32.6	0.61	0.21	37.4	7.41	5.31
		SKH6	28.8	10.5	21.3	1.12	0.31	22.3	5.18	3.08

Table 7c. Variable quantitative morphological characters of *Phyllanthus* accessions in central zone

Zone	Temporal sites	Acc. No.	Stem length (cm)	No. of branchlets per plant	No. of leaflets per compound leaf	Leaflet length (cm)	LeafLet width (cm)	No. of capsules per branch	Fresh weight (g)	Dry weight (g)
Central Kerala (CK)	Midlands (M)	CKM1	34.1	16.3	32.2	0.81	0.41	25.1	5.79	3.69
		CKM2	54.2	23.2	39.1	0.92	1.19	32.9	9.75	7.45
		CKM3	87.1	30.3	40.9	1.21	0.47	34.3	15.67	13.27
		CKM4	26.7	10.2	21.1	1.01	0.44	12.8	4.81	2.79
		CKM5	44.1	21.1	36.1	0.83	0.42	27.4	7.93	5.73
	Coastal regions (C)	CKC1	34.7	16.5	32.4	1.01	0.44	22.5	6.25	4.45
		CKC2	23.5	9.7	20.2	1.11	0.62	11.7	3.99	2.09
		CKC3	24.1	9.9	20.8	1.10	0.61	12.9	4.33	2.23
	Plains (P)	CKP1	22.7	9.5	20.4	0.81	0.31	12.1	4.09	1.99
		CKP2	54.2	22.8	38.8	0.93	1.12	27.6	9.75	7.45
		CKP3	36.3	17.2	33.1	1.02	0.52	23.2	6.53	4.33
	High ranges (H)	CKH1	33.5	15.7	31.2	0.91	0.41	21.2	6.03	4.13
		CKH2	22.9	09.8	20.6	1.04	0.53	10.6	3.89	1.98
		CKH3	25.2	10.1	20.9	1.03	0.51	11.9	4.53	2.43

For the rest of the quantitative characters, the accession NKM3 recorded maximum values (Table 7d). The accession, NKH4 from high ranges, recorded maximum mean number of capsules as well (33.1). Lowest values were recorded by the accessions, NKP2, for mean stem length, mean number of branchlets per plant and mean number of leaflets per compound leaf, NKP3 for mean leaf length, NKP3 and NKP5 for mean leaf width, NKP5 for mean number of capsules per branch and NKC1 for mean fresh and dry weights (Table 7d).

4.1.3. Decoding of collected *Phyllanthus* accessions

The collected *Phyllanthus* accessions were decoded into *Phyllanthus* species (Table 8), using key characters of herbaceous *Phyllanthus* species reported from Kerala (Gamble and Fischer, 1915-1936) as given below.

4.1.3.1. *Phyllanthus amarus* Schum and Thonn.

A branching annual herb reaching 12-18 inches high. Leaves are usually broadly obtuse at apex, elliptic - obovate or oblong, prominently distichous so that the branchlets resemble pinnate leaves; anthers are transversely dehiscing.

4.1.3.2. *Phyllanthus rheedei* Wight.

A slender branching erect herb. The calyx lobes are usually white-margined. Leaves are glabrous, membranous, elliptic or ovate, acute; stipules are lanceolate and decurrent; male flowers minute and fascicled. Female flowers are larger, sessile; anthers are sessile.

4.1.3.3. *Phyllanthus urinaria* L.

An annual or perennial erect herb, with more or less sensitive leaflets, which are sometimes pink when young. Capsules are verrucose, the seeds prominently transversely ridged and with faint cross-bars; leaves glabrous or hispid on the margins, chartaceous, oblong, apiculate. Stipules subulate; male flowers very minute, female flower larger, sessile; anthers sessile.

Table 7d. Variable quantitative morphologicl characters of *Phyllanthus* accessions in northern zone

Zone	Temporal sites	Acc. No.	Stem length (cm)	No. of branchlets per plant	No. of leaflets per compound leaf	Leaflet length (cm)	Leaflet width (cm)	No. of capsules per branch	Fresh weight (g)	Dry weight (g)
Northern Kerala (NK)	Midlands (M)	NKM1	37.5	16.9	31.9	1.02	0.51	24.2	6.57	4.47
		NKM2	43.1	21.1	34.7	1.21	0.62	27.4	7.75	5.45
		NKM3	56.2	23.9	39.6	0.92	1.03	33.1	9.55	7.35
	Coastal region (C)	NKC1	26.1	10.1	21.1	1.09	0.42	16.4	4.43	2.13
		NKC2	43.8	21.3	36.1	0.52	0.23	31.1	7.88	5.99
		NKC3	33.1	15.3	30.9	0.83	0.54	25.2	6.28	4.23
	Plains (P)	NKP1	32.5	14.6	30.7	0.54	0.31	26.4	6.15	3.95
		NKP2	25.6	09.7	20.6	0.92	0.41	16.4	4.35	2.05
		NKP3	42.1	19.9	34.3	0.42	0.22	29.6	7.15	5.05
		NKP4	87.4	30.6	36.5	1.51	0.63	30.2	15.71	13.21
		NKP5	27.6	09.9	20.8	0.84	0.22	15.6	4.96	2.98
		NKP6	46.1	21.8	36.6	0.52	0.34	29.6	8.29	6.09
	High ranges (H)	NKH1	35.1	15.6	31.2	1.21	0.53	24.8	6.61	4.51
		NKH2	85.4	30.2	36.4	1.71	0.62	31.5	14.15	12.15
		NKH3	42.1	20.1	34.4	1.03	0.31	29.2	7.57	5.37
		NKH4	90.1	31.5	36.8	2.08	0.54	33.1	16.21	13.81

Table 8. Decoded of *Phyllanthus* accessions

Sl. No.	Accession	Name of the species
1	SKM1	*P. urinaria*
2	SKM2	*P. amarus*
3	SKM3	*P. rheedei*
4	SKM4	*P. airy-shawii*
5	SKC1	*P. amarus*
6	SKC2	*P. airyshawii*
7	SKC3	*P. maderaspatensis*
8	SKC4	*P. urinaria*
9	SKP1	*P. amarus*
10	SKP2	*P. urinaria*
11	SKP3	*P. urinaria*
12	SKH1	*P. amarus*
13	SKH2	*P. airy-shawii*
14	SKH3	*P. rheedei*
15	SKH4	*P. urinaria*
16	SKH5	*P. airy-shawii*
17	SKH6	*P. urinaria*
18	NKM1	*P. amarus*
19	NKM2	*P. airy-shawii*
20	NKM3	*P. rheedei*
21	NKC1	*P. urinaria*
22	NKC2	*P. airy-shawii*
23	NKC3	*P. amarus*
24	NKP1	*P. amarus*
25	NKP2	*P. urinaria*
26	NKP3	*P. airy-shawii*
27	NKP4	*P. virgatus* var. *virgatus*
28	NKP5	*P. urinaria*
29	NKP6	*P. airy-shawii*
30	NKH1	*P. amarus*
31	NKH2	*P. virgatus* var. *virgatus*
32	NKH3	*P. airy-shawii*
33	NKH4	*P. virgatus* var. *gardnerianus*
34	CKM1	*P. amarus*
35	CKM2	*P. rheedei*
36	CKM3	*P. virgatus* var. *virgatus*
37	CKM4	*P. urinaria*
38	CKM5	*P. airy-shawii*
39	CKC1	*P. amarus*
40	CKC2	*P. urinaria*
41	CKC3	*P. urinaria*
42	CKP1	*P. urinaria*
43	CKP2	*P. rheedei*
44	CKP3	*P. amarus*
45	CKH1	*P. amarus*
46	CKH2	*P. urinaria*
47	CKH3	*P. urinaria*

4.1.3.4. *Phyllanthus virgatus* G. Forst. *var.virgatus.*

Leaves are distichous; stems usually ascending; female pedicel 5 mm long. disk orbicular, undivided and capsules with scale like protuberances.

4.1.3.5. *Phyllanthus virgatus* G. Forst. *var.gardnerianus*

A slender under shrub with woody rootstock and long branches, the leaves are smaller in size upwards, plants of dry hill tops often dwarfed and with very small leaves. Leaves of upper branchlets are elliptic, obtuse, of lower stems, elliptic-oblong.

4.1.3.6. *Phyllanthus maderaspatensis* L.

An erect or decumbent herb, sometimes nearly an under-shrub. Leaves are glabrous, subcoriaceous, obovate or oblanceolate, cuneate, rounded or retuse at apex, mucronate, glaucous; stipules lanceolate, peltate; male flowers minute. fascicled, female solitary, on filiform pedicels and anthers sub sessile.

4.1.3.7. *Phyllanthus airy-shawii* Brunel & Roux.

An erect slender herb or under shrub with several very slender branchlets. Stipules are lanceolate, long-acuminate; flowers rather large, the calyx-lobes with prominent scarious margins; disk of male flowers with star-like glands, of male saucer-shaped, crenulate or lobed; stamina column long; styles erect, shortly bifid.

4.1.3.8. *Phyllanthus rotundifolius* Klein ex Wild.

A prostrate or slightly ascending fleshy herb with stout rootstock and long trailing branches. Leaves are coriaceous or fleshy, orbicular or obovate. obtuse or apiculate, scarely 0.25 in. in diameter; style arms are recurved with short lobes.

4.1.4. Range of variable quantitative characters in *Phyllanthus* spp.

Comparison of range of variable quantitative morphological parameters like, plant height (cm), number of branchlets per plant, number of leaflets per compound leaf, leaf length (cm), leaf width (cm), number of capsules per branch, fresh weight (g) and dry weight (g), of seven *Phyllanthus* spp. viz., *P. amarus, P. rheedei, P. urinaria, P. airy-shawii, P. virgatus var. virgatus, P. maderaspatensis,* and *P. virgatus* var. *gardnerianus,* is presented in Table 9.

P. virgatus var. *gardnerianus* recorded maximum plant height (90.1 cm), number of branchlets per plant (31.5), fresh weight (16.21 g) and dry weight (13.81 g). *P. virgatus* var. *virgatus* recorded maximum number of leaflets per compound leaf (36.4 – 40.9) and leaf length (2.01 – 2.21 cm). Minimum values for plant height (22.1 – 28.8 cm), number of branchlets per plant (9.5 – 10.3), number of leaflets per compound leaf (20.1 – 21.2), number of capsules per branch (10.6 – 22.3), fresh weight (3.62 – 5.18g) and dry weight (1.42 – 3.08) were recorded for *P. urinaria. P.airy-shawii* recorded least values for leaf length (0.42 – 1.21 cm) and leaf width (0.21 – 0.62 cm) (Table 9).

4.1.5. Distribution of herbaceous *Phyllanthus* spp. in various zones of Kerala.

The species wise distribution of *Phyllanthus* spp. in various zones of Kerala is given in Table 10a.

Among the 17 accessions collected from southern zone, four accessions each represented *P. amarus* and *P. airy-shawii*, six, *P. urinaria,* two, *P. rheedei* and one, *P. maderaspatensis*. *P. virgatus* var. *virgatus* and *P. virgatus var. gardnerianus* were absent in the zone. In central zone also four accessions represented *P. amarus*, six, *P. urinaria*, and two, *P. rheedei*. *P. airy-shawii* and *P. virgatus* var. *virgatus* were represented by single accessions each. In northern zone, *P. amarus* was represented by four accessions, *P. urinaria*, three, *P. airy-shawii*, five, *P. virgatus* var. *virgatus*, two, and *P. virgatus* var. *gardnerianus* and

Table 9. Range of variable morphological quantitative characters in *Phyllanthus* spp.

Phyllanthus spp.	Plant height (cm)	No. of branchlets per plant	No. of leaflets per compound leaf	Leaf length (cm)	Leaf width (cm)	No. of capsules per branch	Fresh weight (g)	Dry weight (g)
P. airy-shawii	41.1 – 46.1	19.9 – 21.8	32.3 – 36.6	0.42 – 1.21	0.21 – 0.62	27.4 – 38.3	6.98 - 8.29	6.09 – 4.78
P. amarus	32.5 - 37.5	15.4 – 17.2	31.1 – 33.1	0.71 – 1.21	0.31 – 0.54	21.2 – 29.2	5.62 – 6.71	3.34 - 4.51
P. maderaspatenis	60.1	25.2	37.5	1.51	0.63	52.7	10.82	8.62
P. rheedei	51.2 – 56.2	22.7 – 23.9	38.1 – 39.5	1.21 – 1.71	1.03 – 1.32	27.6 – 44.9	8.70 – 9.98	6.60 - 7.88
P. urinaria	22.1 – 28.8	9.5 – 10.3	20.1 – 21.2	0.81 – 1.42	0.22 – 0.62	10.6 – 22.3	3.62 – 5.18	1.42 – 3.08
P. virgatus var. gardnerianus	90.1	31.5	36.8	2.08	0.54	33.1	16.21	13.81
P. virgatus var. *virgatus*	85.4 – 87.4	30.2 – 30.6	36.4 – 40.9	2.01 – 2.21	0.47 – 0.63	30.2 – 34.3	14.15 – 15.71	12.15 –13.27

Table 10a. Species wise distribution of *Phyllanthus* spp. in various zones of Kerala

***Phyllanthus* spp.**	**Southern zone**	**Central zone**	**Northern zone**	**Total**
Phyllanthus airy-shawii	4	1	5	10
Phyllanthus amarus	4	4	4	12
Phyllanthus maderaspatensis	1	-	-	1
Phyllanthus rheedei	2	2	1	5
Phyllanthus urinaria	6	6	3	15
Phyllanthus virgatus var. garderianus	-	-	1	1
Phyllanthus virgatus var. *virgatus*	-	1	2	3

Table 10b. Species wise distribution of *Phyllanthus* spp. in altitudinal areas of Kerala

***Phyllanthus* spp.**	**Coastal regions**	**Plains**	**Midlands**	**High ranges**	**Total**
Phyllanthus airy-shawii	2	2	3	3	10
Phyllanthus amarus	3	3	3	3	12
Phyllanthus maderaspatensis	1	-	-	-	1
Phyllanthus rheedei	-	1	3	1	5
Phyllanthus urinaria	4	5	2	4	15
Phyllanthus virgatus var. *gardnerianus*	-	-	-	1	1
Phyllanthus virgatus var. *virgatus*	-	1	1	1	3
Total	10	12	12	13	47

and *P. rheedei* were represented by single accessions each (Table 10a). *P. virgatus* var. *virgatus* was absent in southern zone, *P. virgatus var. gardnerianus* in southern and central zone and *P. maderaspatensis*, in central as well as northern zones.

P. amarus was distributed equally in the three zones and *P. urinaria* was predominantly observed in southern and central zones. The northern zone of Kerala, had representations of all herbaceous species of *Phyllanthus* under study, except *P. maderaspatensis*. The only accession representing *P. maderaspatensis* was observed in southern zone. *P. virgatus* var. *gardnerianus* was represented only from northern zone. *P. urinaria* was the species with maximum distribution in the state.

4.1.6. Distribution of herbaceous *Phyllanthus* spp. in altitudinal areas of Kerala

The species wise distribution of *Phyllanthus* spp. in various altitudinal areas of Kerala is given in Table 10b.

Among the 10 accessions collected from coastal region, four accessions represented *P. urinaria*, three, *P. amarus,* two, *P. airy-shawii* and one, *P. maderaspatensis*. *P. virgatus* var. *virgatus* and *P. virgatus var. gardnerianus* and *P. rheedei* were absent in the region. Maximum accessions (13 numbers) were observed in high ranges followed by plains and midlands (12 numbers) each. In plains, *P. urinaria* dominated (five numbers), followed by *P. amarus* (three numbers). *P. virgatus* var. *virgatus* and *P. rheedei* were represented by single accessions each. In midlands, three accessions each represented *P. amarus, P. airy-shawii* and *P. rheedei*, two accessions, *P. urinaria* and a single accession. *P .virgatus* var. *virgatus*. The dominant species in high ranges was *P. urinaria* (four numbers), followed by *P. airy-shawii* and *P. amarus* (three numbers each). Rest of the species except *P. maderaspatensis*, were represented by single accessions each.

P. amarus was equally distributed in coastal regions, plains, midlands and in high ranges. *P. virgatus* var. *gardnerianus* had representation only in high ranges, while *P. maderaspatensis* was represented only in coastal region (Table 10b). Coastal region represented fewer species of *Phyllanthus* (four numbers), while, high ranges registered maximum representation of herbaceous *Phyllanthus* spp. (Table 10b).

4.1.7. Cluster analysis of *Phyllanthus* accessions

4.1.7.1. *Cluster analysis of Phyllanthus accessions from southern zone based on variable quantitative characters*

Fig 1 represents the dendrogram of *Phyllanthus* accessions from southern zone based on variable quantitative characters. Cluster analysis of seventeen accessions from southern zone, from high ranges, plains, midlands and coastal regions in southern zone, based on quantitative characters, representing *Phyllanthus* spp. are given in Table 11a.

The *Phyllanthus* accessions collected from southern zone could be grouped into nine clusters at 10 per cent similarity. Cluster I representing *P. urinaria* comprised of a single accession SKM1. Cluster II and cluster VIII also comprised of single accessions each, SKH2 from high ranges and SKC3 from coastal region representing *P. airy-shawii* and *P. maderaspatensis* respectively. Cluster III comprising of SKP1 from plains and SKH1 from high ranges and cluster VI comprising of SKC1 from coastal and SKM2 from midlands represent *P. amarus*. *P. urinaria* is included in both cluster IV (SKH4 from high range and SKP2 from plains) and cluster IX (SKP3 from plains, SKH6 from high range and SKC4 from coastal region). SKC2, SKM4 and SKH5 were included in cluster VII representing *P. airy-shawii* from coastal area, midlands and high ranges respectively. *P. rheedei* was represented in a single cluster (cluster V), by two accessions, SKM3 from midlands and SKH3 from high ranges (Table 11a).

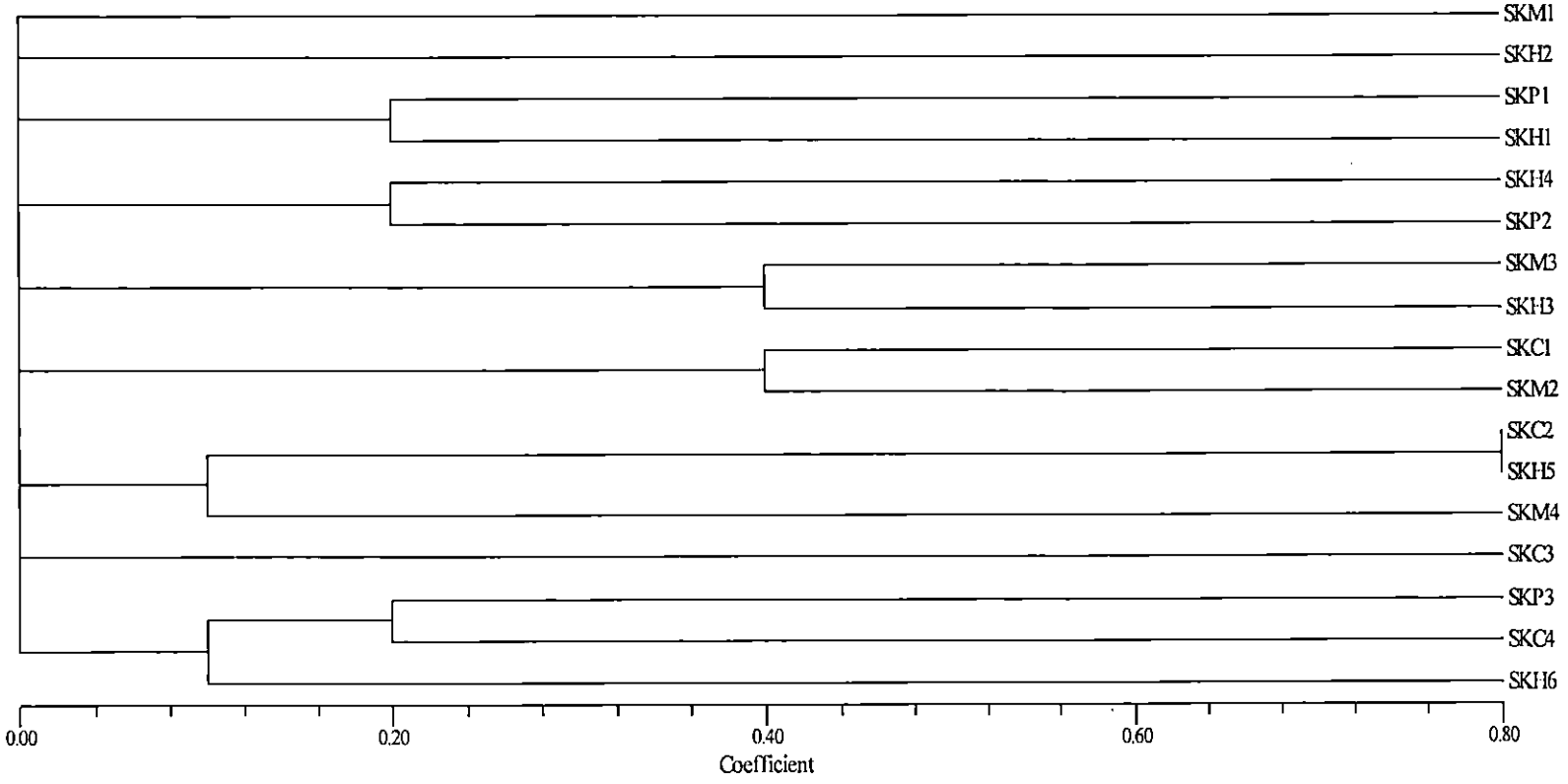


Fig 1. Dendrogram based on quantitative morphological characters of *Phyllanthus* accessions from southern zone of Kerala

Table 11a. Clusters of *Phyllanthus* accessions from southern zone based on quantitative morphological characters

Sl No.	Cluster number	Accessions	No. of accessions	Name of the accessions
1	Cluster I	SKM1	1	*Phyllanthus urinaria*
2	Cluster II	SKH2	1	*Phyllanthus airy-shawii*
3	Cluster III	SKP1, SKH1	2	*Phyllanthus amarus*
4	Cluster IV	SKH4, SKP2	2	*Phyllanthus urinaria*
5	Cluster V	SKM3, SKH3	2	*Phyllanthus rheedei*
6	Cluster VI	SKC1, SKM2	2	*Phyllanthus amarus*
7	Cluster VII	SKC2, SKM4, SKH5	3	*Phyllanthus airy-shawii*
8	Cluster VIII	SKC3	1	*Phyllanthus maderaspatensis*
9	Cluster IX	SKP3, SKH6, SKC4	3	*Phyllanthus urinaria*

The summary statistics for variable quantitative characters of accessions from southern zone is given in Table 11b. Maximum mean stem length (60.1 cm) and mean number of branchlets per compound leaf (25.2), mean number of capsules per branch (52.7), mean fresh weight (10.82 g) and mean dry weight (8.62 g) were noted in *P. maderaspatensis* (cluster VIII), represented by a single accession SKC3, from coastal region of southern zone. Highest values for mean number of leaflets per compound leaf (38.3), leaf length (2.06) and leaf width (1.31 cm) were recorded in cluster V including SKM3 from midlands and SKH3 from high ranges representing *P. rheedei* (Table 11b).

4.1.7.2. *Cluster analysis of Phyllanthus accessions from central zone based on variable quantitative characters*

Fig 2 represents the dendrogram of *Phyllanthus* accessions from southern zone based on variable quantitative characters. Cluster analysis of fourteen accessions from coastal, plains, midland and high ranges representing species/morphotypes of *Phyllanthus* from central zone, based on quantitative character is given in Tables 11c and 11d. The collected *Phyllanthus* accessions from central zone, representing high ranges, plains, midlands and coastal region could be grouped into seven clusters at 15 per cent similarity.

Cluster I representing *P. amarus* include the accessions CKM1, CKC1 and CKH1 from midlands, coastal regions and high ranges, respectively. The four accessions CKM4 from midlands, CKH3 from high ranges and CKC2 and CKC3 from coastal area were grouped in cluster VII, representing *P. urinaria* . Cluster IV, V and. VI had only one accession each, CKM3, CKM5 and CKP3 respectively, representing *P. virgatus* var. *virgatus*; *P. airy-shawii* and *P. amarus* respectively. Cluster II and cluster III comprising of two accessions each, CKP2 and CKM2 in cluster II and CKH2 and CKP1 in cluster III, representing *P. rheedei* and *P. urinaria* respectively (Table 11c).

The summary statistics for variable quantitative characters of accessions from central zone is presented in Table 11d, wherein, the cluster mean values of

Table 11b. Summary statistics for quantitative morphological characters of *Phyllanthus* accessions from southern zone

Sl No.	Cluster number	Cluster mean values							
		Stem length (cm)	No. of branchlets per plant	No. of leaflets per compound leaf	Leaf length (cm)	Leaf width (cm)	No. of capsules per branch	Fresh weight (g)	Dry weight (g)
1	Cluster I	24.9	10.1	20.6	1.11	0.32	15.9	04.23	2.13
2	Cluster II	45.6	20.9	32.9	0.63	0.21	38.3	07.75	5.65
3	Cluster III	33.9	15.5	31.2	0.76	0.52	27.3	05.93	3.69
4	Cluster IV	21.7	9.8	20.2	1.06	0.32	17.1	03.68	1.48
5	Cluster V	51.9	22.8	38.3	2.06	1.31	44.2	9.34	7.24
6	Cluster VI	36.1	16.2	31.8	0.77	0.52	28.6	06.42	4.27
7	Cluster VII	42.4	20.3	32.4	0.61	0.21	36.5	07.35	5.28
8	Cluster VIII	60.1	25.2	37.5	1.51	0.63	52.7	10.82	8.62
9	Cluster IX	27.3	10.3	21.2	1.12	0.31	22.3	05.18	3.08

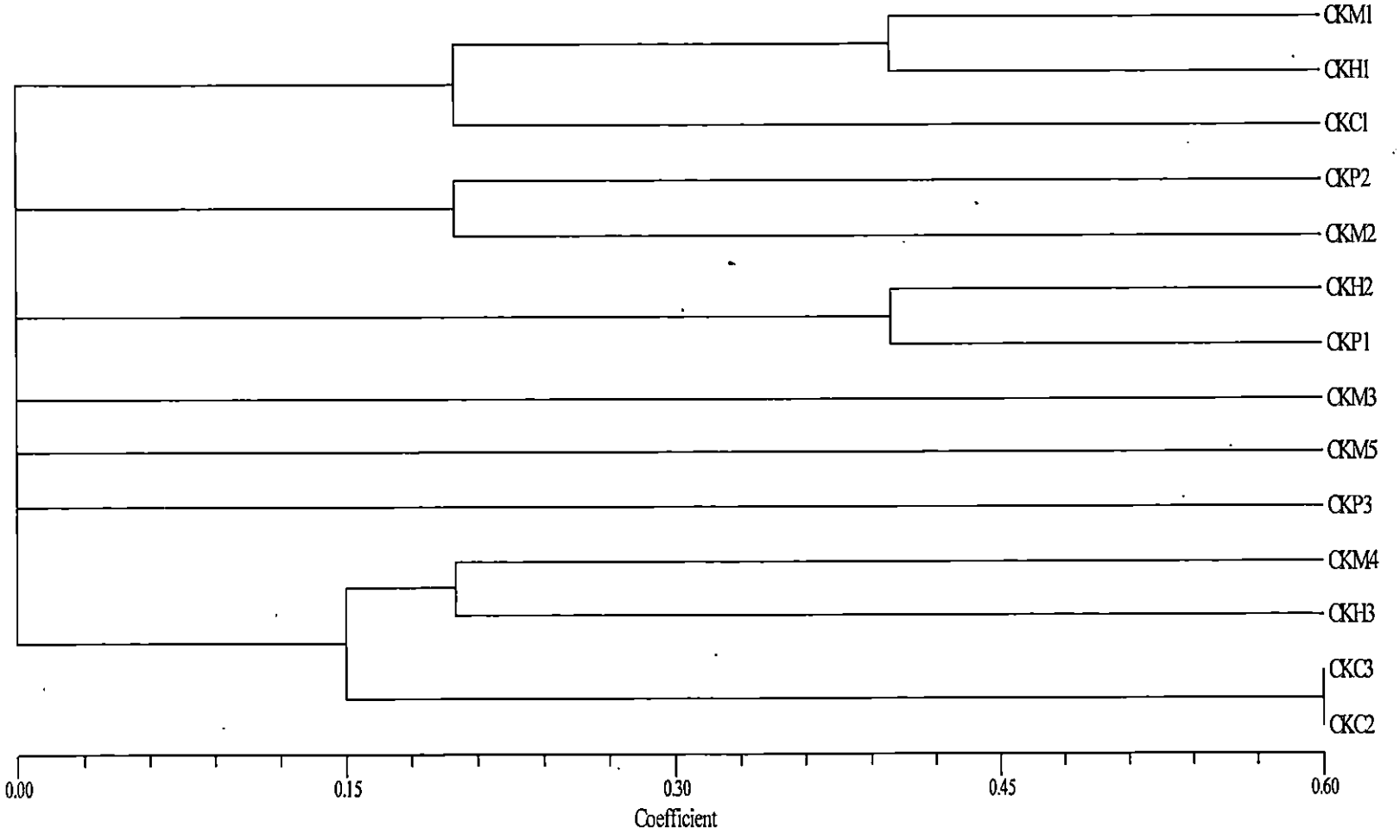


Fig 2. Dendrogram based on quantitative morphological characters of *Phyllanthus* accessions from central zone of Kerala

Table 11c. Clusters of *Phyllanthus* accessions from central zone based on quantitative morphological characters

Sl No.	Cluster number	Accessions	No. of accessions	Name of the accessions
1	Cluster I	CKM1, CKC1, CKH1	3	*Phyllanthus amarus*
2	Cluster II	CKP2, CKM2	2	*Phyllanthus rheedei*
3	Cluster III	CKH2, CKP1	2	*Phyllanthus urinaria*
4	Cluster IV	CKM3	1	*Phyllanthus virgatus* var. *virgatus*
5	Cluster V	CKM5	1	*Phyllanthus airy-shawii*
6	Cluster VI	CKP3	1	*Phyllanthus amarus*
7	Cluster VII	CKM4, CKH3, CKC2, CKC3,	4	*Phyllanthus urinaria*

Table 11d. Summary statistics for variable quantitative morphological characters of *Phyllanthus* accessions from central zone

Sl No.	Cluster number	Cluster mean values							
		Stem length (cm)	No. of branchlets per plant	No. of leaflets per compound leaf	Leaf length (cm)	Leaf width (cm)	No. of capsules per branch	Fresh weight (g)	Dry weight (g)
1	Cluster I	34.1	16.1	31.9	0.91	0.42	22.9	6.02	4.09
2	Cluster II	49.1	21.9	37.4	1.21	0.81	27.5	8.84	6.59
3	Cluster III	22.8	9.6	20.5	1.11	0.42	11.3	3.99	1.98
4	Cluster IV	87.1	30.3	40.9	1.52	0.4	34.3	15.67	13.27
5	Cluster V	44.1	21.1	36.1	0.83	0.42	27.4	7.93	5.73
6	Cluster VI	36.3	17.2	33.1	1.02	0.52	23.2	6.53	4.33
7	Cluster VII	24.8	09.9	20.7	1.13	0.54	12.3	4.41	2.38

quantitative characters employed for grouping the collected accessions into clusters, representing various species of *Phyllanthus* are given. Significantly higher mean stem length of 87.1 cm was noted in a single accession from midlands (CKM3) in cluster IV, which was identified as *P. virgatus* var. *virgatus*, followed by cluster II, comprising of two accessions of *P. rheedei*, CKP2 and CKM2. Significantly higher mean number of branchlets per plant (30.3), mean number of leaflets per compound leaf (40.9) and mean leaf length (1.52 cm) were also observed in the accession CKM3, identified as *P. virgatus* var *virgatus*. The least mean stem length of 22.8 cm was recorded in accessions CKH2 and CKP1 in cluster III, representing *P. urinaria*. Lowest values for mean number of branchlets per plant (9.6), mean number of leaflets per compound leaf (20.5) and mean number of capsules per branch (11.3 cm) were also observed in the same accession. Maximum mean leaf width of 0.81 cm was recorded in cluster II, comprising of accessions CKP2 and CKM2 from plains and midlands representing *P. rheedei*. Highest values for yield contributing characters like fresh weight (15.67 g) and dry weight (13.27 g) were registered in the accession CKM3, representing *P. virgatus* var. *virgatus*. Another morphotype of *P. urinaria* of cluster III, represented by accessions CKH2 and CKP1 registered least values for mean fresh weight (3.99 g) and mean dry weight (1.98 g).

4.1.7.3. *Cluster analysis of Phyllanthus accessions from northern zone based on variable quantitative characters*

Fig 3 represents the dendrogram of *Phyllanthus* accessions from northern zone based on variable quantitative characters. The cluster analysis of sixteen accessions of northern zone, from high ranges, plains, midlands and coastal region based on quantitative characters, representing *Phyllanthus* spp. are given in Table 11e.The *Phyllanthus* collections collected from northern zone could be grouped into nine clusters at nine per cent similarity.

Cluster I representing *P. rheedei* comprised of a single accession from midlands NKM3. Cluster II and Cluster VII also comprised of single accessions

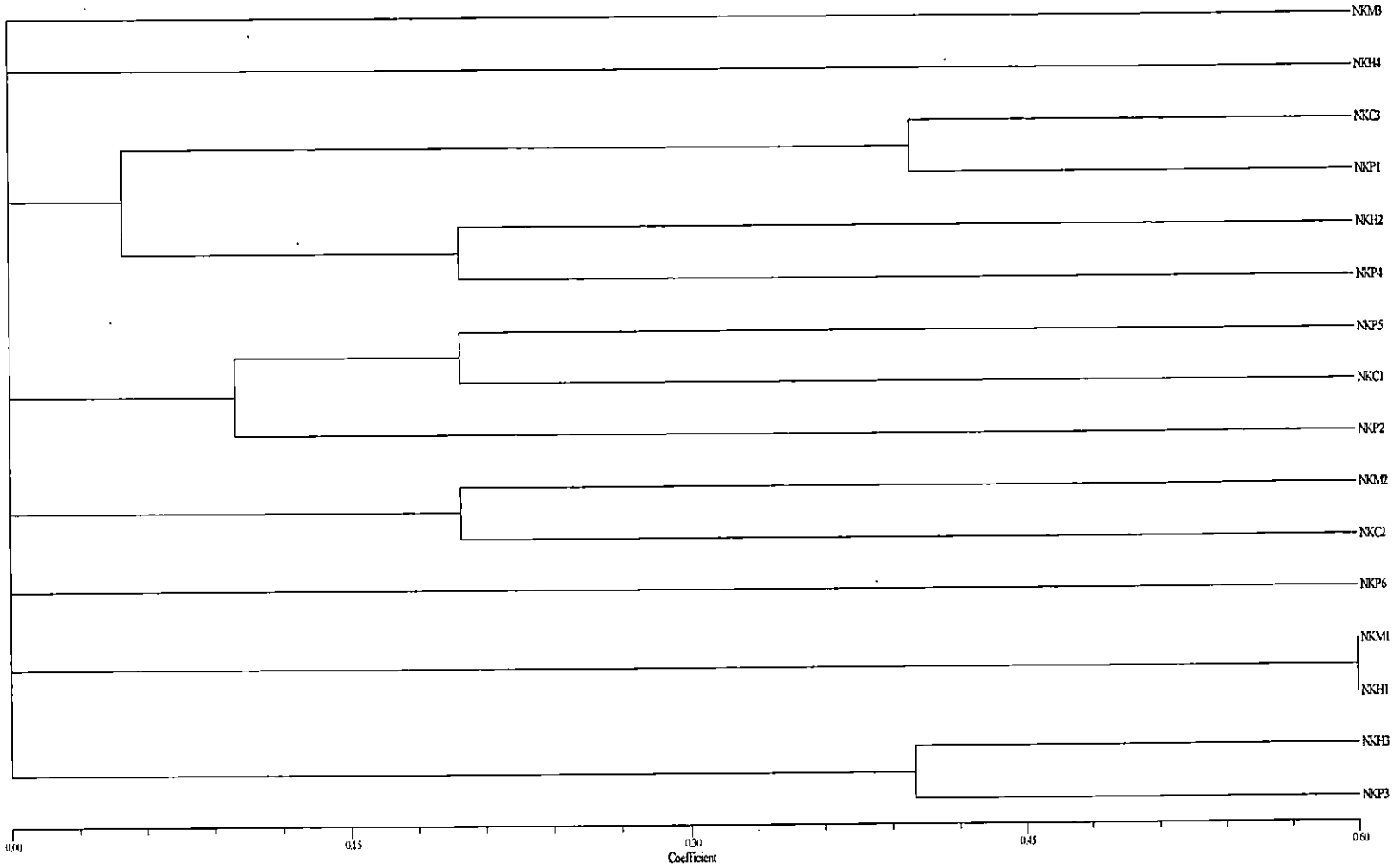


Fig 3. Dendrogram based on quantitative morphological characters of *Phyllanthus* accessions from northern zone of Kerala

Table 11e. Clusters of *Phyllanthus* accessions from northern zone based on quantitative morphological characters

Sl No.	Cluster number	Accessions	No. of accessions	Name of the accessions
1	Cluster I	NKM3	1	*Phyllanthus rheedei*
2	Cluster II	NKH4	1	*Phyllanthus virgatus* var.*gardnerianus*
3	Cluster III	NKC3, NKP1	2	*Phyllanthus amarus*
4	Cluster IV	NKH2, NKP4	2	*Phyllanthus virgatus* var. *virgatus*
5	Cluster V	NKP5, NKP2, NKC1	3	*Phyllanthus urinaria*
6	Cluster VI	NKM2, NKC2	2	*Phyllanthus airy-shawii*
7	Cluster VII	NKP6	1	*Phyllanthus airy-shawii*
8	Cluster VIII	NKM1,NKH1	2	*Phyllanthus amarus*
9	Cluster IX	NKH3, NKP3	2	*Phyllanthus airy-shawii*

each, NKH4 from high range and NKP6 from plains respectively, the former representing *P. virgatus* var. *gardnerianus* and the latter, *P. airy-shawii*. NKH2 and NKP4 of cluster IV represented *P. virgatus* var. *virgatus*. Cluster III comprising of NKC3 from coastal area and NKP1 from plains and cluster VIII comprising of NKM1 from midlands and NKH1 from high ranges represented two different morphotypes of *P. amarus*. NKM2 and NKC2 of cluster VI, from midlands and coastal area respectively and NKH3 and NKP3 of cluster IX from high ranges and plains respectively, represented *P. airy-shawii*. Maximum number of accessions was grouped in cluster V (NKP5, NKP2, and NKC1) representing *P. urinaria*.

The cluster mean values of quantitative characters employed for grouping the collected accessions from northern zone into clusters, representing various *Phyllanthus* spp. are given in Table 11f.

Maximum mean values for stem length (90.1 cm), number of branchlets per plant (31.5), number of leaflets per compound leaf (36.8), leaf length (2.16 cm), fresh weight (16.21g) and dry weight (13.81g) were recorded in the accession NKH4 from high range in cluster II representing *P. virgatus* var. *gardnerianus*. NKM3 representing *P. rheedei* (cluster I) registered maximum leaf width (1.03 cm). Greatest number of capsules per branch of 33.1 each was noted in NKM3 (cluster I) and NKH4 (cluster II) representing *P. rheedei* and *P. virgatus* var. *gardnerianus* respectively. Least values for mean stem length (26.4 cm), number of branchlets per plant (9.9) and number of leaflets per compound leaf (20.8), were recorded in accessions NKP5, NKP2 and NPC1 of cluster V, representing *P. urinaria*. Minimum mean leaf length (0.52 cm) and mean leaf width (0.26 cm) were recorded in accession NKP6 of cluster VII and NKH3 and NKP3 of cluster IX, both clusters representing *P. airy-shawii*. Lowest values for number of mean number of capsules per branch (16.1), mean fresh weight (4.58g) and mean dry weight (2.38g) were registered in cluster V, comprising of NKP5, NKP2 and NKC1, representing *P. urinaria*.

Table 11f. Summary statistics for quantitative morphological characters of accessions *Phyllanthus* from northern zone

Sl No.	Cluster number	Cluster mean values							
		Stem length (cm)	No. of branchlets per plant	No. of leaflets per compound leaf	Leaf length (cm)	Leaf width (cm)	No. of capsules per branch	Fresh weight (g)	Dry weight (g)
1	Cluster I	56.2	23.9	39.6	2.08	1.03	33.1	9.55	7.35
2	Cluster II	90.1	31.5	36.8	2.16	0.54	33.1	16.21	13.81
3	Cluster III	32.8	14.9	30.8	0.68	0.42	25.8	6.21	4.09
4	Cluster IV	86.4	30.4	36.4	1.61	0.62	30.8	14.93	12.68
5	Cluster V	26.4	9.9	20.8	0.95	0.35	16.1	4.58	2.38
6	Cluster VI	43.4	21.2	35.4	0.86	0.42	29.2	7.81	5.72
7	Cluster VII	46.1	21.8	36.6	0.52	0.34	29.6	8.29	6.09
8	Cluster VIII	36.3	16.2	31.5	1.16	0.52	24.5	6.59	4.49
9	Cluster IX	42.1	20.0	34.3	0.72	0.26	29.4	7.36	5.21

4.1.7.4. *Cluster analysis of Phyllanthus accessions from coastal region based on variable quantitative characters*

Fig 4 represents the dendrogram of *Phyllanthus* accessions from coastal regions of southern, central and northern zones of Kerala, based on variable quantitative characters. Cluster analysis of ten accessions collected from northern, central and southern zones of coastal regions, based on quantitative characters, representing various species of *Phyllanthus* is given in Table 11g. As seen in the table, the collected *Phyllathus* accession from coastal regions representing southern, central and northern zone, could be grouped into five clusters at 16.8 per cent similarity.

Cluster I representing *P. amarus* consisted of three accessions from various zones. Clusters II, III and V, consisted of two accessions each, cluster II representing *P. airy-shawii* and clusters III and V representing *P. urinaria* (Table 11g). The one accession in cluster IV i.e., SKC3 represented *P. maderspatensis*.

The cluster mean values of quantitative characters employed for grouping the collected accessions into clusters of various species of *Phyllanthus* are given in Table 11h.

Significantly higher mean stem length (60.1 cm), mean number of branchlets per plant (25.2), number of leaflets per compound leaf (37.5), mean leaf length (1.51 cm), mean leaf width (0.63), number of capsules/branch (52.7), mean fresh weight (10.82) and dry weight (8.62) were recorded in the single accession SKC3 of cluster IV, representing *P. maderaspatensis*. Significantly lower values for mean plant height (23.8 cm), number of branchlets per plant (9.8), number of leaflets per compound leaf (20.5), mean number of capsules per branchlets (12.3), mean fresh weight (4.16 g) and mean dry weight (2.16) were recorded by accessions of cluster III representing *P. urinaria*. Significantly lower mean leaf length and mean leaf width were recorded by accessions included in cluster II representing *P. airy-shawii*.

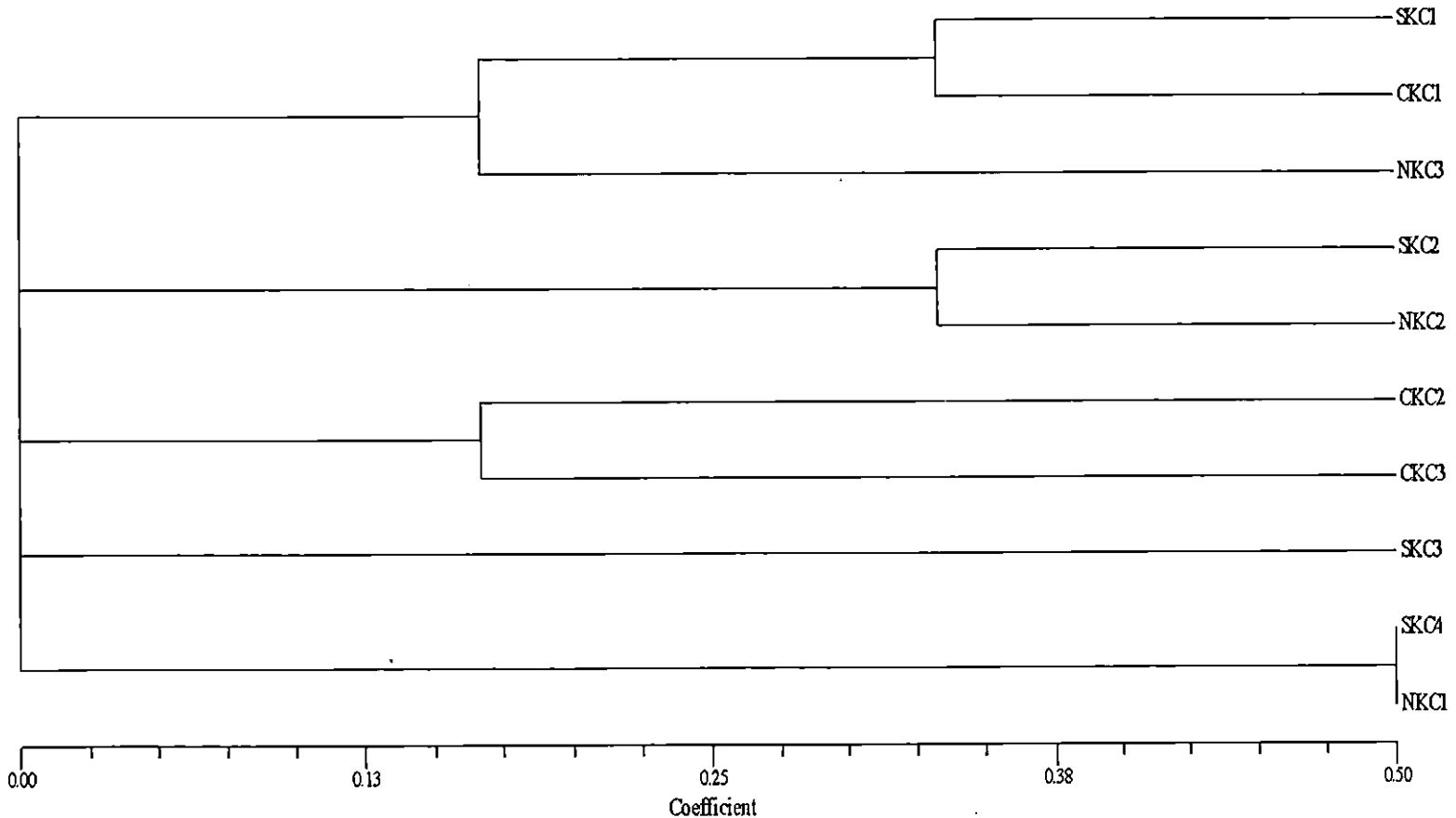


Fig 4. Dendrogram based on quantitative morphological characters of *Phyllanthus* accessions from coastal regions of Kerala

Table 11g. Clusters of *Phyllanthus* accessions collected from coastal regions based on quantitative morphological characters

Sl No.	Cluster number	Accessions	No. of accessions	Name of the accessions
1	Cluster I	SKC1, CKC1, NKC3	3	*Phyllanthus amarus*
2	Cluster II	SKC2, NKC2	2	*Phyllanthus airy-shawii*
3	Cluster III	CKC2, CKC3	2	*Phyllanthus urinaria*
4	Cluster IV	SKC3	1	*Phyllanthus maderaspatensis*
5	Cluster V	SKC4, NKC1	2	*Phyllanthus urinaria*

Table 11h. Summary statistics for quantitative morphological characters of *Phyllanthus* accessions from coastal regions

Sl No.	Cluster number	Cluster mean values							
		Stem length (cm)	No. of branchlets per plant	No. of leaflets per compound leaf	Leaf length (cm)	Leaf width (cm)	No. of capsules per branch	Fresh weight (g)	Dry weight (g)
1	Cluster I	34.63	15.96	31.73	0.853	0.503	25.6	6.22	4.23
2	Cluster II	43.2	20.8	34.25	0.57	0.22	34.4	7.77	5.87
3	Cluster III	23.8	9.8	20.5	1.10	0.61	12.3	4.16	2.16
4	Cluster IV	60.1	25.2	37.5	1.51	0.63	52.7	10.82	8.62
5	Cluster V	26.8	10.2	21.1	1.05	0.37	18.1	4.68	2.48

4.1.7.5. *Cluster analysis of Phyllanthus accessions from plains based on variable quantitative characteristics*

Fig 5 represents the dendrogram of *Phyllanthus* accessions from plains, based on variable quantitative characters

Cluster analysis of 12 accessions from midlands, representing southern, central and northern zones of Kerala is given in Table 11i. The accessions were grouped into six clusters at 17 per cent similarity, with cluster I and cluster IV consisting of three accessions each, representing *P. amarus* and *P. urinaria* respectively (Table 11i). Single accessions each were observed in cluster II (CKP2) and cluster III (NKP4), representing *P. rheedei* and *P. virgatus* var. *virgatus* respectively. Cluster V and VI consisted of two accessions each viz., NKP3 and NKP6 in cluster V representing *P. airy-shawii* and CKP1 and SKP2 representing *P. urinaria.*

Cluster mean values of quantitative characters employed for grouping the collected accessions from plains into various clusters, representing different species of *Phyllanthus* is given in Table 11j. Accessions of cluster III representing *P. virgatus* var. *virgatus* were superior for all the parameters studied. Accessions of cluster VI representing *P. urinaria* recorded significantly lower values for all the above parameters (Table 11j).

4.1.7.6. *Cluster analysis of Phyllanthus accessions from midlands based on variable quantitative characters*

Fig 6 represents the dendrogram of *Phyllanthus* accessions from midlands, based on variable quantitative characters

Cluster analysis of 12 accessions from midlands, representing southern, central and northern zones of Kerala is given in Table 11k. The various accessions from midlands could be grouped into six clusters at 8.5 per cent similarity, two

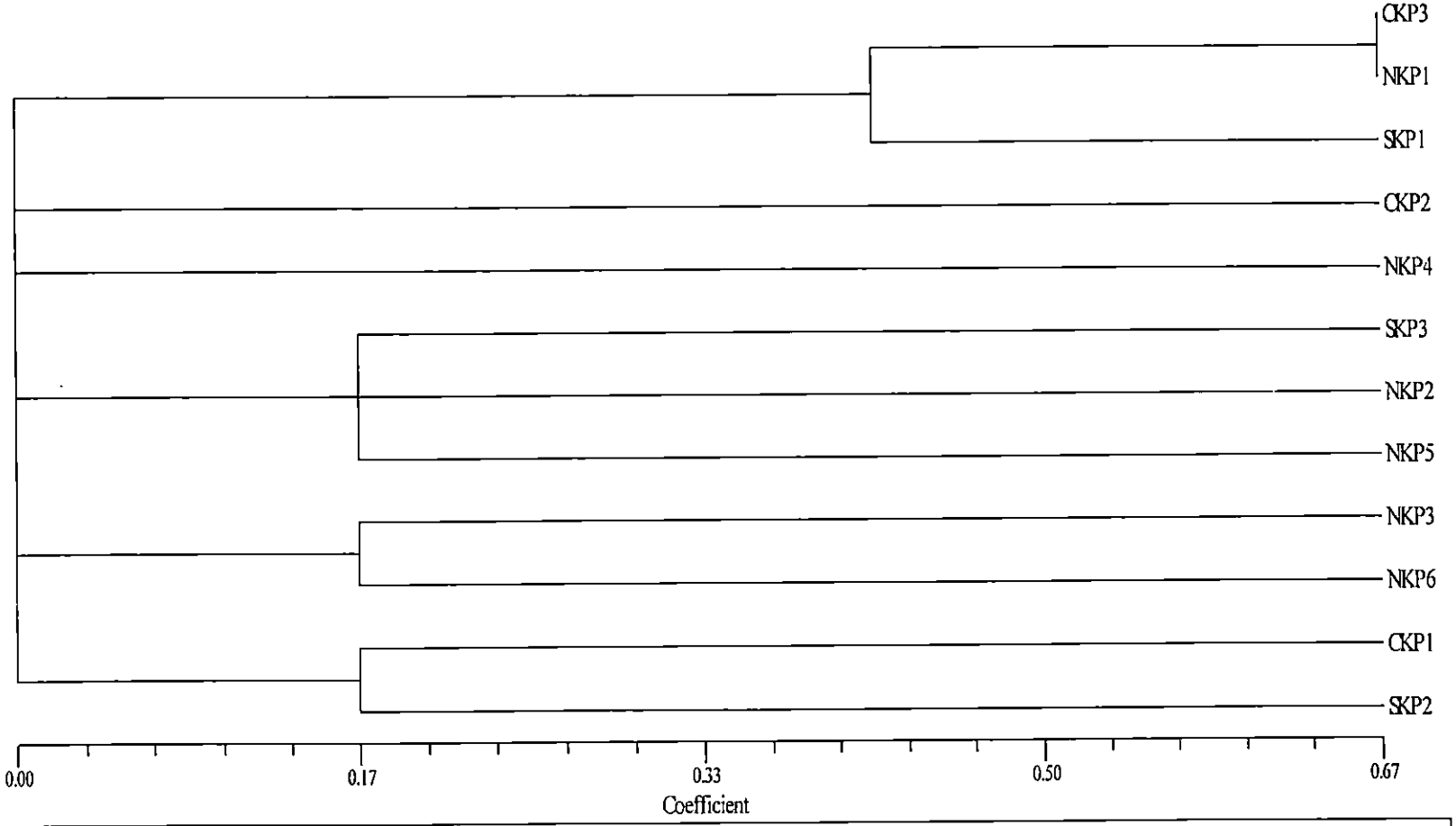


Fig 5. Dendrogram based on quantitative morphological characters of *Phyllanthus* accessions from plains of Kerala

Table 11i. Clusters of *Phyllanthus* accessions collected from plains based on quantitative morphological characters

Sl No.	Cluster number	Accessions	No. of accessions	Name of the accessions
1	Cluster I	CKP3, SKP1, NKP1	3	*Phyllanthus amarus*
2	Cluster II	CKP2	1	*Phyllanthus rheedei*
3	Cluster III	NKP4	1	*Phyllanthus virgatus* var. *virgatus*
4	Cluster IV	SKP3, NKP2, NKP5	3	*Phyllanthus urinaria*
5	Cluster V	NKP3, NKP6	2	*Phyllanthus airy-shawii*
6	Cluster VI	CKP1, SKP2	2	*Phyllanthus urinaria*

Table 11j. Summary statistics for variable quantitative morphological characters of *Phyllanthus* accessions from plain region

Sl No.	Cluster number	Cluster mean values							
		Stem length (cm)	No. of branchlets per plant	No. of leaflets per compound leaf	Leaf length (cm)	Leaf width (cm)	No. of capsules per branch	Fresh weight (g)	Dry weight (g)
1	Cluster	34.5	15.8	31.7	0.793	0.456	25.6	6.30	4.10
2	Cluster II	36.3	17.2	33.1	1.02	0.52	23.2	06.53	4.33
3	Cluster III	87.4	30.6	36.5	1.51	0.63	30.2	15.71	13.21
4	Cluster IV	26.7	9.93	20.86	0.93	0.32	17.2	4.71	2.59
5	Cluster V	44.1	20.85	35.45	0.47	0.28	29.6	7.72	5.57
6	Cluster VI	22.0	9.6	20.25	0.91	0.32	14.4	3.85	1.70

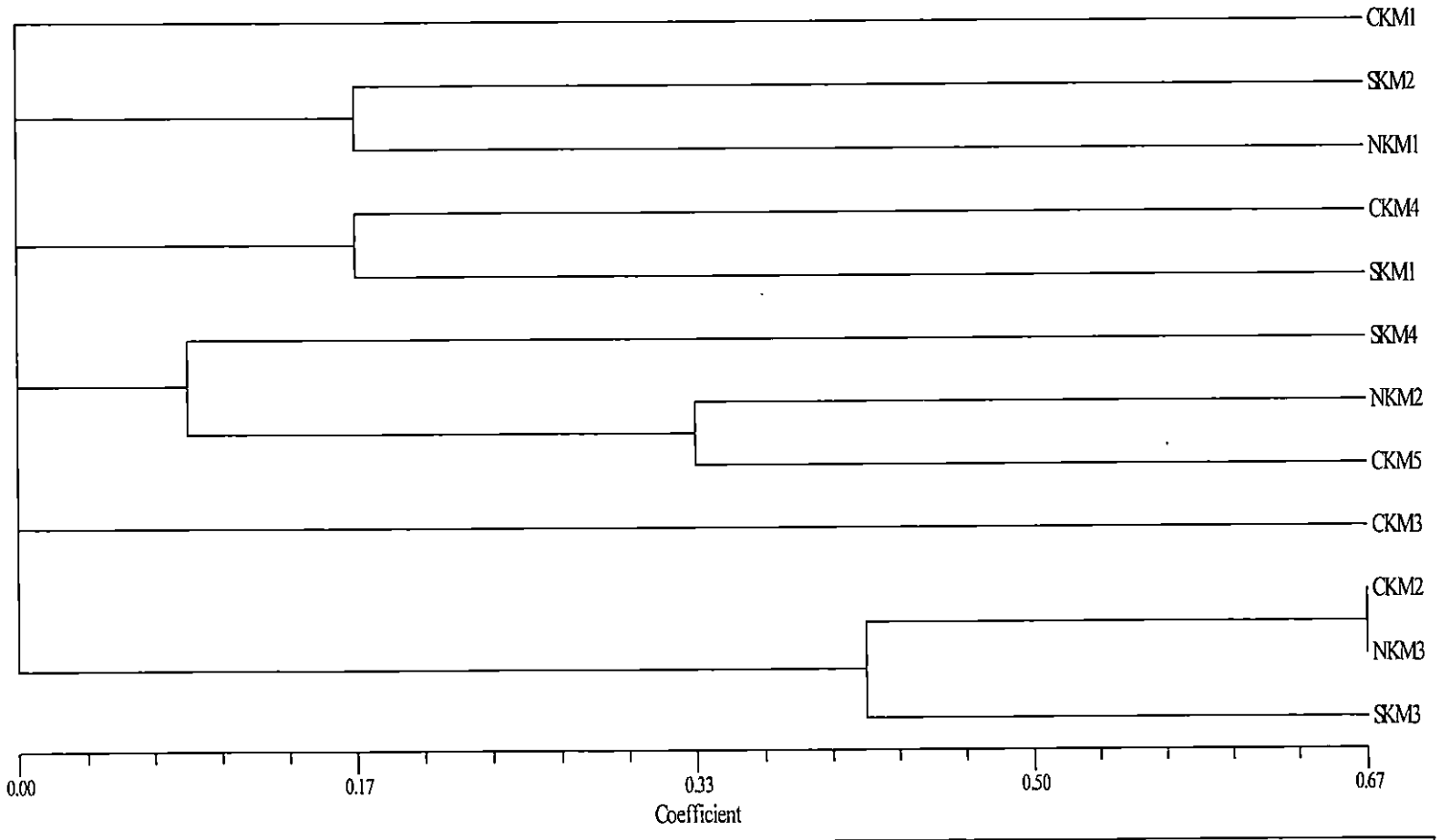


Fig 6. Dendrogram based on quantitative morphological characters of *Phyllanthus* accessions from midlands of Kerala

Table 11k. Clusters of *Phyllanthus* accessions collected from midlands based on quantitative morphological characters

Sl No.	Cluster number	Accessions	No. of accessions	Name of the accessions
1	Cluster I	CKM1	1	*Phyllanthus amarus*
2	Cluster II	SKM2, NKM1	2	*Phyllanthus amarus*
3	Cluster III	CKM4, SKM1	2	*Phyllanthus urinaria*
4	Cluster IV	CKM5, SKM4, NKM2	3	*Phyllanthus airy-shawii*
5	Cluster V	CKM3	1	*Phyllanthus virgatus* var. *virgatus*
6	Cluster VI	CKM2, SKM3, NKM3	3	*Phyllanthus rheedei*

clusters representing *P. amarus*, and one cluster each represent *P. urinaria, P. airy-shawii, P. virgatus* var. *virgatus* and *P. rheedei*. Three accessions each were grouped in cluster IV (CKM5, SKM4 and NKM2 representing *P. airy-shawii*) and cluster VI (CKM2, SKM3 and NKM3 representing *P. rheedei*). Two accessions each were grouped in cluster II (SKM2 and NKM1) and cluster III (CKM4 and SKM1), representing *P. amarus* and *P. urinaria* respectively. Cluster I and V had single accession each , CKM1 in cluster I representing *P. amarus* and CKM3 in cluster V representing, *P. virgatus* var. *virgatus* based on variable quantitative characters (Table 11k).

Cluster mean values of quantitative characters employed for grouping the collected accessions from midlands into various clusters, representing different species of *Phyllanthus* is given in Table 11l.

Significantly higher mean plant height (87.1 cm) mean number of branchlets per plant (30.3), mean number of leaflets per compound leaf (40.9), mean fresh weight (15.67 g) and mean dry weight (13.27 g) were recorded by accessions of cluster V representing *P. virgatus* var. *virgatus*. Accessions included in cluster VI, representing *P. rheedei* registered significantly higher mean leaf length (2.13 cm), mean leaf width (1.18 cm) and mean number of capsules per branch (36.54). Significantly lower values for all parameters except mean leaf length were registered by accessions grouped in cluster III representing *P. urinaria.*

4.1.7.7. *Cluster analysis of Phyllanthus accessions from high ranges based on variable quantitative characters*

Fig 7 represents the dendrogram of *Phyllanthus* accessions from high ranges, based on variable quantitative characters. Cluster analysis of thirteen accessions from high ranges representing southern, central and northern zones of Kerala were grouped into seven clusters at 9.1 per cent similarity (Table 11m). From the table it is evident that, Cluster I representing *P. amarus* consisted of three accessions NKM1, SKH1 and CKH1, cluster II and cluster III, representing

Table 11l. Summary statistics for quantitative morphological characters of *Phyllanthus* accessions from midland region

Sl No.	Cluster number	Cluster mean values							
		Stem length (cm)	No. of branchlets per plant	No. of leaflets per compound leaf	Leaf length (cm)	Leaf width (cm)	No. of capsules per branch	Fresh weight (g)	Dry weight (g)
1	Cluster I	34.7	16.5	32.41	1.01	0.44	22.51	06.22	4.45
2	Cluster II	37.4	16.6	31.85	0.92	0.51	26.15	6.64	4.49
3	Cluster III	25.8	10.15	20.85	1.06	0.38	14.35	4.52	2.46
4	Cluster IV	42.76	20.76	34.36	0.88	0.42	29.76	7.55	5.32
5	Cluster V	87.1	30.3	40.9	1.21	0.47	34.3	15.67	13.27
6	Cluster VI	54.33	23.33	39.1	2.13	1.18	36.54	9.76	7.56

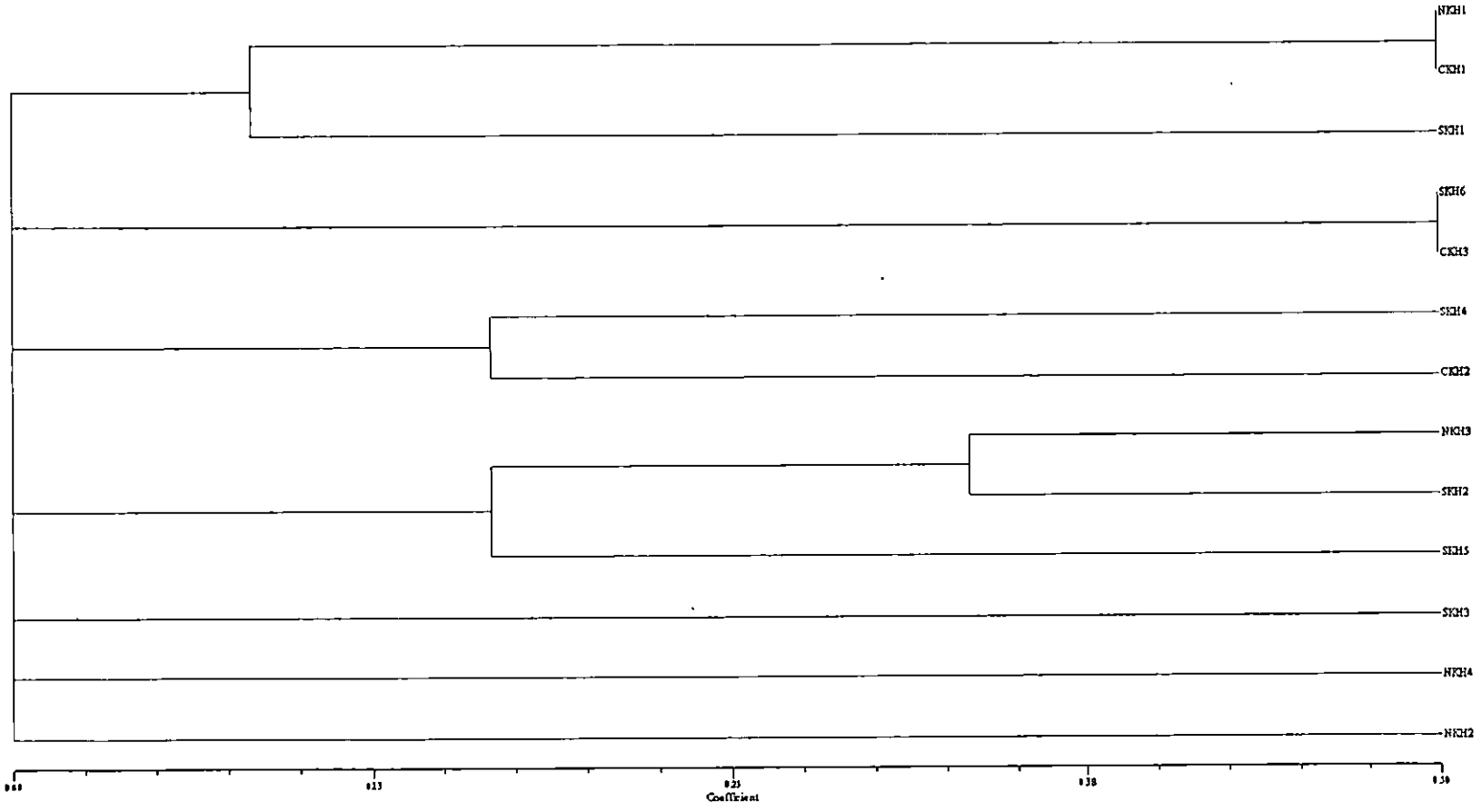


Fig 7. Dendrogram based on quantitative morphological characters of *Phyllanthus* accessions from high ranges of Kerala

Table 11m. Clusters of *Phyllanthus* accessions collected from high ranges based on quantitative morphological characters

Sl No.	Cluster number	Accessions	No. of accessions	Name of the accessions
1	Cluster I	NKH1, SKH1, CKH1	3	*Phyllanthus amarus*
2	Cluster II	SKH6, CKH3	2	*Phyllanthus urinaria*
3	Cluster III	SKH4, CKH2	2	*Phyllanthus urinaria*
4	Cluster IV	NKH3, SKH2, SKH5	3	*Phyllanthus airy-shawii*
5	Cluster V	SKH3	1	*Phyllanthus rheedei*
6	Cluster VI	NKH4	1	*Phyllanthus virgatus* var. *gardnerianus*
7	Cluster VII	NKH2	1	*Phyllanthus virgatus* var. *virgatus*

different morphotypes of *P .urinaria*, consisted of two accessions each, SKH6 and CKH3 and SKH4 and CKH2, respectively. Single accessions each were included in cluster V (SKH3) representing *P. rheedei*, cluster VI (NKH4) representing *P. virgatus* var. *gardnerianus*) and cluster VII (NKH2) representing *P. virgatus* var. *gardnerianus*). Three accessions NKH3, SKH2 and SKH5 representing *P. airy-shawii* were included in cluster IV.

Cluster mean values of quantitative characters employed for grouping the collected accessions from high ranges into various clusters, representing different species of *Phyllanthus* is given in Table 11n.

The lone accession NKH4 belonging to cluster VI representing *P. virgatus* var. *gardnerianus*, recorded significantly higher mean stem length (90.1 cm), mean number of branchlets per plant (31.5), mean leaf length (2.08 cm), mean fresh weight (16.21 g) and mean dry weight (13.81 g). Significantly higher mean number of leaflets per compound leaf (38.1), mean leaf width (1.31 cm) and mean number of capsules per branchlet (44.9) were recorded in accession SKH3, grouped in cluster V, representing *P. rheedei*. Significantly lower values for all characters except mean leaf length and leaf width were recorded by the accessions SKH4 and CKH2 grouped in cluster III, representing *P. urinaria*.

Table 11n. Summary statistics for quantitative morphological characters of *Phyllanthus* accessions from high ranges

Sl No.	Cluster number	Cluster mean values							
		Stem length (cm)	No. of branchlets per plant	No. of leaflets per compound leaf	Leaf length (cm)	Leaf width (cm)	No. of capsules per branch	Fresh weight (g)	Dry weight (g)
1	Cluster I	33.9	15.56	31.16	0.94	0.48	24.46	6.08	3.99
2	Cluster II	27.0	10.3	21.1	1.21	0.41	17.1	4.85	2.75
3	Cluster III	22.5	9.85	20.45	1.26	0.42	14.05	3.82	1.77
4	Cluster IV	43.76	20.5	33.3	0.756	0.243	34.96	7.57	5.44
5	Cluster V	51.2	22.7	38.1	2.01	1.31	44.9	08.70	06.60
6	Cluster VI	90.1	31.5	36.8	2.08	0.54	33.1	16.21	13.81
7	Cluster VII	85.4	30.2	36.4	1.71	0.62	31.5	14.15	12.15

4.1.8. Performance of *Phyllanthus* accessions within altitudinal regions in pot culture with respect to growth characters.

Results of the performance of collected *Phyllanthus* accessions in pot culture, zone wise, within each altitudinal region is given below.

4.1.8.1. *Coastal region*

Southern zone

Significant variation was observed among the accessions of southern zone of coastal region with respect to mean plant height, mean number of branchlets, mean number of leaflets and east-west spread (Table 12a). In the zone, significantly higher mean plant height (60.1 cm), mean number of branchlets (25.2), number of leaflets (37.5) and east- west spread (24.2 cm) were registered by accessions SKC3 followed by SKC2, recording 42.6 cm, 20.3, 32.4 and 13.4 cm respectively for mean plant height, mean number of branchlets, mean number of leaflets and east-west spread. Least values for all the biometric characters observed, were recorded by accession SKC4 (Table 12a).**Central zone**

For the same altitudinal area under observation, significant variation was noted among accessions of central zone, for the biometric characters observed (Table 12b). In the zone, significantly higher mean plant height (34.7 cm). mean number of branchlets (16.5), mean number of leaflets (32.4) and mean east-west spread (13.2 cm) were recorded by the accession CKC1. The accessions CKC2 and CKC3 were on par with each other with respect to the parameters studied (Table 12b).

Northern zone

Among the accessions of northern zone of coastal region, significant variation was noted for mean plant height, mean number of branchlets, mean number of leaflets and east-west spread (Table 12c). NKC2 recorded the highest mean plant height (43.8 cm), mean number of branchlets (21.3), number of leaflets (36.1) and east-west spread (13.9 cm). the values being significantly superior.

Table 12a. Performance of collected *Phyllanthus* accessions from coastal region of southern zone in pot culture, with respect to growth characteristics

Biometric characters	Accessions			
	SKC1	SKC2	SKC3	SKC4
Mean plant height (cm)	36.1^{c}	42.6^{b}	60.1^{a}	27.5^{d}
Mean number of branchlets	16.1^{c}	20.3^{b}	25.2^{a}	10.3^{d}
Mean number of leaflets	31.9^{b}	32.4^{b}	37.5^{a}	21.1^{c}
Mean east-west spread (cm)	13.1^{b}	13.4^{b}	24.2^{a}	9.1^{c}

Table 12b. Performance of collected *Phyllanthus* accessions from coastal region of central zone in pot culture, with respect to growth characteristics

Biometric characters	Accessions		
	CKC1	CKC2	CKC3
Mean plant height (cm)	34.7^{a}	23.5^{b}	24.1^{b}
Mean number of branchlets	16.5^{a}	9.7^{b}	9.9^{b}
Mean number of leaflets	32.4^{a}	20.2^{b}	20.8^{b}
Mean east-west spread (cm)	13.2^{a}	8.6^{b}	8.8^{b}

Table 12c. Performance of collected *Phyllanthus* accessions from coastal region of northern zone in pot culture, with respect to growth characteristics

Biometric characters	Accessions		
	NKC1	NKC2	NKC3
Mean plant height (cm)	26.1^{c}	43.8^{a}	33.1^{b}
Mean number of branchlets	10.1^{c}	21.3^{a}	15.3^{b}
Mean number of leaflets	21.1^{c}	36.1^{a}	30.9^{b}
Mean east-west spread (cm)	9.1^{b}	13.9^{a}	12.3^{a}

4.1.8.2. *Plains*

Southern zone

The accessions of southern zone in plains recorded values significantly different from one another with respect to the parameters studied (Table 12d). SKP1 recorded significantly higher values for mean plant height (34.7 cm), mean number of branchlets (15.6), mean number of leaflets (31.3) and mean east-west spread (12.6 cm). SKP2 and SKP3 recorded values on par with each other, with respect to mean number of branchlets (9.7 and 10.2 respectively), mean number of leaflets (20.1 and 21.2 respectively) and mean east-west spread (8.2 and 9.3 cm respectively).

Central zone

Significant variation was noted among accessions of central zone in plains, with respect to the parameters studied (Table 12e). CKP2 was significantly superior, registering higher values for mean plant height (54.2 cm), mean number of branchlets (22.8), mean number of leaflets (38.8) and mean east-west spread (15.7 cm), followed by the accession CKP3 registering a mean plant height of 36.3 cm, with 17.2 number of branchlets, 33.1 number of leaflets and an east-west spread of 14.1 cm, the last value, being on par with that of CKP2.

Northern zone

The accessions from northern zone of plains were significantly different from one another, with respect to the characters studied (Table 12f). The accession NKP4 recorded highest mean plant height (87.4 cm), mean number of branchlets (30.6), mean number of leaflets (36.5) and greatest mean east-west spread (37.5cm). The accessions NKP2 and NKP5 were on par with each other with respect to the characters studied.

4.1.8.3. *Midlands*

Southern zone

The accessions from the midlands of southern zone varied significantly in characters like mean plant height, mean number of branchlets, mean number of leaflets and mean east-west spread (Table 12g). The accession SKM3 recorded

Table 12d. Performance of collected *Phyllanthus* accessions from plains of southern zone in pot culture, with respect to growth characteristics

Biometric characters	Accessions		
	SKP1	SKP2	SKP3
Mean plant height (cm)	34.7^{a}	21.3^{c}	26.9^{b}
Mean number of branchlets	15.6^{a}	9.7^{b}	10.2^{b}
Mean number of leaflets	31.3^{a}	20.1^{b}	21.2^{b}
Mean east-west spread (cm)	12.6^{a}	8.2^{b}	9.3^{b}

Table 12e. Performance of collected *Phyllanthus* accessions from plains of central zone in pot culture, with respect to growth characteristics

Biometric characters	Accessions		
	CKP1	CKP2	CKP3
Mean plant height (cm)	22.7^{c}	54.2^{a}	36.3^{b}
Mean number of branchlets	9.5^{c}	22.8^{a}	17.2^{b}
Mean number of leaflets	20.4^{c}	38.8^{a}	33.1^{b}
Mean east-west spread (cm)	8.5^{b}	15.7^{a}	14.1^{a}

Table 12f. Performance of collected *Phyllanthus* accessions from plains of northern zone in pot culture, with respect to growth characteristics

Biometric characters	Accessions					
	NKP1	NKP2	NKP3	NKP4	NKP5	NKP6
Mean plant height (cm)	32.5^{d}	25.6^{e}	42.1^{c}	87.4^{a}	27.6^{e}	46.1^{b}
Mean number of branchlets	14.6^{c}	9.7^{d}	19.9^{b}	30.6^{a}	9.9^{d}	21.8^{b}
Mean number of leaflets	30.7^{b}	20.6^{e}	34.3^{a}	36.5^{a}	20.8^{c}	36.6^{a}
Mean east-west spread (cm)	11.8^{c}	8.7^{d}	13.3^{bc}	37.5^{a}	8.9^{d}	14.9^{b}

Table 12g. Performance of collected *Phyllanthus* accessions from midlands of southern zone in pot culture, with respect to growth characteristics

Biometric characters	Accessions			
	SKM1	SKM2	SKM3	SKM4
Mean plant height (cm)	24.9^{c}	37.3^{b}	52.6^{a}	41.1^{b}
Mean number ofbranchlets	10.1^{c}	16.3^{b}	22.9^{a}	20.1^{a}
Mean number of leaflets	20.6^{c}	31.8^{b}	38.6^{a}	32.3^{b}
Mean east-west spread (cm)	8.8^{c}	12.9^{b}	15.6^{a}	13.2^{b}

Table 12h. Performance of collected accessions from midlands of central zone in pot culture, with respect to growth characteristics

Biometric characters	Accessions				
	CKM1	CKM2	CKM3	CKM4	CKM 5
Mean plant height (cm)	34.1^{d}	54.2^{b}	87.1^{a}	26.7^{e}	44.1^{c}
Mean number of branchlets	16.3^{c}	23.2^{b}	30.3^{a}	10.2^{d}	21.1^{b}
Mean number of leaflets	32.2^{b}	39.1^{a}	40.9^{a}	21.1^{c}	36.1^{ab}
Mean east-west spread (cm)	13.1^{b}	15.7^{b}	36.6^{a}	9.3^{c}	14.2^{b}

Table 12i. Performance of collected *Phyllanthus* accessions from midlands of northern zone in pot culture, with respect to growth characteristics

Biometric characters	Accessions		
	NKM1	NKM2	NKM3
Mean plant height (cm)	37.5^{b}	43.1^{b}	56.2^{a}
Mean number of branchlets	16.9^{b}	21.1^{a}	23.9^{a}
Mean number of leaflets	31.9^{b}	34.7^{ab}	39.5^{a}
Mean east-west spread (cm)	12.8^{b}	13.7^{b}	15.9^{a}

significantly higher values for mean plant height (52.6 cm), mean number of branchlets (22.9), mean number of leaflets (38.6) and mean east-west spread (15.6 cm). SKM2 and SKM4 were on par with each other, with respect to mean plant height, mean number of leaflets and mean east-west spread. Lowest values were registered by SKM1 for all the characters studied (Table 12g).

Central zone

Significant variation was noted among the accessions of central zone in midlands with respect to mean plant height, mean number of branchlets, mean number of leaflets and mean east-west spread (Table 12h). For all the characters under observation, CKM3 recorded significantly higher values (87.1 cm for mean plant height, 30.3 for mean number of branchlets, 40.9 for mean number of leaflets and 36.6 cm for mean east-west spread. The accessions CKM2 was also significantly superior with respect to mean number of leaflets (39.1).The accessions CKM2 and CKM5 recorded values on par with each other with respect to mean number of branchlets (23.2 and 21.1 respectively) and mean east- west spread (15.7 and 14.2 cm respectively). CKM1 recorded lowest values for all the parameters studied (Table 10h).

Northern zone

Significant variation was noted among the accessions of northern zone in midlands with respect to mean plant height, mean number of branchlets, mean number of leaflets and mean east-west spread (Table 12i). Significantly higher values for mean plant height (56.2 cm), mean number of branchlets (23.9), mean number of leaflets (39.5) and mean east-west spread (15.9 cm) were recorded by the accession NKM3. The accession NKM2 also recorded values on par with NKM3 with respect to mean number of branchlets. NKM1 recorded lowest values for mean number of branchlets (16.9) and mean number of leaflets (31.9).

4.1.8.4. *High ranges*

Southern zone

Experimental results indicate that mean plant height, mean number of branchlets, mean number of leaflets and east-west spread, vary significantly among the six accessions from high ranges of southern zone of Kerala (Table 12j).

Table 12j. Performance of collected *Phyllanthus* accessions from high ranges of southern zone in pot culture, with respect to growth characteristics

Biometric characters	Accessions					
	SKH1	SKH2	SKH3	SKH4	SKH5	SKH6
Mean plant height (cm)	33.1^{c}	45.6^{b}	51.2^{a}	22.1^{e}	43.6^{b}	28.8^{d}
Mean number of branchlets	15.4^{b}	20.9^{a}	22.7^{a}	9.9^{c}	20.5^{a}	10.5^{c}
Mean number of leaflets	31.1^{b}	32.9^{b}	38.1^{a}	20.3^{c}	32.6^{b}	21.3^{c}
Mean east-west spread (cm)	12.4^{a}	13.9^{ab}	15.4^{a}	8.6^{c}	13.6^{ab}	9.3^{c}

Table 12k. Performance of collected *Phyllanthus* accessions from high ranges of central zone in pot culture, with respect to growth characteristics

Biometric characters	Accessions		
	CKH1	CKH2	CKH3
Mean plant height (cm)	33.5^{a}	22.9^{b}	25.2^{b}
Mean number of branchlets	15.7^{a}	9.8^{b}	10.1^{b}
Mean number of leaflets	31.2^{a}	20.6^{b}	20.9^{b}
Mean east-west spread(cm)	12.5^{a}	8.6^{b}	8.9^{b}

Table 12l. Performance of collected *Phyllanthus* accessions from high ranges of northern zone in pot culture, with respect to growth characteristics

Biometric characters	Accessions			
	NKH1	NKH2	NKH3	NKH4
Mean plant height (cm)	35.1^{d}	85.4^{b}	42.1^{c}	90.1^{a}
Mean number of branchlets	15.6^{c}	30.2^{a}	20.1^{b}	31.5^{a}
Mean number of leaflets	31.2^{b}	36.4^{a}	34.4^{a}	36.8^{a}
Mean east-west spread(cm)	12.6^{b}	37.4^{a}	14.1^{b}	37.8^{a}

The accessions SKH3 recorded significantly higher values for mean plant height (51.2 cm), mean number of branchlets (22.7), mean number of leaflets (38.1) and mean east- west spread (15.4 cm). The accessions SKH2 and SKH5 recorded values on par with each other for the parameters studied and were noted as the second best (Table 12j). The accession SKH6 was inferior with respect to mean plant height.

Central zone

Accessions from central zone of high ranges also recorded significant variation among one another, for mean plant height, mean number of branchlets, mean number of leaflets and mean east- west spread, with CKH1 registering significantly higher values for all the parameters studied. (Table 12k). The accessions CKH2 and CKH3 were on par with each other, with respect to mean plant height, mean number of branchlets, mean number of leaflets and mean east-west spread.

Northern zone

Significant variation was noted among the accessions of northern zone of high ranges, with respect to mean plant height, mean number of branchlets, mean number of leaflets and mean east-west spread, with NKH4 recording significantly higher mean plant height (90.1 cm). For the rest of the characters, NKH4 recorded value on par with NKH2. Significantly lower values were registered by NKH1 for all the characters studied (Table 12l).

4.1.9. Performance of *Phyllanthus* accessions within altitudinal regions in pot culture with respect to yield parameters

4.1.9.1. *Coastal region*

Accessions from southern, central and northern zone of coastal region recorded significant variation among one another, for mean fresh and dry weights. In southern zone, SKC3 registered significantly higher values for the parameters studied, followed by SKC2. Lowest values were registered by SKC4 (Table 13a).

Table 13a. Performance of collected *Phyllanthus* accessions from coastal region of southern zone in pot culture, with respect to yield characteristics

Biometric characters	Accessions			
	SKC1	SKC2	SKC3	SKC4
Mean fresh weight per plant (g)	6.13^{c}	7.66^{b}	10.82^{a}	4.94^{d}
Mean dry weight per plant (g)	4.03^{c}	5.76^{b}	8.62^{a}	2.84^{d}

Table 13b. Performance of collected *Phyllanthus* accessions from coastal region of central zone in pot culture, with respect to yield characteristics

Biometric characters	Accessions		
	CKC1	CKC2	CKC3
Mean fresh weight per plant (g)	6.25^{a}	3.99^{c}	4.33^{b}
Mean dry weight per plant (g)	4.45^{a}	2.09^{c}	2.23^{b}

Table 13c. Performance of collected *Phyllanthus* accessions from coastal region of northern zone in pot culture, with respect to yield characteristics

Biometric characters	Accessions		
	NKC1	NKC2	NKC3
Mean fresh weight per plant (g)	4.43^{c}	7.88^{a}	6.28^{b}
Mean dry weight per plant (g)	2.13^{c}	5.99^{a}	4.23^{b}

With respect to mean fresh and dry weights, the accession CKC1 registered significantly higher values for the parameters studied, followed by CKC3 in central zone. Lowest values were recorded by CKC3 (Table 13b).

In northern zone, the accession NKC2 recorded significantly higher value with respect to mean fresh (7.88 g) and mean dry weight (5.99 g). Accession NKC3 recorded the second best value for the parameters studied (Table 13c).

4.1.9.2. *Plains*

Accessions from southern, central and northern zone of plains recorded significant variation among one another, for mean fresh and dry weights. In southern zone, SKP1 registered significantly higher values for all the parameters studied, followed by SKP3. Lowest values were registered by SKP2 (Table 13d).

The accession CKP2 registered significantly higher values, with respect to mean fresh (9.75 g) and dry weight (7.45 g), followed by CKP3 in central zone. Lowest values were registered by CKP1 (Table 13e).

The accession NKP4 registered significantly higher values, with respect to mean fresh (15.71 g) and dry weight (13.21 g), followed by NKP6 in northern zone. Lowest values were registered by NKP2 (Table 13f).

4.1.9.3. *Midlands*

Accessions from southern, central and northern zone of midlands recorded significant variation among one another, for mean fresh and dry weights. In southern zone, SKM3 registered significantly higher values for the parameters studied, followed by SKM4. Lowest values were registered by SKM1 (Table 13g).

With respect to mean fresh and dry weights, the accession CKM3 registered significantly higher values for the parameters studied, followed by CKM2 in central zone. Lowest values were recorded by CKM4 (Table 13h).

In northern zone, the accession NKM3 recorded significantly higher value with respect to mean fresh (9.55 g) and dry weight (7.35 g). Accession NKM2 recorded the second best values for the parameters studied (Table 13i).

Table 13d. Performance of collected *Phyllanthus* accessions from plains of southern zone in pot culture, with respect to yield characteristics

Biometric characters	Accessions		
	SKP1	SKP2	SKP3
Mean fresh weight per plant (g)	6.24[a]	3.62[c]	4.84[b]
Mean dry weight per plant (g)	4.04[a]	1.42[c]	2.74[b]

Table 13e. Performance of collected *Phyllanthus* accessions from plains of central zone in pot culture, with respect to yield characteristics

Biometric characters	Accessions		
	CKP1	CKP2	CKP3
Mean fresh weight per plant (g)	4.09[c]	9.75[a]	6.53[b]
Mean dry weight per plant (g)	1.99[c]	7.45[a]	4.33[b]

Table 13f. Performance of collected *Phyllanthus* accessions from plains of northern zone in pot culture, with respect to yield characteristics

Biometric characters	Accessions					
	NKP1	NKP2	NKP3	NKP4	NKP5	NKP6
Mean fresh weight per plant (g)	6.15[e]	4.35[f]	7.15[c]	15.71[a]	4.96[d]	8.29[b]
Mean dry weight per plant (g)	3.95[e]	2.05[f]	5.05[c]	13.21[a]	2.98[d]	6.09[b]

Table 13g. Performance of collected *Phyllanthus* accessions from midlands of southern zone in pot culture, with respect to yield characteristics

Biometric characters	Accessions			
	SKM1	SKM2	SKM3	SKM4
Mean fresh weight per plant (g)	4.23^{d}	6.71^{c}	9.98^{a}	6.98^{b}
Mean dry weight per plant (g)	2.13^{d}	4.51^{c}	7.88^{a}	4.78^{b}

Table 13h. Performance of collected *Phyllanthus* accessions from midlands of central zone in pot culture, with respect to yield characteristics

Biometric characters	Accessions				
	CKM1	CKM2	CKM3	CKM4	CKM5
Mean fresh weight per plant (g)	5.79^{d}	9.75^{b}	15.67^{a}	4.81^{e}	7.93^{c}
Mean dry weight per plant (g)	3.69^{d}	7.45^{b}	13.27^{a}	2.79^{e}	5.73^{c}

Table 13i. Performance of collected *Phyllanthus* accessions from midlands of northern zone in pot culture, with respect to yield characteristics

Biometric characters	Accessions		
	NKM1	NKM2	NKM3
Mean fresh weight per plant (g)	6.57^{c}	7.75^{b}	9.55^{a}
Mean dry weight per plant (g)	4.47^{c}	5.45^{b}	7.35^{a}

4.1.9.4. *High ranges*

Accessions from southern, central and northern zone of high ranges recorded significant variation among one another, for mean fresh and dry weight. In southern zone, SKH3 registered significantly higher values for all the parameters studied, followed by SKH2. Lowest values were registered by SKH4 (Table 13j).

The accession CKH1 registered significantly higher values, with respect to mean fresh (6.03 g) and dry (4.13 g) weights, followed by CKH3 in central zone. Lowest values were registered by CKH2 (Table 13k).

The accession NKH4 registered significantly higher values, with respect to mean fresh (16.21 g) and dry (13.81 g) weights, followed by NKH2 in northern zone. Lowest values were registered by NKH1 (Table 13l).

4.1.10. Altitudinal influence on growth parameters of *Phyllanthus* spp. in pot culture

The accessions of *P. amarus* and *P. urinaria* collected from various altitudes of different zones of Kerala recorded significant differences with respect to mean plant height, (Tables 14a and 14b) wherein, accessions from coastal regions and midlands were rated superior in the former species and those from high ranges, in the latter. For the rest of the characters the two species did not exhibit significant differences based on the temporal sites of collection. The accessions of *P. airy-shawii* and *P. rheedei* did not show significant differences with respect to mean plant height, mean number of branchlets, mean number of leaflets and east-west spread (Tables 14c and 14d). With respect to number of leaflets, accessions of *P. virgatus* var. *virgatus* collected from midlands in the central zone of Kerala was superior (Table 14e). Since there is only one accession each for *P. maderaspatensis* and *P. virgatus* var. *gardnerianus*, species wise comparison based on temporal sites is not possible.

Table 13j. Performance of collected *Phyllanthus* accessions from high ranges of southern zone in pot culture, with respect to yield characteristics

Biometric characters	Accessions					
	SKH1	SKH2	SKH3	SKH4	SKH5	SKH6
Mean fresh weight per plant (g)	5.62[d]	7.75[b]	8.70[a]	3.75[f]	7.41[c]	5.18[e]
Mean dry weight per plant (g)	3.34[d]	5.65[b]	6.60[a]	1.55[f]	5.31[c]	3.08[e]

Table 13k. Performance of collected *Phyllanthus* accessions from high ranges of central zone in pot culture, with respect to yield characteristics

Biometric characters	Accessions		
	CKH1	CKH2	CKH3
Mean fresh weight per plant (g)	6.03[a]	3.89[c]	4.53[b]
Mean dry weight per plant (g)	4.13[a]	1.98[c]	2.43[b]

Table 13l. Performance of collected *Phyllanthus* accessions from high ranges of northern zone in pot culture, with respect to yield characteristics

Biometric characters	Accessions			
	NKH1	NKH2	NKH3	NKH4
Mean fresh weight per plant (g)	6.61[d]	14.15[b]	7.57[c]	16.21[a]
Mean dry weight per plant (g)	4.51[d]	12.15[b]	5.37[c]	13.81[a]

Table 14a. Altitudinal influence on growth parameters of *Phyllanthus amarus* accessions in pot culture

Zones	Temporal sites	Accession number	Mean plant height (cm)	Mean number of branchlets	Mean number of leaflets	Mean east-west spread (cm)
Southern zone	Coastal	SKC1	36.1^{ab}	16.1	31.9	13.1
	Plains	SKP1	34.7^{ab}	15.6	31.3	12.6
	Midlands	SKM2	33.1^{b}	16.3	31.8	12.9
	High ranges	SKH1	34.7^{ab}	15.4	31.1	12.4
Central zone	Coastal	CKC1	36.3^{ab}	16.5	32.4	13.2
	Plains	CKP3	32.5^{b}	17.2	33.1	14.1
	Midlands	CKM1	37.3^{a}	16.3	32.2	13.1
	High ranges	CKH1	34.1^{ab}	15.7	31.2	12.5
Northern zone	Coastal	NKC3	37.5^{a}	15.3	30.9	12.3
	Plains	NKP1	33.1^{b}	14.6	30.7	11.8
	Midlands	NKM1	33.5^{b}	16.9	31.9	12.8
	High ranges	NKH1	35.1^{ab}	15.6	31.2	12.6

Table 14b. Altitudinal influence on growth parameters of *Phyllanthus urinaria* accessions in pot culture

Zones	Temporal sites	Accession number	Mean plant height (cm)	Mean number of branchlets	Mean number of leaflets	Mean east-west spread (cm)
Southern zone	Coastal	SKC4	27.5^{ab}	10.3	21.1	9.1
	Plains	SKP2	21.3^{e}	9.7	20.1	8.2
		SKP3	26.9^{abc}	10.2	21.2	9.3
	Midlands	SKM1	24.9^{bcde}	10.1	20.6	8.8
	High ranges	SKH4	22.1^{ef}	9.9	20.3	8.6
		SKH6	28.8^{a}	10.5	21.3	9.3
Central zone	Coastal	CKC2	23.5^{cdef}	9.7	20.2	8.6
		CKC3	24.1^{bcdef}	9.9	20.8	8.8
	Plains	CKP1	22.7^{def}	9.5	20.4	8.5
	Midlands	CKM4	26.7^{abc}	10.2	21.1	9.3
	High ranges	CKH2	22.9^{def}	9.8	20.6	8.6
		CKH3	25.2^{bcde}	10.1	20.9	8.9
Northern zone	Coastal	NKC1	26.1^{abcd}	10.1	21.1	9.1
	Plains	NKP2	25.6^{abcde}	9.7	20.6	8.7
		NKP5	$27..6^{ab}$	9.9	20.8	8.9
	Midlands	NP	NP	NP	NP	NP
	High ranges	NP	NP	NP	NP	NP

*NP – Not Present

Table 14c. Altitudinal influence on growth parameters of *Phyllanthus airy-shawii* accessions in pot culture

Zones	Temporal sites	Accession number	Mean plant height (cm)	Mean number of branchlets	Mean number of leaflets	Mean east-west spread (cm)
Southern zone	Coastal	SKC2	42.6	20.3	32.4	13.4
	Plains	NP	NP	NP	NP	NP
	Midlands	SKM4	41.1	20.1	32.3	13.2
	High ranges	SKH2	45.6	20.9	32.9	13.9
		SKH5	43.6	20.5	32.6	13.6
Central zone	Coastal	NP	NP	NP	NP	NP
	Plains	NP	NP	NP	NP	NP
	Midlands	CKM5	44.1	21.1	36.1	14.2
	High ranges	NP	NP	NP	NP	NP
Northern zone	Coastal	NKC2	43.8	21.3	36.1	13.9
	Plains	NKP3	42.1	19.9	34.3	13.3
		NKP6	46.1	21.8	36.6	14.9
	Midlands	NKM2	43.1	21.1	34.7	13.7
	High ranges	NKH3	42.1	20.1	34.4	14.1

Table 14d. Altitudinal influence on growth parameters of *Phyllanthus virgatus* var. *virgatus* accessions in pot culture

Zones	Temporal sites	Accession number	Mean plant height (cm)	Mean number of branchlets	Mean number of leaflets	Mean east-west spread (cm)
Southern zone	Coastal	NP	NP	NP	NP	NP
	Plains	NP	NP	NP	NP	NP
	Midlands	NP	NP	NP	NP	NP
	High ranges	NP	NP	NP	NP	NP
Central zone	Coastal	NP	NP	NP	NP	NP
	Plains	NP	NP	NP	NP	NP
	Midlands	CKM3	87.1	30.3	40.9[a]	36.6
	High ranges	NP	NP	NP	NP	NP
Northern zone	Coastal	NP	NP	NP	NP	NP
	Plains	NKP4	87.4	30.6	36.5[b]	37.5
	Midlands	NP	NP	NP	NP	NP
	High ranges	NKH2	85.4	30.2	36.4[b]	37.4

*NP – Not Present

Table 14c. Altitudinal influence on growth parameters of *Phyllanthus rheedei* accessions in pot culture

Zones	Temporal sites	Accession number	Mean plant height (cm)	Mean number of branchlets	Mean number of leaflets	Mean east west sprea (cm)
Southern zone	Coastal	NP	NP	NP	NP	NP
	Plains	NP	NP	NP	NP	NP
	Midlands	SKM3	52.6	22.9	38.6	15.6
	High ranges	SKH3	51.2	22.7	38.1	15.4
Central zone	Coastal	NP	NP	NP	NP	NP
	Plains	CKP2	54.2	22.8	38.8	15.7
	Midlands	CKM2	54.2	23.2	39.1	15.7
	High ranges	NP	NP	NP	NP	NP
Northern zone	Coastal	NP	NP	NP	NP	NP
	Plains	NP	NP	NP	NP	NP
	Midlands	NKM3	56.2	23.9	39.5	15.9
	High ranges	NP	NP	NP	NP	NP

*NP – Not Present

4.1.11. Altitudinal influence on yield parameters of *Phyllanthus* accessions in pot culture

The accessions of *P. amarus* exhibited significant difference among one another, with respect to mean fresh weight and dry weights (Table 15a). The accessions from plains (CKP3), midlands (NKM1) and high ranges (NKH1) were significantly superior with respect to mean fresh weight. The accessions from midlands (SKM2) and high ranges (NKH1) were rated superior with respect to mean dry weight.

In *P. urinaria,* the accession SKH6 from high ranges was significantly superior with respect to yield parameters (Table 15b). The accession SKP2 from plains was significantly inferior with respect to yield parameters. In *P. airy-shawii,* the accession NKP6 from plains was significantly superior with respect to yield parameters (Table 15c). The accession SKM4 from midlands was inferior with respect to yield parameters. In *P. virgatus* var. *virgatus*, the accession NKP4 from plains and the accession, CKM3 from midlands registered significantly higher values for mean fresh (15.71g) and dry weights (13.27 g) respectively. Accession from high ranges was inferior with respect to yield parameters studied (Table 15d). In *P. rheedei*, the accession SKM3 from midlands was significantly superior with respect to mean fresh (9.98 g) and dry weights (7.88 g). The accession SKH3 from high ranges was inferior with respect to yield parameters studied (Table 15e).

Table 15a. Altitudinal influence on yield parameters of *Phyllanthus amarus* accessions in pot culture

Zones	Temporal sites	Accession number	Mean fresh weight per plant (g)	Mean dry weight per plant (g)
Southern zone	Coastal	SKC1	6.13^{ab}	4.03^{cd}
	Plains	SKP1	6.24^{ab}	4.04^{cd}
	Midlands	SKM2	6.17^{ab}	4.51^{a}
	High ranges	SKH1	5.62^{b}	3.34^{h}
Central zone	Coastal	CKC1	6.25^{ab}	4.45^{ab}
	Plains	CKP3	6.53^{a}	4.33^{c}
	Midlands	CKM1	5.79^{ab}	3.69^{g}
	High ranges	CKH1	6.03^{ab}	4.13^{de}
Northern zone	Coastal	NKC3	6.28^{ab}	4.23^{c}
	Plains	NKP1	6.15^{ab}	3.95^{f}
	Midlands	NKM1	6.57^{a}	4.47^{ab}
	High ranges	NKH1	6.61^{a}	4.51^{a}

Table 15b. Altitudinal influence on yield parameters of *Phyllanthus urinaria* accessions in pot culture

Zones	Temporal sites	Accession number	Mean fresh weight per plant (g)	Mean dry weight per plant (g)
Southern zone	Coastal	SKC4	4.94^{c}	2.84^{c}
	Plains	SKP2	3.62^{o}	1.42^{m}
		SKP3	4.84^{d}	2.74^{e}
	Midlands	SKM1	4.23^{j}	2.13^{h}
	High ranges	SKH4	3.75^{n}	1.55^{l}
		SKH6	5.18^{a}	3.08^{a}
Central zone	Coastal	CKC2	3.99^{l}	2.09^{i}
		CKC3	4.33^{i}	2.23^{g}
	Plains	CKP1	4.09^{k}	1.99^{k}
	Midlands	CKM4	4.81^{e}	2.79^{d}
	High ranges	CKH2	3.89^{m}	1.98^{k}
		CKH3	4.53^{f}	2.43^{f}
Northern zone	Coastal	NKC1	4.43^{g}	2.13^{h}
	Plains	NKP2	4.35^{h}	2.05^{j}
		NKP5	4.96^{b}	2.98^{b}
	Midlands	NP	NP	NP
	High ranges	NP	NP	NP

*NP – Not Present

Table 15c. Altitudinal influence on yield parameters of *Phyllanthus airy-shawii* accessions in pot culture

Zones	Temporal sites	Accession number	Mean fresh weight per plant (g)	Mean dry weight per plant (g)
Southern zone	Coastal	SKC2	7.66^{e}	5.76^{c}
	Plains	NP	NP	NP
	Midlands	SKM4	6.98^{i}	4.78^{j}
	High ranges	SKH2	7.75^{d}	5.65^{e}
		SKH5	7.41^{g}	5.31^{h}
Central zone	Coastal	NP	NP	NP
	Plains	NP	NP	NP
	Midlands	CKM5	7.93^{b}	5.73^{d}
	High ranges	NP	NP	NP
Northern zone	Coastal	NKC2	7.88^{c}	5.99^{b}
	Plains	NKP3	7.15^{h}	5.05^{i}
		NKP6	8.29^{a}	6.09^{a}
	Midlands	NKM2	7.75^{d}	5.45^{f}
	High ranges	NKH3	7.57^{f}	5.37^{g}

Table 15d. Altitudinal influence on yield parameters of *Phyllanthus virgatus* var. *virgatus* accessions in pot culture

Zones	Temporal sites	Accession number	Mean fresh weight per plant (g)	Mean dry weight per plant (g)
Southern zone	Coastal	NP	NP	NP
	Plains	NP	NP	NP
	Midlands	NP	NP	NP
	High ranges	NP	NP	NP
Central zone	Coastal	NP	NP	NP
	Plains	NP	NP	NP
	Midlands	CKM3	15.67^{b}	13.27^{a}
	High ranges	NP	NP	NP
Northern zone	Coastal	NP	NP	NP
	Plains	NKP4	15.71^{a}	13.21^{b}
	Midlands	NP	NP	NP
	High ranges	NKH2	14.15^{c}	12.15^{c}

*NP – Not Present

Table 15e. Altitudinal influence on yield parameters of *Phyllanthus rheedei* accessions in pot culture

Zones	Temporal sites	Accession number	Mean fresh weight per plant (g)	Mean dry weight per plant (g)
Southern zone	Coastal	NP	NP	NP
	Plains	NP	NP	NP
	Midlands	SKM3	9.98[a]	7.88[a]
	High ranges	SKH3	8.70[d]	6.60[d]
Central zone	Coastal	NP	NP	NP
	Plains	CKP2	9.75[b]	7.45[b]
	Midlands	CKM2	9.75[b]	7.45[b]
	High ranges	NP	NP	NP
Northern zone	Coastal	NP	NP	NP
	Plains	NP	NP	NP
	Midlands	NKM3	9.55[c]	7.35[c]
	High ranges	NP	NP	NP

*NP – Not Present

4.1.12. Biochemical characterization of collected *Phyllanthus* accessions from various zones of Kerala.

The biochemical parameters viz., content of total extractives, total phenol content, phyllanthin content and antioxidant capacity were analysed for the collected *Phyllanthus* accessions. Fig. 8, Fig. 9 and Fig. 10 represent the chromatogram of phyllanthin in HPLC analysis.

Table 16a, reveals the quantitative profile of the biochemical parameters studied in collected *Phyllanthus* accessions, from southern zone of Kerala. Maximum content of total extractives (0.58 g) was obtained in the accession SKH1 from high ranges and SKM2 from midlands of southern zone representing *P. amarus*. The accession SKH5 representing *P. airy- shawii* from high ranges recorded maximum total phenol content (255.2 mg g^{-1}). Maximum phyllanthin content (0.46%) was observed in the accession SKM2, from midlands representing *P. amarus*. Minimum Effective Concentration (EC_{50}) value (211.3 µg ml^{-1}) was noted in the accession, SKH5 from high ranges representing *P. airy-shawii*, denoting its greater antioxidant capacity. Lowest values for content of total extractives (0.39 g) and total phenol content (117.2 mg g^{-1}) were registered by accessions, SKC3 representing *P. maderaspatensis* and SKC1 representing *P. amarus* respectively, both accessions, being from coastal region. Phyllanthin was absent in accessions, SKC4 from coastal region, SKP2 and SKP3 from plains and SKH4 and SKH6 from high ranges, all accessions representing *P. urinaria*. Maximum EC_{50} value (337.4 µg ml^{-1}), indicative of its least antioxidant capacity was observed in the accession SKC1, from coastal region representing *P. amarus*.

Table 16b, reveals the content of various biochemical parameters studied, in collected *Phyllanthus* accessions, from central zone of Kerala. The accession CKM1 from midlands representing *P. amarus* recorded maximum content of total extractives (0.61 g) in central zone of Kerala, while, maximum total phenol content (251.2 mg g^{-1}) was obtained in the accession CKM5 representing *P. airy-shawii*, from midlands of central zone

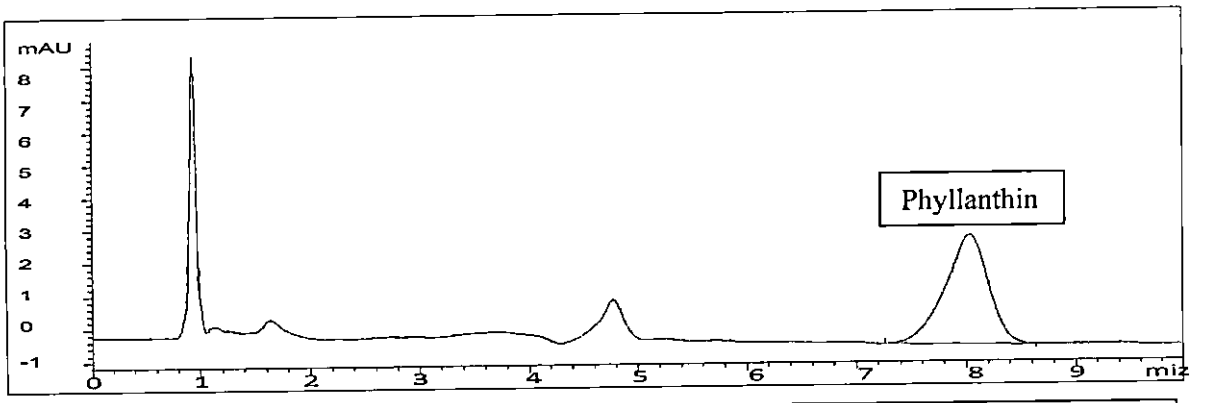


Fig. 8. Chromatogram depicting presence of phyllanthin in standard sample during HPLC analysis

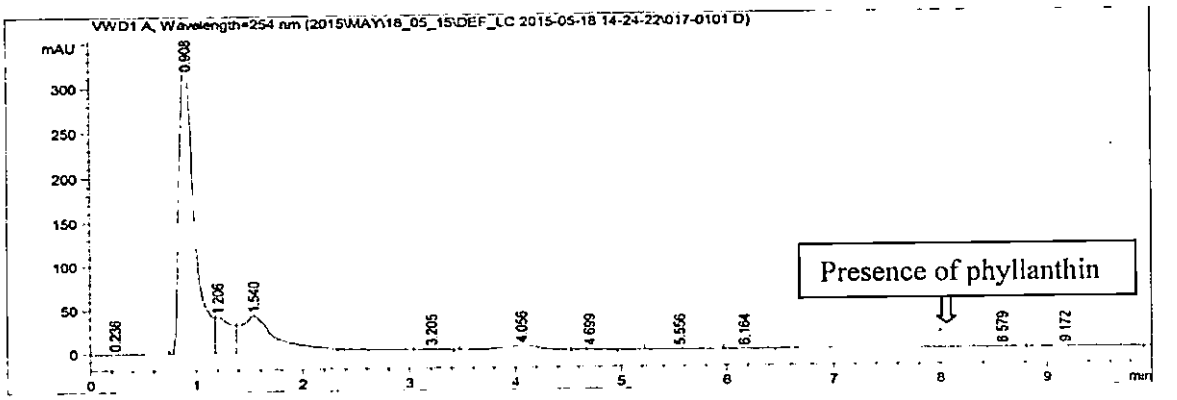


Fig. 9. Chromatogram depicting presence of phyllanthin during HPLC analysis

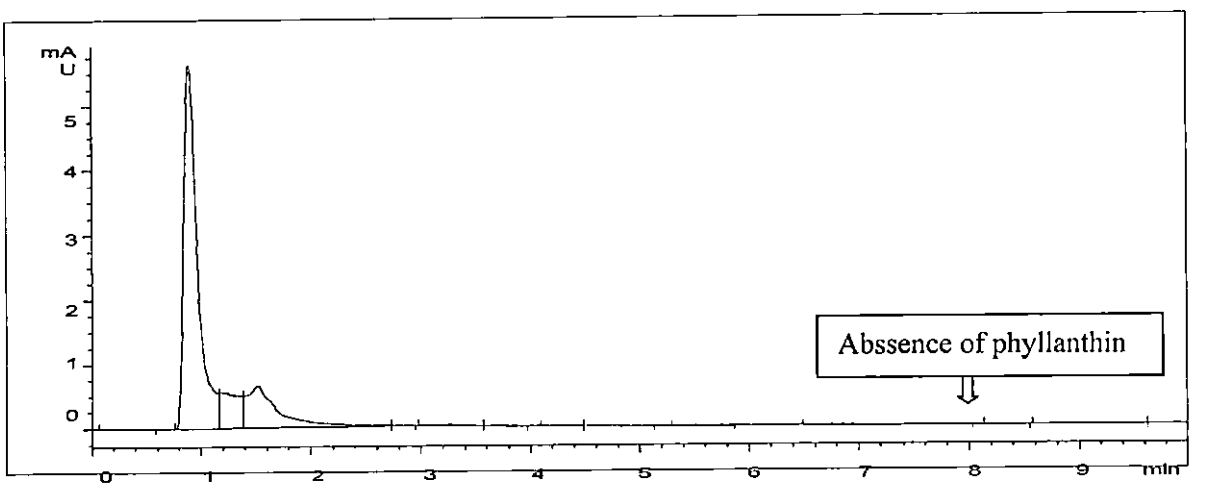


Fig. 10. Chromatogram depicting absence of phyllanthin during HPLC analysis

Table 16a.Biochemical characterization of *Phyllanthus* accessions from southern zone of Kerala

Zone	Temporal site	Acc. No.	Content of total extractives (g)	Total phenol content (mg/g)	Phyllanthin Content (%)	Anti oxidant capacity (EC_{50}) (µg/ml)
Southern Kerala (SK)	Midland	SKM1	0.51	215.3	-	234.4
		SKM2	0.58	136.2	0.46	324.2
		SKM3	0.43	174.2	0.01	282.1
		SKM4	0.49	252.1	0.01	214.2
	Coastal	SKC1	0.57	117.2	0.43	337.4
		SKC2	0.45	234.1	0.02	222.3
		SKC3	0.39	144.1	0.01	313.1
		SKC4	0.50	200.2	-	244.2
	Plain	SKP1	0.55	129.1	0.42	327.1
		SKP2	0.52	217.2	-	230.2
		SKP3	0.48	211.3	-	236.3
	High range	SKH1	0.58	126.1	0.40	331,4
		SKH2	0.50	242.2	0.01	218.4
		SKH3	0.40	169.3	0.01	286.1
		SKH4	0.51	209.1	-	239.2
		SKH5	0.49	254.2	0.01	211.3
		SKH6	0.50	207.3	-	240.2

The accession CKH1 representing *P. amarus* from high ranges, registered the highest phyllanthin content (0.45%). Minimum effective concentration of 215.1 μg ml^{-1} was noted in the accession CKM5 representing *P. airy-shawii,* from midlands, revealing its highest antioxidant capacity. Accession CKM2 from midlands representing *P. rheedei* registered lowest content of total extractives (0.47 g), while lowest total phenol content (119.2 mg g^{-1}) was obtained in accession CKH1 from high ranges representing *P. amarus*. Phyllanthin content was absent in accessions, CKM4 from midlands, CKC2 and CKC3 from coastal region, CKP1 from plains and CKH2 and CKH3 from high ranges, all accessions representing *P. urinaria*. Highest EC_{50} value of 335.6 μg ml^{-1} was observed in CKH1 representing *P. amarus* from high ranges denoting its least antioxidant activity.

Table 16c, reveals the content of various biochemical parameters studied, in collected *Phyllanthus* accessions, from northern zone of Kerala.

The accessions, NKM1 from midlands and NKP1 from plains representing *P. amarus,* recorded maximum content of total extractives (0.59 g) followed by the accession NKH1 from high ranges. Maximum total phenol content 248.2 mg g^{-1} was registered by the accession NKM2, from midlands of northern zone of Kerala representing *P. airy-shawii*. Highest phyllanthin content 0.39 per cent was obtained in the accession NKC3 from coastal region of northern zone representing *P. amarus*. Minimum EC_{50} value of 216.2 μg ml^{-1} was recorded in the accession NKM2 representing *P. airy-shawii* from midlands, indicative of its highest antioxidant capacity. Least content of total extractives (0.41 g), was obtained in the accession NKH2, from high ranges of northern zone representing *P. urinaria*, while, the accession NKH1 representing *P. amarus*, also from high ranges recorded the lowest total phenol content (112.3 mg g^{-1}). Phyllanthin content was absent in *P. urinaria* accessions NKC1, NKP2 and NKP5, while the accessions NKM3, NKP4, NKH2 and NKH4 representing *P. rheedei*, *P. virgatus* var. *virgatus*, *P. virgatus* var. *gardenerianus* recorded low phyllanthin content (0.01 % each).

Table 16b. Biochemical characterization of *Phyllanthus* accessions from central zone of Kerala

Zone	Temporal site	Acc. No.	Content of total extractives (g)	Total phenol Content (mg/g)	Phyllanthin Content (%)	Anti oxidant capacity (EC_{50}) (µg/ml)
Central Kerala (CK)	Midlands (M)	CKM1	0.61	123.2	0.43	333.1
		CKM2	0.47	188.1	0.01	274.1
		CKM3	0.49	178.2	0.01	281.3
		CKM4	0.54	213.1	_	235.4
		CKM5	0.50	251.2	0.01	215.1
	Coastal region (C)	CKC1	0.59	139.4	0.41	320.2
		CKC2	0.55	196.2	_	242.3
		CKC3	0.53	199.3	_	248.2
	Plains (S)	CKP1	0.51	221.2	_	227.1
		CKP2	0.48	185.1	0.01	278.2
		CKP3	0.60	132.3	0.39	325.4
	High ranges (H)	CKH1	0.58	119.2	0.45	335.6
		CKH2	0.57	197.2	_	251.2
		CKH3	0.55	215.1	_	243.2

Table 16c. Biochemical characterization of *Phyllanthus* accessions from northern zone of Kerala

Zone	Temporal zone	Acc. No.	Content of total extractives (g)	Total phenol Content (mg/g)	Phyllanthin Content (%)	Anti oxidant capacity (EC_{50})(µg/ml)
Northern Kerala (NK)	Midlands (M)	NKM1	0.59	119.7	0.32	335.3
		NKM2	0.49	248.2	0.02	216.2
		NKM3	0.40	174.3	0.01	282.3
	Coastal region (C)	NKC1	0.51	217.2	-	230.2
		NKC2	0.50	239.3	0.02	219.2
		NKC3	0.58	128.5	0.39	329.3
	Plains (P)	NKP1	0.59	115.4	0.37	338.4
		NKP2	0.52	213.3	-	235.3
		NKP3	0.47	232.1	0.02	223.3
		NKP4	0.45	187.2	0.01	276.4
		NKP5	0.54	207.2	-	240.2
		NKP6	0.48	236.2	0.02	220.4
	High ranges (H)	NKH1	0.57	112.3	0.36	340.2
		NKH2	0.41	182.4	0.01	279.2
		NKH3	0.49	246.1	0.02	217.2
		NKH4	0.47	191.1	0.01	271.1

4.1.13. Cluster analysis of *Phyllanthus* accessions collected from southern zone based on biochemical parameters

Fig 11 represents the dendrogram of *Phyllanthus* accessions from southern zone, based on biochemical parameters. Clustering mean values of biochemical characters employed for grouping the collected accessions from southern zone are given in Table 17a.

The seventeen accessions collected from southern zone, could be grouped into five clusters, at 20 per cent similarity, representing five species, *P. rheedei*, *P. airy-shawii*, *P. urinaria*, *P. maderaspatensis* and *P. amarus*. Cluster I consisting of two accessions, (SKH3 and SKM3), represented *P. rheedei* and cluster two consisting of four accessions (SKH5, SKH2, SKC2 and SKM4), represented *P. airy-shawii*. Cluster III was the largest group comprising of six accessions, viz., SKM1, SKH6, SKC4, SKP2, SKP3 and SKH4 representing *P. urinaria*. Cluster IV comprising of lone accession, SKC3 and cluster V comprising of four accessions, SKM2, SKP1, SKC1 and SKH1 represented *P. maderapatensis* and *P. amarus* respectively.

The cluster mean values of biochemical characters employed for grouping the collected accessions from southern zone into clusters, representing various species of *Phyllanthus* are given in Table 17b.

Cluster V, representing, *P. amarus*, recorded highest mean values for content of total extractives (0.57 g), phyllanthin content (0.42 %), EC_{50} value (329.9 μg ml^{-1}) and lowest value for mean total phenol content (127.1 mg g-1). The same cluster recorded the least antioxidant capacity as is evident from the high EC value (329.9 μg ml^{-1}) recorded by the accessions of the cluster. The EC50 value was least (216.4 μg ml^{-1}) for cluster II representing *P. airy-shawii*, indicating its high antioxidant capacity. Phyllanthin content was absent in cluster III, representing *P. urinaria*. Clusters I, II and IV representing *P. rheedei*, *P. airy-shawii* and *P. maderaspatensis* registered 0.01 per cent phyllanthin content.

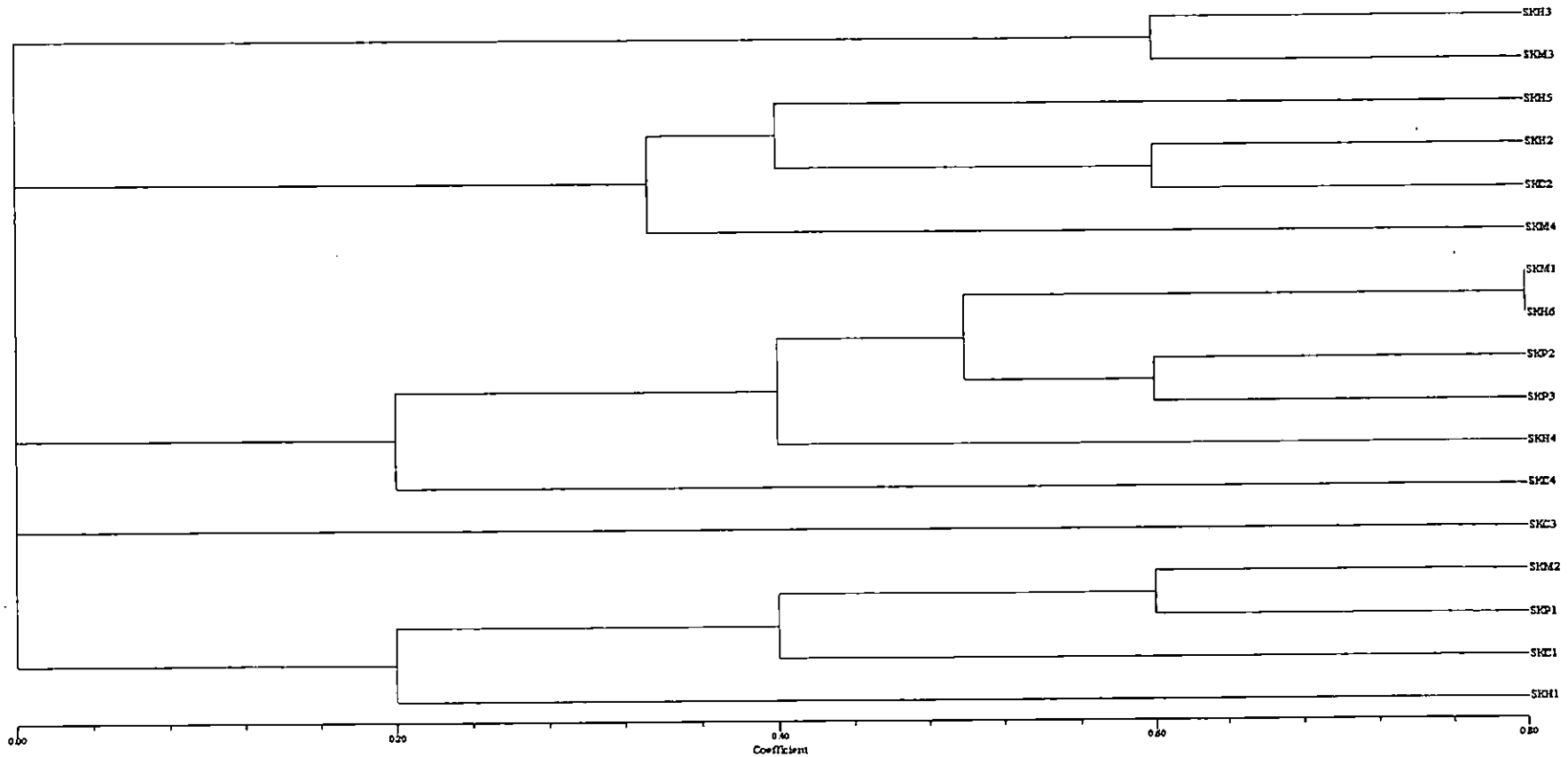


Fig 11. Dendrogram based on biochemical parameters of *Phyllanthus* accessions from southern zone of Kerala

Table 17a. Clusters of *Phyllanthus* accessions collected from southern zone based on biochemical parameters

Sl No.	Cluster number	Cluster mean values			
		Content of total extractives (g)	Total phenol content (mg/g)	Phyllanthin content (%)	Anti oxidant capacity (EC_{50}) (µg/ml)
1	Cluster I	0.41	171.65	0.01	284.1
2	Cluster II	0.48	245.7	0.01	216.4
3	Cluster III	0.50	210.0	-	237.3
4	Cluster IV	0.39	144.2	0.01	313.2
5	Cluster V	0.57	127.1	0.42	329.9

Table 17b. Summary statistics for biochemical parameters of *Phyllanthus* accessions from southern zone

Sl No.	Cluster number	Accessions	No. of accessions	Name of the accessions
1	Cluster I	SKH3, SKM3	2	*Phyllanthus rheedei*
2	Cluster II	SKH5, SKH2, SKC2, SKM4	4	*Phyllanthus airy-shawii*
3	Cluster III	SKM1, SKH6, SKC4 SKP2,SKP3, SKH4	6	*Phyllanthus urinaria*
4	Cluster IV	SKC3	1	*Phyllanthus maderaspatensis*
5	Cluster V	SKM2, SKP1, SKC1, SKH1	4	*Phyllanthus amarus*

4.1.14. Clustering of *Phyllanthus* accessions collected from central zone based on biochemical parameters

Fig 12 represents the dendrogram of *Phyllanthus* accessions from central zone, based on biochemical parameters. Cluster analysis of fourteen accessions from central zone, representing high ranges, plains, midlands and coastal regions of central zone, based on biochemical parameters, are given in Table 17c. The fourteen accessions from central zone could be grouped into four clusters, at 4.7 per cent similarity, representing *P. rheedei* and *P. virgatus* var *virgatus* in a single cluster (cluster I), *P. airy-shawii* (cluster II), *P. amarus* (cluster III) and *P. urianria* (cluster IV). Cluster I representing *P. rheedei* and *P. virgatus* var *virgatus,* included three accessions viz., CKM2, CKM3 and CKP2. CKM5 was the lone accession in Cluster II, representing *P. airy-shawii*. *P. amarus* in central zone was represented by cluster III consisting of four accessions, CKM1, CKC1, CKP3 and CKH1. Cluster IV contained the maximum number of accessions (six numbers) viz., CKH3, CKH2, CKP1, CKC3, CKC2 and CKM4 representing *P. urinaria.*

The cluster mean values of biochemical characters employed for grouping the collected accessions from central zone into clusters, representing various species of *Phyllanthus* are given in Table 17d.

Highest content of total extractives (0.59 g), phyllanthin content (0.42 %) and EC_{50} value (328.5 μg ml^{-1}) were recorded by the accessions of cluster III representing *P. amarus*. The second cluster, representing *P. airy-shawii* recorded highest antioxidant capacity as indicated by the lowest EC_{50} value (241.2 μg ml^{-1}). As observed in the other two zones, cluster IV representing *P. urinaria*, did not contain the lignan, phyllanthin. Lowest total phenol content (128.5 mg g^{-1}) was recorded by cluster III, representing *P. amarus*.

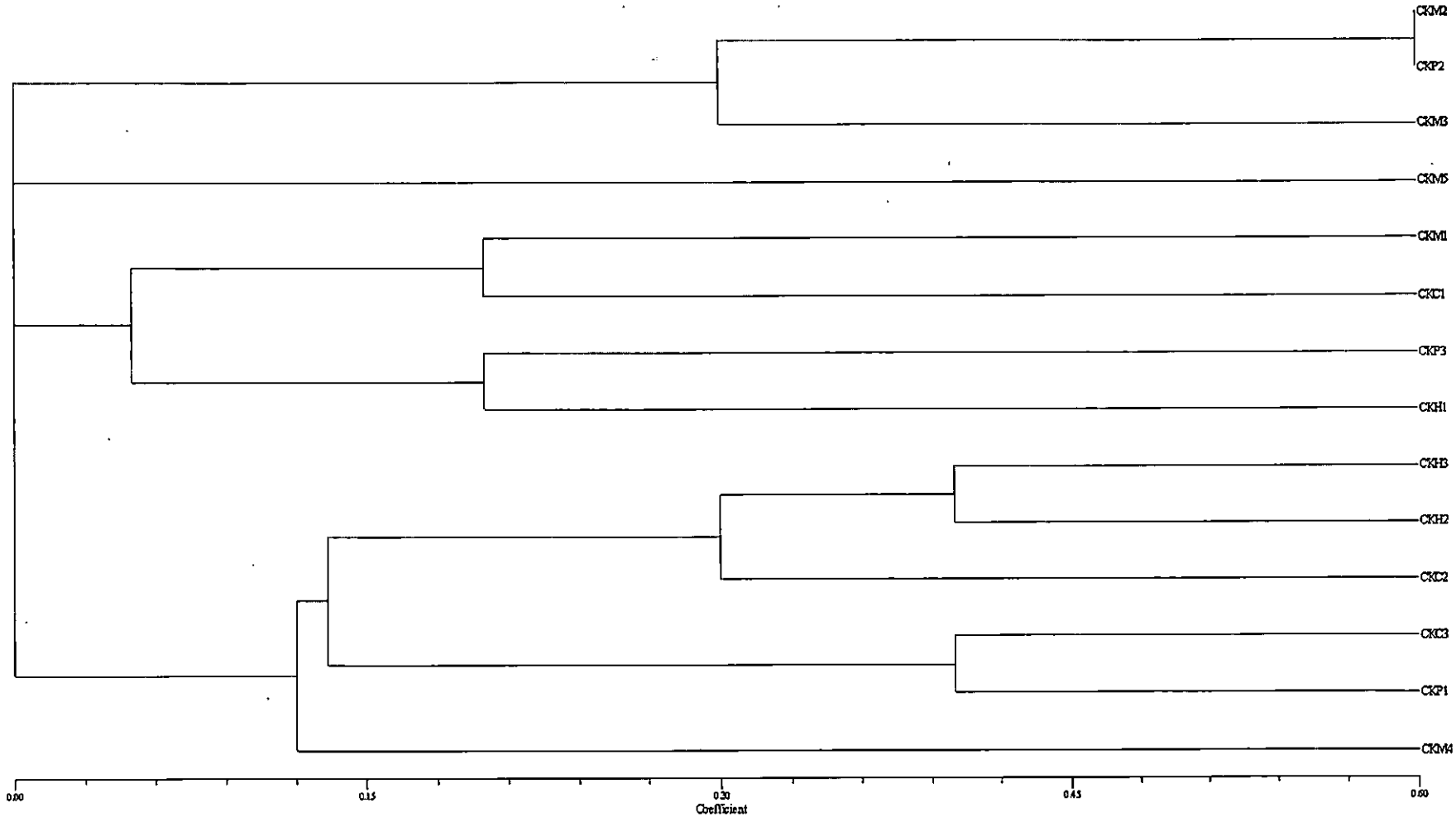


Fig 12. Dendrogram based on biochemical parameters of *Phyllanthus* accessions from central zone of Kerala

Table 17c. Clusters of *Phyllanthus* accessions collected from central zone based on biochemical parameters

Sl No.	Cluster number	Accessions	No. of accessions	Name of the accessions
1	Cluster I	CKM2, CKM3, CKP2	3	*Phyllanthus rheedei* *Phyllanthus virgatus* var *virgatus*
2	Cluster II	CKM5	1	*Phyllanthus airy-shawii*
3	Cluster III	CKM1, CKC1, CKP3,CKH1	4	*Phyllanthus amarus*
4	Cluster IV	CKH3, CKH2, CKP1, CKC3,CKC2, CKM4	6	*Phyllanthus urinaria*

Table 17d. Summary statistics for biochemical parameters of *Phyllanthus* accessions from central zone

Sl No.	Cluster number	Cluster mean values			
		Content of total extractives (g)	Total phenol content (mg/g)	Phyllanthin content (%)	Anti oxidant capacity (EC50) (µg/ml)
1	Cluster I	0.48	183.8	0.01	277.8
2	Cluster II	0.54	207.0	0.01	241.2
3	Cluster III	0.59	128.5	0.42	328.5
4	Cluster IV	0.54	201.2	-	245.3

4.1.14. Clustering of *Phyllanthus* accessions collected from northern zone based on biochemical parameters

Fig 13 represents the dendrogram of *Phyllanthus* accessions from northern zone, based on biochemical parameters. Cluster analysis of fourteen accessions from high ranges, plains, midlands and coastal regions of northern zone, based on biochemical parameters, is given in Table 17e.

The accessions collected from northern zone were grouped into four clusters at 5 per cent similarity. Cluster I representing *P. urinaria* included three accessions, NKC1, NKP2 and NKP5. Maximum number of accessions (five numbers) viz., NKM2, NKC2, NKP3, NKP6 and NKH3 were grouped under cluster III representing *P. airy-shawii*. Four accessions each, were grouped in cluster II (NKM1, NKC3, NKP1, NKH1) and cluster IV (NKM3, NKP4, NKH2 and NKH4), with the former representing *P. amarus* and the latter, three species, *P. rheedei, P. virgatus* var. *virgatus* and *P. virgatus* var. *gardnerianus*.

The cluster mean value of biochemical character employed for grouping the collected accessions from northern zone into clusters, representing various species of *Phyllanthus* are given in Table 17f.

Cluster II representing *P. amarus* recorded highest value for total extractives (0.58 g), phyllanthin content (0.36%), exhibiting highest EC_{50} value (335.8 μg ml^{-1}), indicating its least antioxidant capacity. Cluster I, representing *P. urinaria* was the second best with respect to total extractives (0.52 g). Cluster III representing *P. airy-shawii* recorded least EC_{50} value (219.2 μg ml^{-1}), indicating its highest antioxidant capacity. Phyllanthin was absent in accessions NKC1, NKP2, NKP5 of cluster I, representing *P. urinaria* (Table 17f). As compared to the cluster representing *P. amarus*, low values were recorded with respect to phyllanthin content in cluster III, representing *P. airy-shawii* (0.02 %) and cluster IV representing, *P. rheedei, P. virgatus* var. *virgatus* and *P. virgatus* var. *gardnerianus* (0.01 %).

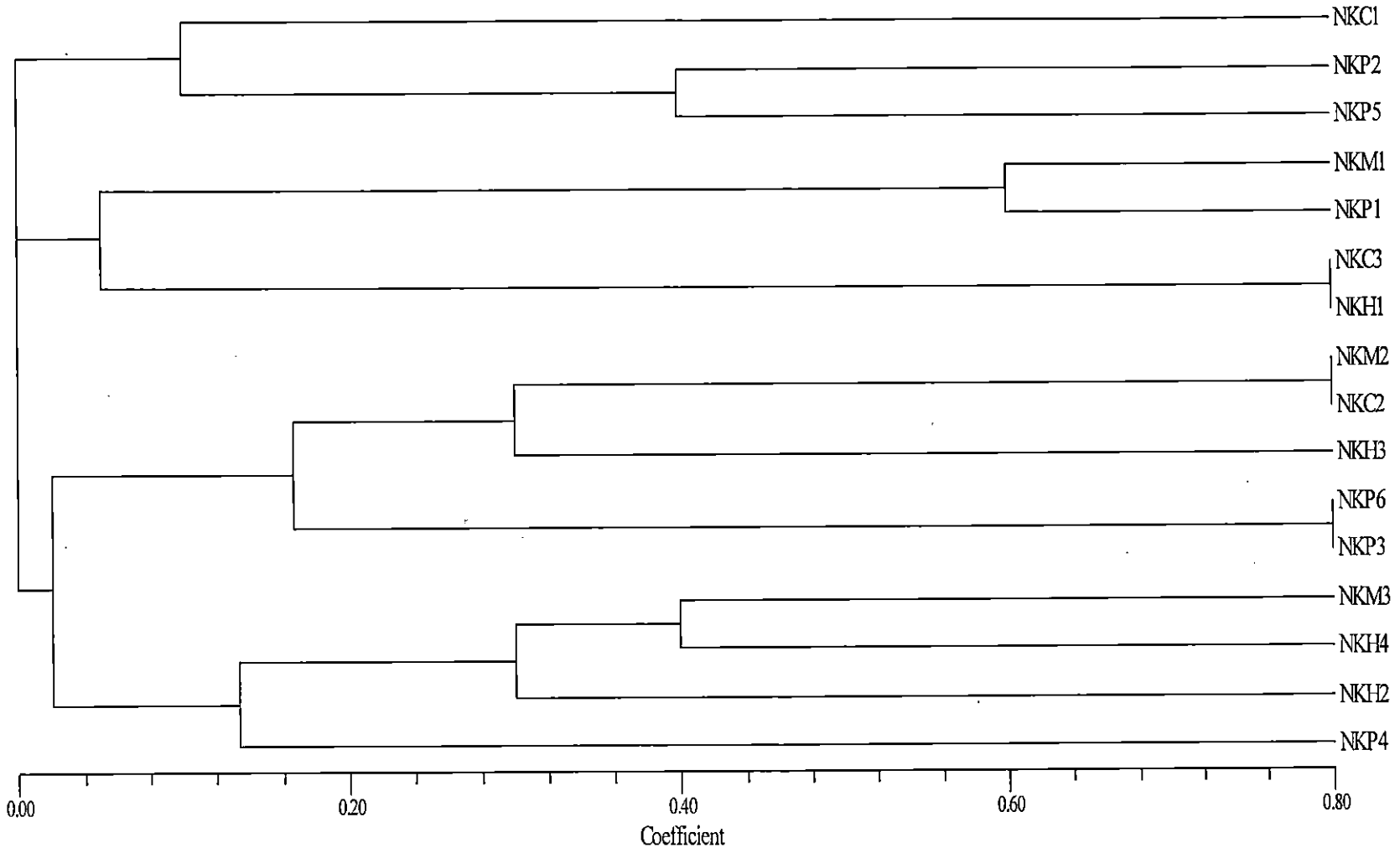


Fig 13. Dendrogram based on biochemical parameters of *Phyllanthus* accessions from northern zone of Kerala

Table 17e. Clusters of *Phyllanthus* accessions collected from northern zone based on biochemical parameters

Sl No.	Cluster number	Accessions	No. of accessions	Name of the accessions
1	Cluster I	NKC1, NKP2, NKP5	3	*Phyllanthus urinaria*
2	Cluster II	NKM1,NKC3,NKP1,NKH1	4	*Phyllanthus amarus*
3	Cluster III	NKM2, NKC2, NKP3, NKP6,NKH3	5	*Phyllanthus airy-shawii*
4	Cluster IV	NKM3 NKP4,NKH2,NKH4	4	*Phyllanthus rheedei* *Phyllanthus virgatus* var. *virgatus* *Phyllanthus virgatus var.gardenarianus*

Table 17f. Summary statistics for biochemical parameters of *Phyllanthus* accessions from northern zone

Sl No.	Cluster number	Cluster mean values			
		Content of total extractives (g)	Total phenol content (mg/g)	Phyllanthin content (%)	Anti oxidant capacity (EC50)(µg/ml)
1	Cluster I	0.52	212.5	-	235.2
2	Cluster II	0.58	118.9	0.36	335.8
3	Cluster III	0.48	240.3	0.02	219.2
4	Cluster IV	0.43	183.7	0.01	277.2

4.1.15. Clustering of *Phyllanthus* accessions collected from coastal region based on biochemical parameters

Fig 14 represents the dendrogram of *Phyllanthus* accessions from coastal region, based on biochemical parameters. Cluster analysis of ten accessions from coastal region of southern, central and northern zones, based on biochemical parameters, is given in Table 17g.

The accessions from coastal region were grouped into four clusters at 15 per cent similarity. Cluster I representing *P. amarus* included three accessions CKC1, NKC3 and SKC1. Cluster II consisting of two accessions (NKC2 and SKC2) represented *P. airy-shawii* and cluster III consisting of a single accession (SKC3) represented *P. maderaspatensis*. Maximum number of accessions (four numbers) viz., CKC2, CKC3, NKC1 and SKC4 were grouped under cluster IV representing *P. urinaria*.

The cluster mean value of biochemical characters employed for grouping the collected accessions from coastal region into clusters, represents various species of *Phyllanthus* are given in Table 17h.

Cluster I representing *P. amarus* recorded highest value for total extractives (0.58 g) and phyllanthin content (0.41 %). Highest total phenol content (236.6 mg g^{-1}) and lowest EC_{50} value (220.6 µg ml^{-1}), indicating highest antioxidant activity was recorded in cluster II, representing *P. airy-shawii*. Phyllanthin content was absent in cluster IV representing *P. urinaria*.

4.1.16. Clustering of *Phyllanthus* accessions collected from plains based on biochemical parameters

Fig 15. represents the dendrogram of *Phyllanthus* accessions from plains, based on biochemical parameters. Cluster analysis of twelve accessions from plains of southern, central and northern zones, based on biochemical parameters, is given in Table 17i. The accessions from plains were grouped into four clusters at 10 per cent similarity. Cluster I representing *P. airy-shawii* and cluster II

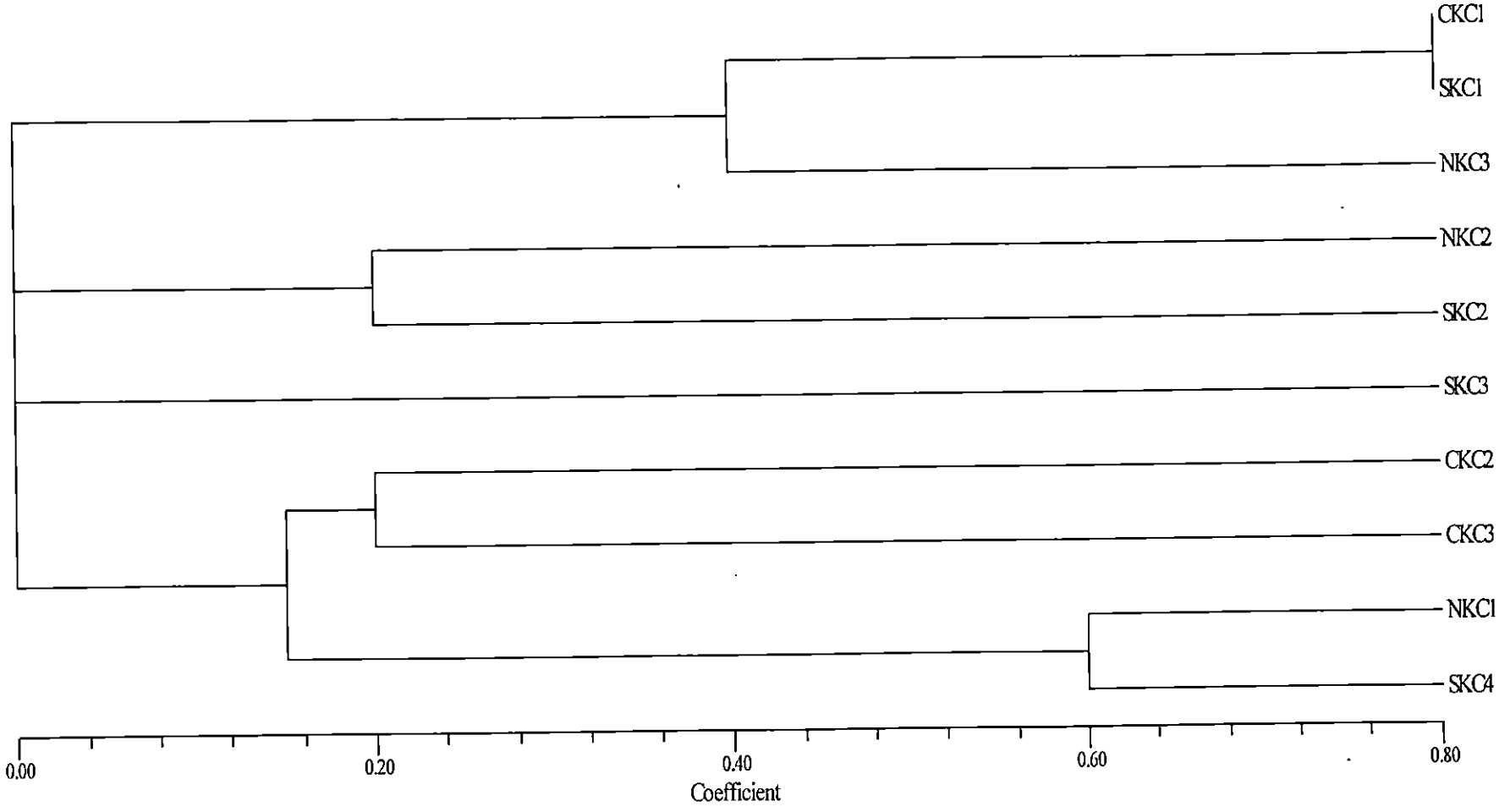


Fig 14. Dendrogram based on biochemical parameters of *Phyllanthus* accessions from coastal regions of Kerala

Table 17g. Clusters of *Phyllanthus* accessions collected from coastal regions based on biochemical parameters

Sl No.	Cluster number	Accessions	No. of accessions	Name of the accessions
1	Cluster I	CKC1, NKC3, SKC1	3	*Phyllanthus amarus*
2	Cluster II	NKC2, SKC2	2	*Phyllanthus airy-shawii*
3	Cluster III	SKC3	1	*Phyllanthus maderaspatensis*
4	Cluster IV	CKC2, CKC3, NKC1, SKC4	4	*Phyllanthus urinaria*

Table 17h. Summary statistics for quantitative characters of *Phyllanthus* accessions from coastal regions

Sl No.	Cluster number	Cluster mean values			
		Content of total extractives (g)	Total phenol content (mg/g)	Phyllanthin content (%)	Anti oxidant capacity (EC_{50}) (µg/ml)
1	Cluster I	0.58	128.3	0.41	328.8
2	Cluster II	0.47	236.6	0.02	220.6
3	Cluster III	0.39	144.1	0.01	313.1
4	Cluster IV	0.52	203.1	-	241.1

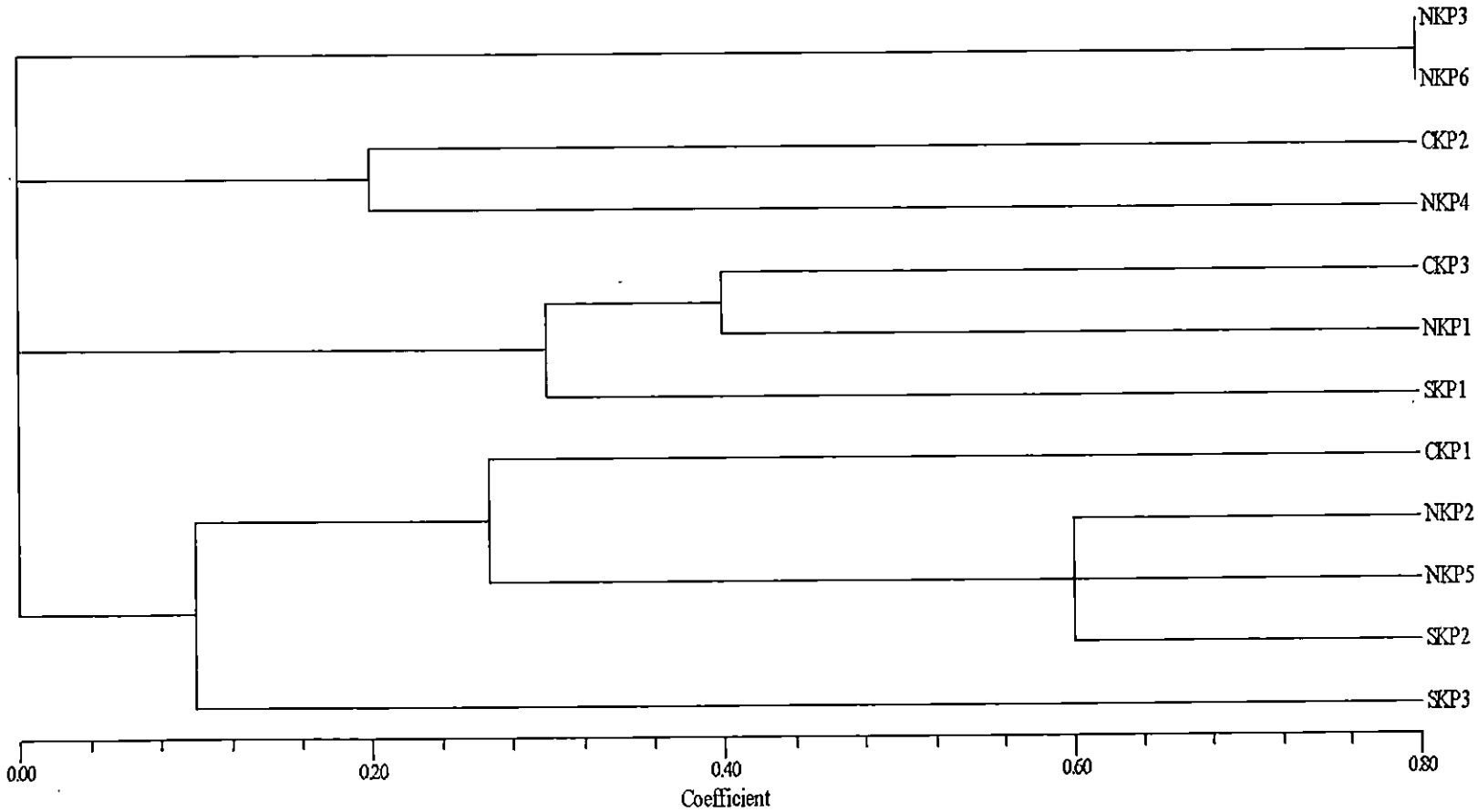


Fig 15. Dendrogram based on biochemical parameters of *Phyllanthus* accessions from plains of Kerala

Table 17i. Clusters of *Phyllanthus* accessions collected from plains based on biochemical parameters

Sl No.	Cluster number	Accessions	No. of accessions	Name of the accessions
1	Cluster I	NKP3, NKP6	2	*Phyllanthus airy-shawii*
2	Cluster II	NKP4, CKP2	2	*Phyllanthus virgatus var.virgatus* *Phyllanthus rheedei*
3	Cluster III	CKP3, NKP1, SKP1	3	*Phyllanthus amarus*
4	Cluster IV	CKP1, NKP2, NKP5, SKP3, SKP2	5	*Phyllanthus urinaria*

Table 17j. Summary statistics for quantitative characters of *Phyllanthus* accessions from plains

Sl No.	Cluster number	Cluster mean values			
		Content of total extractives (g)	Total phenol content (mg/g)	Phyllanthin content (%)	Anti oxidant capacity (EC_{50}) (µg/ml)
1	Cluster I	0.47	234.1	0.02	221.8
2	Cluster II	0.46	186.1	0.01	277.3
3	Cluster III	0.58	125.6	0.39	330.3
4	Cluster IV	0.51	214.0	-	233.8

representing *P. virgatus* var. *virgatus*, *P. rheedei*, consisted of two accessions each, NKP3 and NKP6 representing *P. airyshawii* and NKP4 and CKP2 representing *P. virgatus* var. *virgatus* and *P. rheedei*, respectively. Maximum number of accessions (five numbers) viz., CKP1, NKP2, NKP5, SKP3 and SKP2 were grouped under cluster IV representing *P. urinaria*.

The cluster mean values of biochemical characters employed for grouping the collected accessions from plains into clusters, represents various species of *Phyllanthus* are given in Table 17j.

Highest content of total extractives (0.58 g) and phyllanthin content (0.39 %) and lowest total phenol content (125.6 mg g^{-1}), were recorded by the accessions of cluster III representing *P. amarus*. The same cluster also recorded highest EC_{50} value (330.3 µg ml^{-1}), indicating its lowest antioxidant activity. Lowest EC_{50} value (221.8 µg ml^{-1}) and highest total phenol content (234.1 mg g^{-1}) was recorded in cluster I representing *P. airy-shawii*. Phyllanthin content was absent in cluster IV, representing *P. urinaria*.

4.1.17. Clustering of *Phyllanthus* accessions collected from midlands based on biochemical parameters

Fig.16 represents the dendrogram of *Phyllanthus* accessions from midlands, based on biochemical parameters. Cluster analysis of twelve accessions from midlands of southern, central and northern zones, based on biochemical parameters, is given in Table 17k.

The accessions from midlands were grouped into five clusters at 10 per cent similarity. Cluster I, II and III consisted of three accessions each, SKM4, NKM2 and CKM5 representing *P. airy-shawii*, CKM2, SKM3 and NKM3 representing *P. rheedei* and CKM1, NKM1 and SKM2 representing *P. amarus*. Two accessions, CKM4 and SKM1 were included in cluster IV representing *P. urinaria* and CKM3 was the lone accession in cluster V representing *P. virgatus* var. *virgatus*. The cluster mean values of biochemical characters employed for

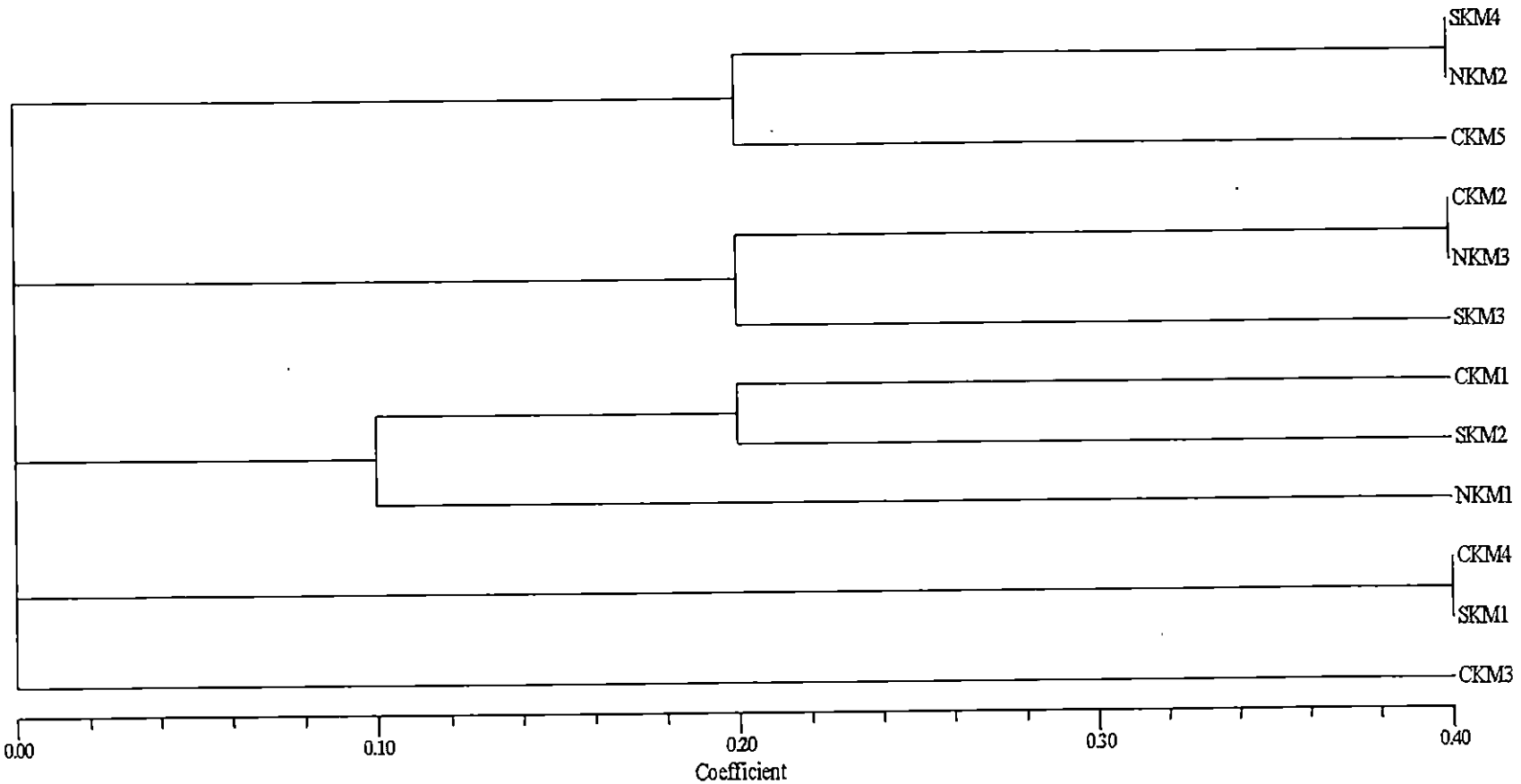


Fig 16. Dendrogram based on biochemical parameters for accessions from midlands of Kerala

Table 17k. Clusters of *Phyllanthus* accessions collected from midlands based on biochemical parameters

Sl No.	Cluster number	Accessions	No. of accessions	Name of the accessions
1	Cluster I	SKM4, NKM2, CKM5	3	*Phyllanthus airyshawii*
2	Cluster II	CKM2,SKM3,NKM3	3	*Phyllanthus rheedei*
3	Cluster III	CKM1,NKM1,SKM2	3	*Phyllanthus amarus*
4	Cluster IV	CKM4,SKM1	2	*Phyllanthus urinaria*
5	Cluster V	CKM3	1	*Phyllanthus virgatus* var. *virgatus*

Table 17l. Summary statistics for quantitative characters of *Phyllanthus* accessions from midlands

Sl No.	Cluster number	Cluster mean values			
		Content of total extractives (g)	Total phenol content (mg/g)	Phyllanthin content (%)	Anti oxidant capacity (EC_{50}) (µg/ml)
1	Cluster I	0.49	250.4	0.01	215.1
2	Cluster II	0.43	178.8	0.01	279.4
3	Cluster III	0.59	126.3	0.40	330.8
4	Cluster IV	0.52	214.0	-	234.7
5	Cluster V	0.49	178.2	0.01	281.3

grouping the collected accessions from midlands into clusters, represents various species of *Phyllanthus* are given in Table 17l.

Cluster I, representing *P. airy-shawii* recorded lowest EC_{50} value (215.1 μg ml^{-1}), and highest total phenol content (250.4 mg g^{-1}) indicating its highest antioxidant activity. Cluster I and II recorded 0.01% phyllanthin contents each. Cluster III representing *P. amarus* recorded highest values for total extractives (0.59 g) and phyllanthin content (0.40%) and lowest content of phenol (126.3 mg g^{-1}). Phyllanthin content was absent in cluster IV, representing *P. urinaria*.

4.1.18. Clustering of *Phyllanthus* accessions collected from high ranges based on biochemical parameters

Fig 17 represents the dendrogram of *Phyllanthus* accessions from high ranges, based on biochemical parameters. Cluster analysis of thirteen accessions from high ranges of southern, central and northern zones, based on biochemical parameters, is given in Table 17m.

The thirteen accessions from high ranges were grouped into five clusters, at nine per cent similarity, of which cluster I and cluster III comprising of three accessions each, CKH1, NKH1 and SKH1 representing *P. amarus* and NKH3, SKH2 and SKH5 representing *P. airy-shawii*. Cluster II included maximum number of accessions (four numbers), representing *P. urinaria*. Two accessions NKH2 and NKH4 were included in cluster IV representing *P. virgatus* var *virgatus* and *P. virgatus* var. *gardnerianus* and a lone accession (SKH3) was included in cluster V, representing *P. rheedei*.

The cluster mean values of biochemical characters employed for grouping the collected accessions from high ranges into clusters, representing various species of *Phyllanthus* are given in Table 17n.

Highest total extractives (0.57 g) and phyllanthin content (0.40 %) were recorded in cluster I, representing *P. amarus*. Lowest EC_{50} value (215.4 μg ml^{-1})

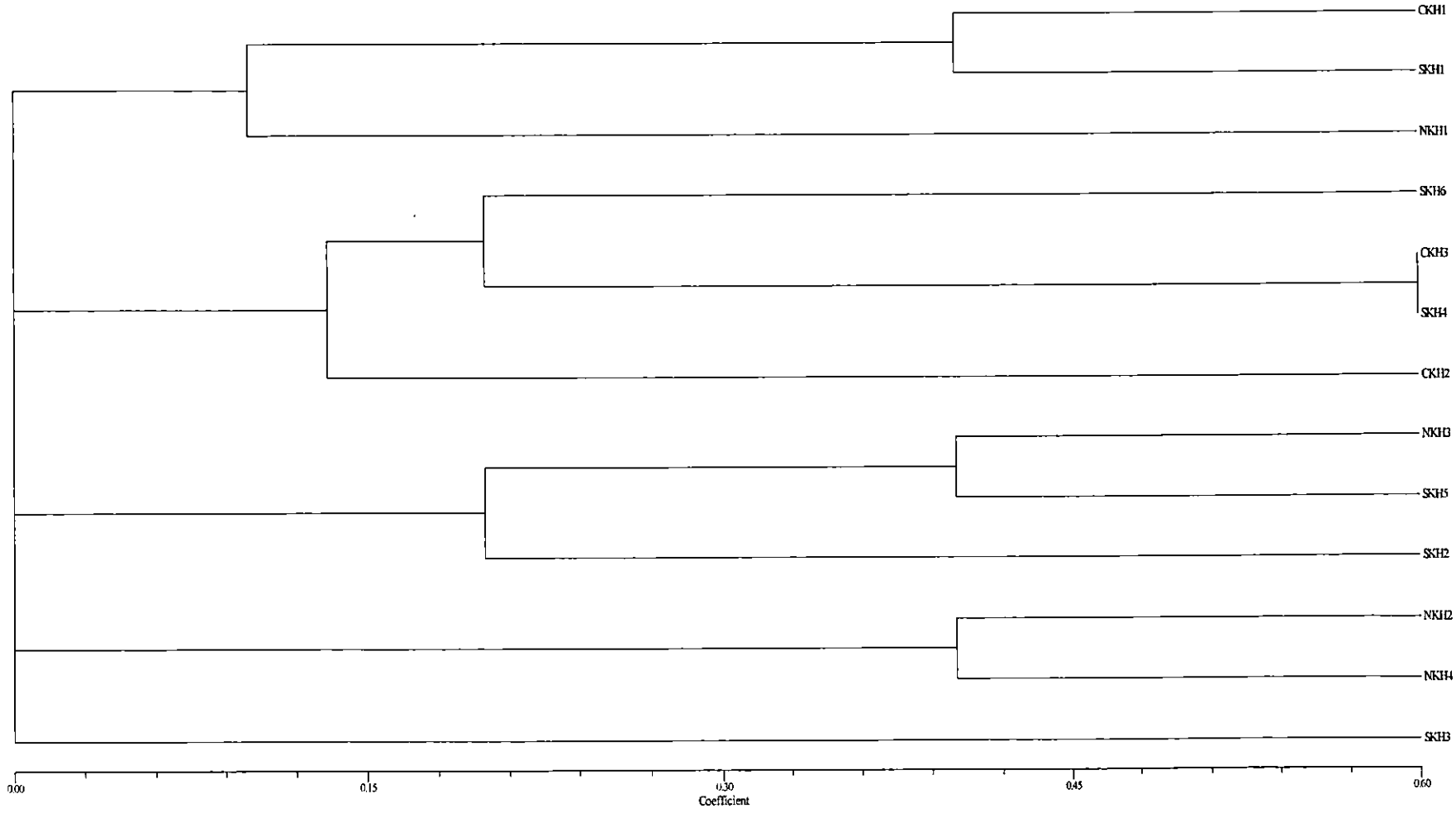


Fig 17. Dendrogram based on biochemical parameters of *Phyllanthus* accessions from high ranges of Kerala

Table 17m. Clusters of *Phyllanthus* accessions collected from high ranges based on biochemical parameters

Sl No.	Cluster number	Accessions	No. of accessions	Name of the accessions
1	Cluster I	CKH1, NKH1, SKH1	3	*Phyllanthus amarus*
2	Cluster II	SKH6, CKH2, CKH3, SKH4	4	*Phyllanthus urinaria*
3	Cluster III	NKH3, SKH2, SKH5	3	*Phyllanthus airy-shawii*
4	Cluster IV	NKH2, NKH4	2	*Phyllanthus virgatus* var. *virgatus* *P.virgatus var. gardnerianus*
5	Cluster V	SKH3	1	*Phyllanthus rheedei*

Table 17n. Summary statistics for quantitative characters of *Phyllanthus* accessions from high ranges

Sl No.	Cluster number	Cluster mean values			
		Content of total extractives (g)	Total phenol content (mg/g)	Phyllanthin Content (%)	Anti oxidant capacity (EC_{50} value) (µg/ml)
1	Cluster I	0.57	119.1	0.40	335.6
2	Cluster II	0.53	207.0	-	243.3
3	Cluster III	0.49	247.3	0.01	215.4
4	Cluster IV	0.44	186.7	0.01	275.1
5	Cluster V	0.40	169.3	0.01	286.1

and highest total phenol content (247.3%) was recorded in cluster III, representing *P. airy-shawii,* revealing its high antioxidant capacity. Cluster III, IV and V recorded 0.01 per cent of phyllanthin content, each. Phyllanthin content was absent in cluster II, representing *P. urinaria.*

4.2. EXPERIMENT II- DETECTION OF SPECIES ADMIXTURES IN TRADED CRUDE DRUG OF *Phyllanthus* spp.

4.2.1 Organoleptic evaluation of raw drug samples of *Phyllanthus*

The results of organoleptic evaluation of raw drug of *Phyllanthus* obtained from two user industries, RD-1 (Plate 11A) and RD-2 (Plate 11B) are presented in Table 18a. When evaluated on a nine point Hedonic scale, the raw drug sample RD-1, proved to be superior, registering a mode value of 8, for overall acceptability and 9 for appearance and colour. The raw drug sample RD-2 registered only a mode value of 5 each, for appearance and extent of species admixtures, lowering its overall acceptability level to a mode value of 5. In the raw drug sample RD-2, though the predominant species was *P. amarus*, presence of *P. airy-shawii* was detected to the extent of seven per cent. The raw drug sample RD-1, represented *P. amarus* alone, devoid of any species admixtures.

As is evident from Table 18b, the two raw drug samples of *Phyllanthus* RD-1 and RD-2 were on par with one another with respect to colour, flavour, texture, odour, taste and after taste. The sample from RD-1 was significantly superior in terms of appearance and overall acceptability.

4.2.2. Biochemical analyses of raw drug samples of *Phyllanthus* collected from user industries

The biochemical parameters of raw drug of *Phyllanthus* collected from RD-1 and RD-2 are presented in Table 18c. The raw drug of *Phyllanthus* from RD-1 registered a total extractive content of 0.55 g, total phenol content 129 mg g^{-1} and a phyllanthin content of 0.42 g, with an EC_{50} value of 327.1 µg ml^{-1} (Table 18c). As is evident from Table 18b, the respective values for raw drug sample of *Phyllanthus,* RD-2 were 0.57 g, 123.3 mg g^{-1}, 0.38 per cent and 332.2 µg ml^{-1}. The samples from user industries did not register any appreciable difference with the reference sample with respect to all biochemical parameters studied (Table 18c).

Plate 11. Raw drug samples of *Phyllanthus* from user industries

(A). Raw drug sample, RD-1

(B). Raw drug sample, RD-2

Table 18a. Organoleptic evaluation of raw drug of *Phyllanthus* spp. collected from user industries

Parameters	RD-1	RD-2
Appearance	9	5
Colour	9	8
Flavour	8	7
Texture	8	7
Odour	8	8
Taste	8	7
After taste	8	8
Species admixtures	9	5
Over all acceptability	8	5

Table 18b. Kendall's concordance test for organoleptic evaluation of raw drug of *Phyllanthus*

Parameters	RD-1	RD-2	Level of significance
	Mean rank		
Appearance	2.00	1.00	1.00**
Colour	1.60	1.40	NS
Flavour	1.65	1.35	NS
Texture	1.65	1.35	NS
Odour	1.75	1.25	NS
Taste	1.65	1.35	NS
After taste	1.50	1.50	NS
Over all acceptability	2.00	1.00	1.00**

Table 18c. Assessment of biomchemical parameters of raw drug of *Phyllanthus* species collected from user industries

Sl No.	Samples	Total extractive (g)	Phenol content (mg/g)	Phyllanthin Content (%)	Anti oxidant capacity (EC_{50} value) (µg/ml)
		Mean values			
1	RD-1	0.55	129.1	0.42	327.1
2	RD-2	0.57	123.3	0.38	332.2
3	Reference sample	(0.59)	(115.4)	(0.37)	(338.4)

Discussion

5. DISCUSSION

Systematic studies elucidating deep level angiosperm relationships is developing rapidly due to rapid advancements in new phylogenetic informations. *Phyllanthus* species of family Euphorbiaceae are reputed for their hepatoprotective activity, and are used widely in traditional Indian medicine. A nomenclatural problem persists among the herbaceous members of the genus, since they closely resemble one another in gross morphology. The medicinally important herb in this group, *Phyllanthus amarus* is often adulterated with allied species (Kandavel *et al.*, 2011).

The present study entitled 'Survey, collection and characterization of *'Kizharnelli'* (*Phyllanthus* spp.) of Kerala' thus attempts to resolve the nomenclatural confusion existing within the genus and to assess the extent of adulteration in traded raw drug of the species by analysing the morphological and biochemical characters. The study also attempted to assess the quality of traded crude drug of the species by detecting admixtures and assessing biochemical parameters. The salient results obtained in the study are discussed here under.

5.1. EXPERIMENT I – SURVEY, COLLECTION AND CHARACTERIZATION OF *Phyllanthus* ACCESSIONS FROM VARIOUS ZONES OF KERALA.

5.1.1. Survey, collection and characterization of *Phyllanthus* accessions from various zones of Kerala.

In the present study, morphological characterization of 47 *Phyllanthus* accessions of seven *Phyllanthus* spp., viz., *P. amarus*, *P. airy-shawii*, *P. maderaspatensis*, *P. rheedei*, *P. urinaria*, *P. virgatus* var. *gardnerianus* and *P. virgatus* var.*virgatus*, collected from coastal regions, plains, midlands and high ranges of southern, central and northern zones of Kerala, was done based on qualitative and quantitative parameters, separately.

5.1.1.1. *Characterization of Phyllanthus accessions based on morphological qualitative characters*

Considerable variations for the qualitative characters, namely, leaflet shapes, leaflet apices, leaflet bases among the *Phyllanthus* accessions. Except two accessions of *P. amarus* collected from high ranges, ten *Phyllanthus* accessions, were found to have oblong leaflet shape, obtuse apex and round base, while, the two accessions from high ranges were observed to have oblong leaflet shape, round leaflet apex and round leaflet base (Tables 6e, 6f and 6g). This variation may be due to the influence of microclimate prevailing at high altitude areas. Dark green, light green and purple green stem colour and faintly mucronate to mucronate leaf apices were observed for the collected accessions of *P. urinaria*. The purple stem colour may be due to the low availability of sunlight in growing premises. Agarwal *et al.* (2013) also reported the influence of environment on the genotype of the species as the reason for morphovariants in *Ocimum* spp. The accession of *P. maderaspatensis* had obcordate leaf apex, which is not noticed in other collected accessions of *Phyllanthus* spp. Hence, this character may be unique to this species. Sasidharan (2004) has also observed this character *in P. maderaspatensis*. *P. amarus*, *P. airy-shawii* and *P. rheedei* were observed to have terete stem shape, while, rest of the collected species had angular stems. These observations are in conformity with the species characterization of *Phyllanthus* based on morphological parameters proposed by Khatoon *et al.* (2006). No considerable variations (zone wise and altitude wise) were observed for characters like, growth habit (erect), branching pattern (spreading), leaf margin (entire), capsule colour (yellowish green), capsule shape (depressed globose), and flower colour (pale green), among the collected accessions of seven *Phyllathus* spp. Khatoon *et al.* (2006), had also recorded similar observations for morphological characters of *Phyllanthus* spp. in their investigations.

5.1.1.2. *Characterization of Phyllanthus accessions based on morphological quantitative characters*

P. virgatus var. *gardnerianus* and *P. virgatus* var. *virgatus* were observed to have longest pedicel length (1.0 cm), while rest of the *Phyllanthus* spp. recorded 0.1 cm for the same character. Highest plant height, fresh weight and dry weight were observed for *P. virgatus* var. *gardnerianus,* collected from high ranges, inferring that, it is the tallest herbaceous *Phyllanthus* spp. Only 36.8 and 36.4 to 40.9 number of leaflets were reported for *P. virgatus* var. *gardnerianus* and *P. virgatus* var. *virgatus* respectively, indicating that the distance between leaflets is higher in these species. Similar observations with regard to *P. virgatus* var. *virgatus* and *P. virgatus* var. *gardnerianus* have been reported by Gamble and Fischer (1915-1936). Broad leaves were observed in *P. rheedei* and longest leaflets in *P. virgatus* var. *gardnerianus* and *P. virgatus* var. *virgatus.* Udayan *et al.* (2005) reported the leaflet size of *P. rheedei* as 2.5 x 1.5 cm, indicating the broad nature of leaves. The accessions of *P. urinaria* reported lowest plant height within and among the zones and altitudes. All *Phyllanthus* spp. except *P. amarus*, recorded six sepals each, while *P. amarus* reported five sepals, indicating this unique characteristic of *P. amarus*. Kandavel *et al.* (2011) also made similar observations in *P. amarus*, *P.urinaria*, *P. airy-shawii*, *P. maderaspatensis*, *P. virgatus* var. *virgatus*.

5.1.2. Distribution of herbaceous *Phyllanthus* spp. in various zones of Kerala.

Fig.18 depicts the distribution of *Phyllanthus* spp. in the zones surveyed. The major species favoured by the drug industry, *P.amarus* was uniformly distributed in southern, central and northern zones of Kerala. Northern zones harboured maximum number of species, where all the species under study, except *P. maderaspatensis* were represented. The zone wise geographical distribution of *Phyllanthus* revealed a species specific pattern, where *P. virgatus* var. *gardnerianus* was seen only in northern zone and *P. virgatus* var. *virgatus* was seen only in central and northern zones. Similarly, *P. maderaspatensis* was

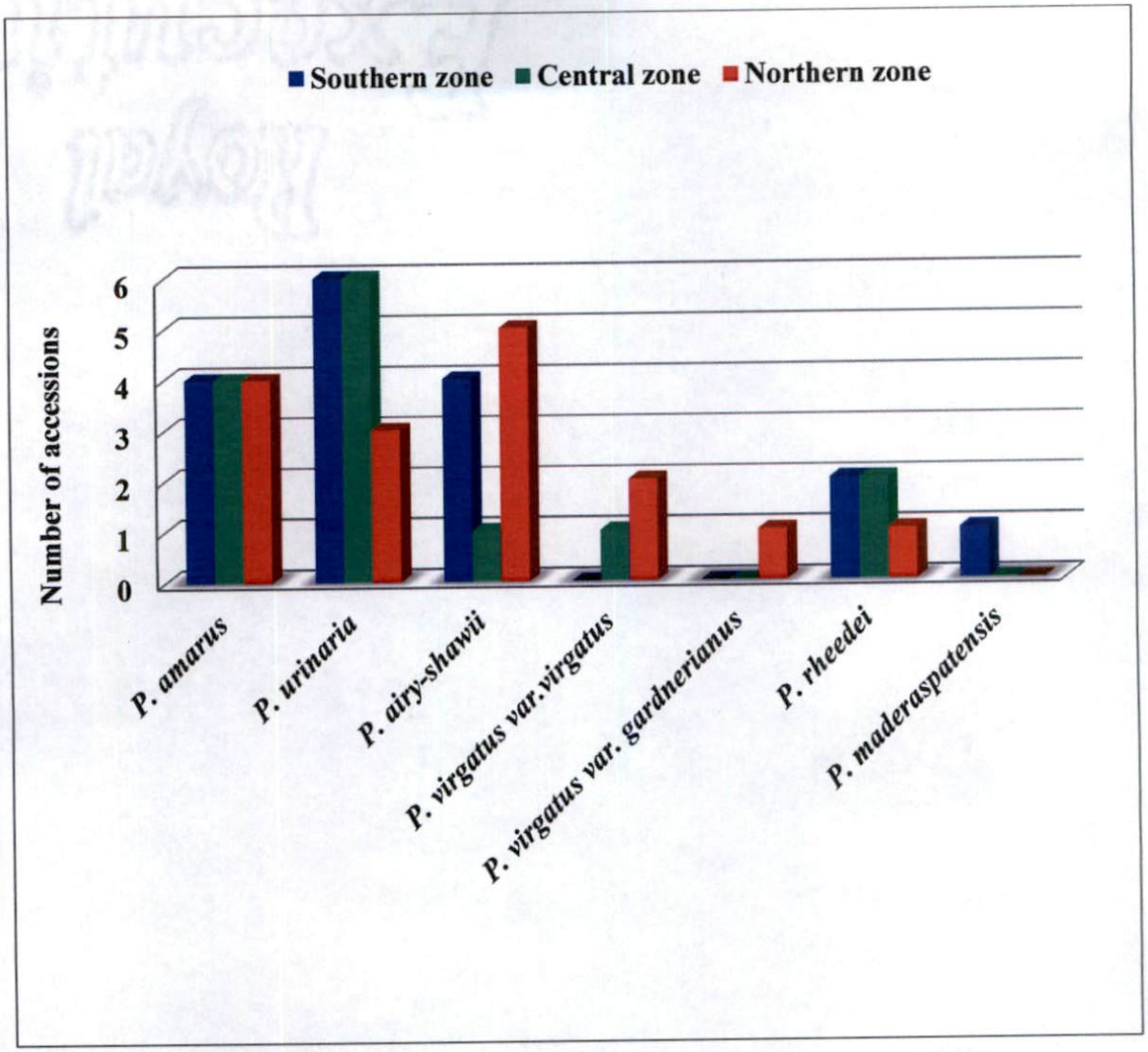


Fig. 18 Species wise distribution of herbaceous *Phyllanthus* in various zones surveyed

confined to southern zone. *P. urinaria* was the most predominant species in southern and central zone. Rana *et al.* (2012) observed a similar species specific distribution of *Tinospora* spp. collected from different ecological regions and altitudes in the north western Himalayan region of India ranging from 400 to 1300 m above mean sea level. Rokaya *et al.* 2012, has also observed that environmental factors influenced the population of medicinal flora in various habitats.

5.1.3. Distribution of herbaceous *Phyllanthus* spp. in altitudinal areas of Kerala

Fig. 19 depicts the distribution of *Phyllanthus* spp. in temporal sites surveyed viz., coastal regions, plains, midlands and high ranges. Maximum number of species, with the exception of *P. maderapatensis* was represented in high ranges and minimum in coastal areas. *P. amarus* was represented equally in coastal regions, plains midlands and high ranges. Among the study areas, coastal region alone harboured *P. maderaspatensis*. Comparatively lesser distribution of *P. maderaspatensis* in Kerala can be considered to be advantageous, as it diminishes the chances of the species being included as a species admixture in traded crude drug of *Phyllanthus*, the species being inferior with respect to biochemical constituents (Table 19a). Gamble and Fischer (1915-1936) has also observed coastal regions as the predominant habitat of *P. maderaspatensis*. Among the altitudinal areas surveyed, predominance of *P. urinaria* was seen in plains, an observation in conformity with the report of Gamble and Fischer (1915-1936).

5.1.4. Clustering of *phyllanthus* accessions based on morphological parameters

Morphological characters of 47 *Phyllanthus* accessions collected from coastal regions, plains, midlands and high ranges of southern, central and northern zones of Kerala, were analysed using UPGMA cluster analysis based on similarity level, using NTSYS software, for finding out the morphological relationship

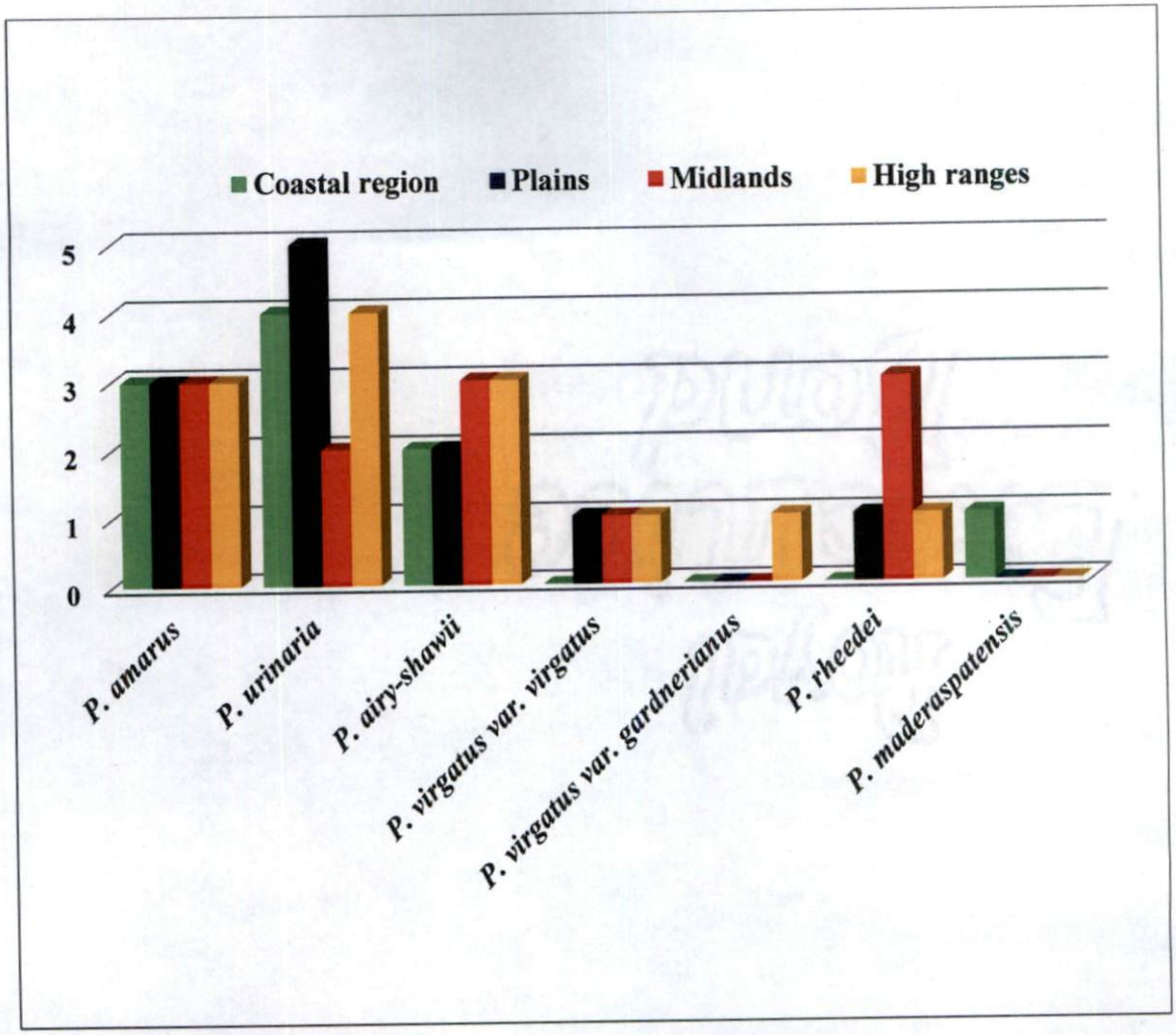


Fig. 19 Species wise distribution of herbaceous *Phyllanthus* in various altitudinal areas surveyed

within and among the *Phyllanthus* spp. Clustering of plant species based on quantitative characters is regularly being done to assess the similarity or dissimilarity among species of a single genus. Based on 31 morphological characters, Shinwari *et al.* (2011) could group the 30 accessions belonging to two different species, *Mentha royaleana* and *Mentha spicata* into two major clusters, at 84 and 88 per cent similarity respectively, using NTSYS software.

In southern zone, based on morphological characterization, the species, *P. urinaria, P. airy-shawii,* and *P. amarus* occurred in more than one cluster, which indicates variants within these species (Table 9a). The other *Phyllanthus* spp., *P. maderaspatensis* and *P. rheedei* formed single separate clusters, indicating their individual morphological identity as well as absence of morphological variants in them. Mahmood *et al.* (2010) also observed accessions of *Caralluma tuberculata* and *Caralluma edulis* collected from different locations, occurring in two separate groups during morphological characterization.

Similar trend wherein, accessions of the same species occurred in different clusters, were observed in central and northern zone as well, as in the case of *P. amarus* and *P. urinaria* in central zone and *P. amarus* and *P. airy-shawii*, in northern zone, indicating the presence of morphological variants in these species, in central and northern zones as well (Table 11c, 11e).

Thus, based on morphological characterization, in all the three zones surveyed, *P. rheedei*, *P. maderaspatensis, P. virgatus* var. *gardnerianus* and *P.virgatus* var. *virgatus* were grouped in separate clusters (Tables 11a, 11c and 11e). As is seen in zone wise clustering, altitude wise clustering based on morphological parameters also presented a similar clustering pattern (Tables 11g, 11i, 11k, 11m). Clustering of accessions from coastal regions, plains and high ranges resulted in *P. urinaria*, occurring in two different clusters in each of the above temporal sites (Tables 11g, 11i, 11m) and *P. amarus* occuring in two separate clusters in midlands (Table 11k). From the indications obtained in the present study during zone wise and altitudinal wise clustering, *P. urinaria* and *P.*

amarus proved to be the species harbouring greater number of morphovariants. Presence of morphovariants in herbaceous *Phyllanthus* spp. can be attributed to the fact that, they are highly cross pollinated (Mitra and Jain, 1985).

5.1.5. Altitudinal influence on growth parameters of *Phyllanthus* spp. in pot culture

During pot culture studies, only in *P. amarus* and *P. urinaria*, significant differences in plant height were observed among accessions collected from various altitudes, wherein, accessions from coastal regions and midlands in the former and those from high ranges in the latter were found to be significantly superior (Tables 14a and 14b). In *P. virgatus* var. *virgatus*, accessions from midlands were significantly superior with respect to number of leaflets (Table 14d). For the rest of the growth characters, altitudinal difference did not exert a significant difference in the species studied. Raghu *et al.* (2007) reported morphological variation in different species of *Tribulus terrestris* collected from various locations under domestication. Altitudinal influence was not significant in *P. airy-shawii* and *P.rheedei* with respect to growth characters.

When assessed for yield parameters like mean fresh and dry weights, the accessions of all species under study, exhibited significant differences, based on altitudinal variations. The influence of altitude on performance of *Phyllanthus* accessions, with respect to yield parameters was found to be species dependent. Accessions from coastal regions did not figure in the high performance group. For the commercially relevant species *P. amarus*, accessions from coastal regions and plains were inferior with respect to yield parameters while, those from plains, midlands and high ranges registered superior performance and were on par with one another (Table 15a). For *P. virgatus var. virgatus* and *P. rheedei*, accessions from midlands were significantly superior with respect to mean dry weight, while, those from high ranges registered inferior performance with respect to mean fresh and dry weight, having failed to acclimatize thoroughly in midlands (Tables 15d and 15e). Nautiyal *et al.* (2005) also reported influence of altitudinal variations on

yield parameters on dry weight basis in threatened medicinal species in Garhal, Western Himalaya, under domestication.

5.1.6. Biochemical characterization of collected *Phyllanthus* accessions from various zones of Kerala

In the present study, biochemical characterization of *Phyllanthus* accessions collected from coastal regions, plains, midlands and high ranges of southern, central and northern zones of Kerala, was done by estimating the contents of total extractives, total phenols, phyllanthin and antioxidant capacity.

Table 19. Content of biochemical constituents in *Phyllanthus* spp. of Kerala

***Phyllanthus* species**	Content of total extractives (g)	Phenol content (mg g^{-1})	Phyllanthin content (%)	Antioxidant capacity (EC_{50} value) (µg/ml)
	Range			
P. amarus	0.55g - 0.61	112.3 - 139.4	0.32 - 0.46	320.2 – 338.4
P. urinaria	0.50 - 0.57	196.2 – 221.2	Absent	227.1 – 251.2
P. airy-shawii	0.45 - 0.50	232.1 – 252.1	0.01 – 0.02	211.3 -222.3
P. virgatus var. virgatus	0.41 - 0.49	178.2 – 187.2	0.01	276.4 – 281.4
P. virgatus var.gardnerianus	0.47	191.2	0.01	271.1
P. rheedei	0.40 - 0.48	169.3 – 188.1	0.01	274.1 -286.1
P. maderaspatensis	0.39	144.1	0.01	313.1

Significant variation in content of total extractives was observed in methanolic extracts of collected *Phyllanthus* spp. Highest content of total extractives was obtained in *P. amarus* (0.55 g to 0.61 g), followed by *P. urinaria* (Fig. 20). The total content of extractives ranged from 0.45 g to 0.50 g, 0.41 g to 0.49 g, 0.47 g, 0.40 g to 0.48 g and 0.39 g in, *P. airy-shawii, P. virgatus, P. virgatus* var. *gardnerianus, P. rheedei, P. maderaspatenis* respectively (Table 19a). The content of total extractives of medicinal species obtained by solvent

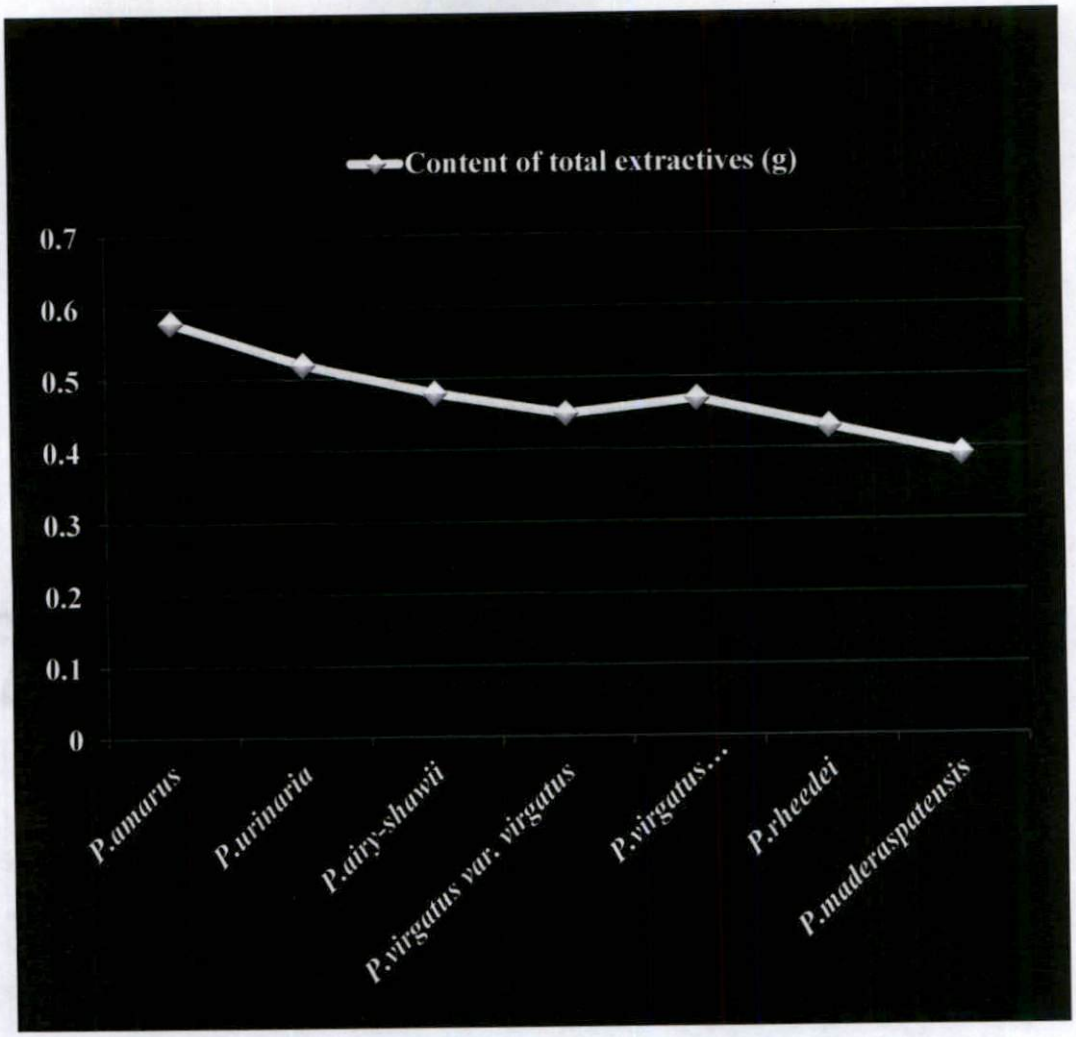


Fig 20. Content of total extractives in herbaceous *Phyllanthus* spp.

extraction is found to vary from species to species. Parekh and Chanda (2007) also made similar observations with regard to content and composition of solvent extracts, which varied among twelve Indian medicinal species studied. As observed in the present study, maximum content of crude extract was obtained in methanolic extract of *P. amarus* (0.695 g) was obtained by Sen and Batra (2013) as well.

The species, *P. amarus* also registered maximum content of the major active ingredient, phyllanthin, within a range of 0.32 - 0.46 per cent. Murali *et al.* (2001) also reported the presence of phyllanthin in *P.amarus* in higher amounts. Other *Phyllanthus* spp. under study registered low amounts of phyllanthin, while, phyllanthin was absent in *P. urinaria* (Fig. 21). Though traces of phyllanthin (0.01 %) were detected in *P. maderaspatensis* in the present study, Sharma *et al.* (2011) have reported the absence of phyllanthin in this species However, absence of phyllanthin in *P. urinara*, as is observed in the study, has been reported by Srirama *et al.* (2010).

Maximum content of phenol was recorded in *P. airy-shawii* (232.1 -252.1 mg g^{-1}) followed by *P. urinaria* (196.2 -221.2 mg g^{-1}). *P. amarus* recorded lowest content of phenol (Fig. 22). The results of this study conform to the observations made by Kumaran and Karunakaran (2007), who also observed *P. airy-shawii* to be the highest phenol containing species among herbaceous *Phyllanthus* spp. *P. airy-shawii* recorded lowest EC_{50} value (211.3 to 222.3 µg ml^{-1}), indicating its highest antioxidant capacity followed by *P. urinaria*, (227.1 to 251.2 µg ml^{-1}). Eldeen *et al.* (2010) has also confirmed the high total phenolic content and the remarkable DPPH scavenging effect of *P. urinaria*. Highest EC_{50} value was observed in the species *P. amarus* (320.2 – 338.4 µg ml^{-1}), is indicative of its low antioxidant capacity (Fig. 23). The observations by Kumaran and Karunakaran, (2007), who reported that the antioxidant activity of *Phyllanthus* spp. are in the order, *P. airy-shawii* (171.15.6 mg g^{-1}) > *P. urinaria* (325±17.8 mg g^{-1}) > *P. virgatus* var. *virgatus* (274±13.5 mg g^{-1}) > *P. maderaspatensis* (230±10.7 mg g^{-1}) > *P. amarus* (171 ±15.6 mg g^{-1}), confirms the results of this study. Low

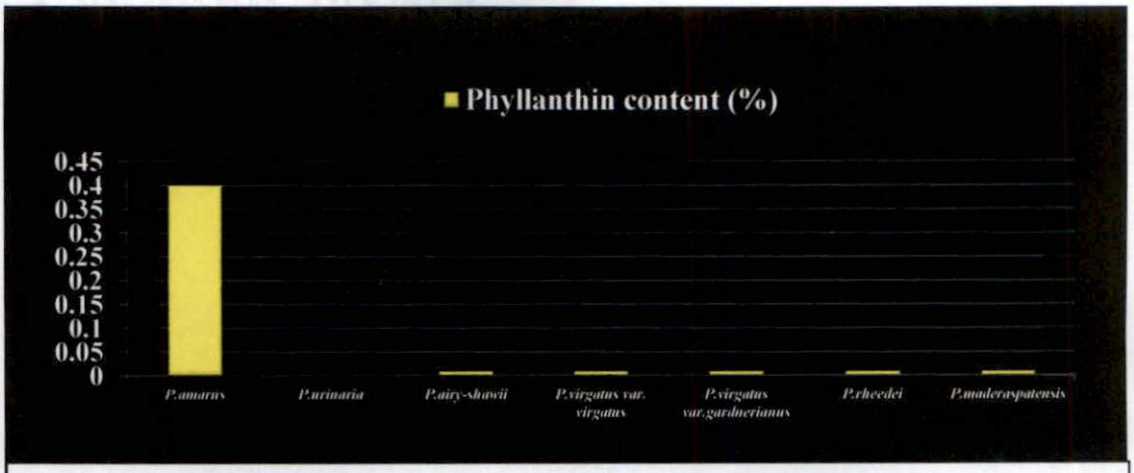


Fig 21. Phyllanthin content in herbaceous *Phyllanthus* spp.

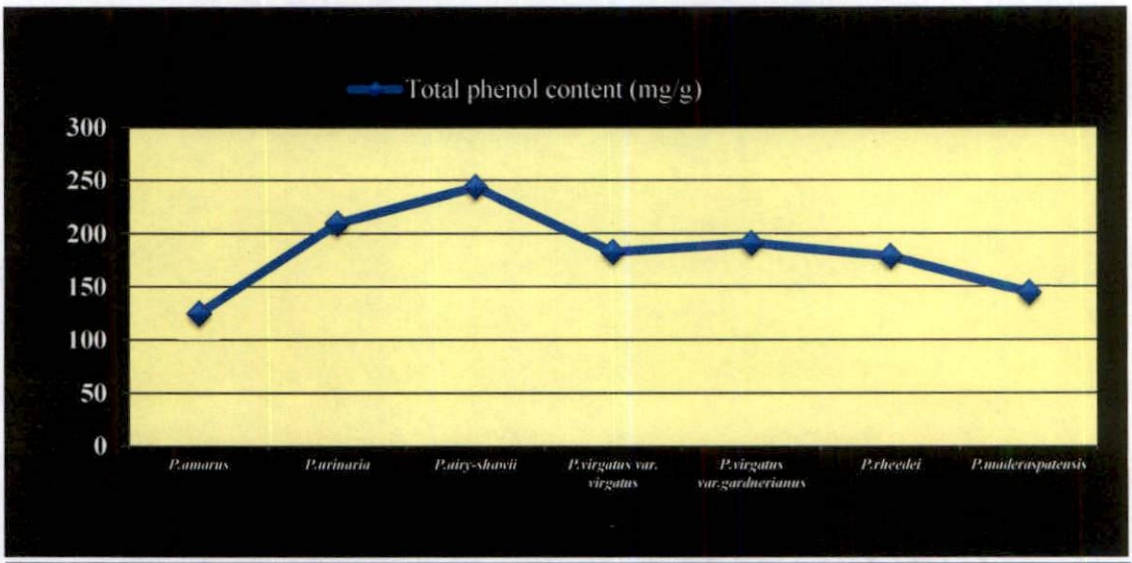


Fig 22. Total phenol content in herbaceous *Phyllanthus* spp.

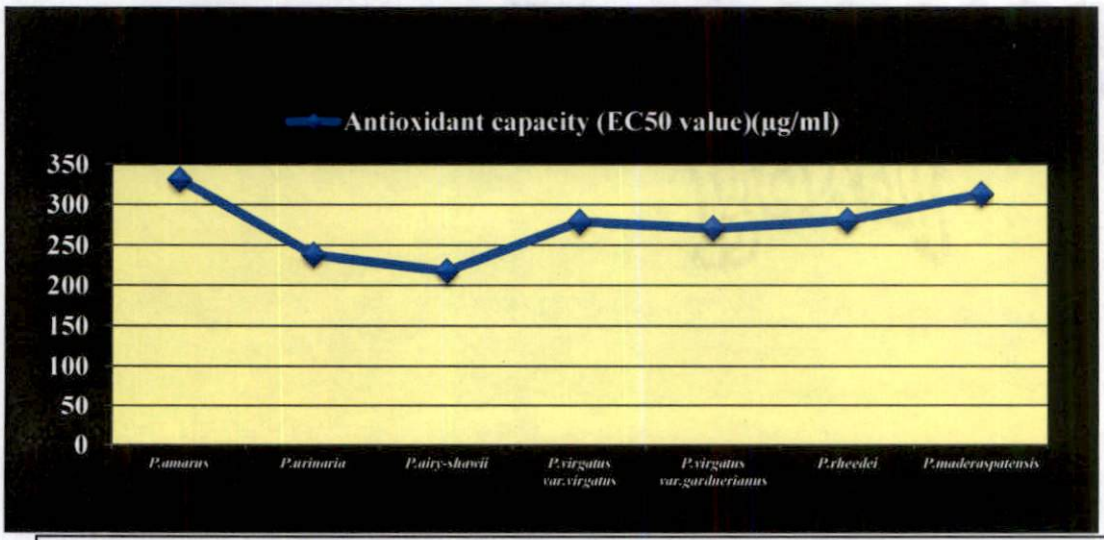


Fig 23. Antioxidant capacity (EC_{50} value) of herbaceous *Phyllanthus* spp.

antioxidant capacity of *P. amarus* extract has also been reported by Sen and Batra (2013). In the present study, the species, *P. virgatus* var. *gardnerianus* also appears to be promising, with respect to its antioxidant capacity (Table 19a). However there are no published reports to confirm this observation.

From the study, it is evident that a positive correlation exists between total phenol content and antioxidant capacity, wherein, higher the phenol content, higher the antioxidant capacity, indicated by low EC_{50} values (Fig. 24). Such a positive correlation has also been observed by Kumaran and Karunakaran (2005). The variation in phenol content among the *Phyllanthus* spp. may be genetic and within the species, may be due to the influence of microclimate. The plants growing in stressed conditions, are observed to produce more amount of secondary metabolites, which include both phenols and the lignan phyllanthin. Pandey *et al.* (2015) reported that podophyllotoxin content in *Podophyllum peltatum* collected from different altitudinal areas varied, under domestication.

5.1.7. Cluster analysis of *Phyllanthus* accessions based on biochemical parameters

The fourteen accessions from central zone were grouped into four clusters, with cluster I, representing *P. rheedei* and *P. virgatus* var. *virgatus*, cluster II representing *P. airy-shawii*, cluster III representing *P. amarus* and cluster IV representing *P. urinaria*. Biochemical parameters have been regularly employed to group crop species into clusters to indicate species similarity based on content of active ingredients. In *Piper longum* Maheshwari (2012), could group 20 accessions into different clusters based on biochemical characters.

In the present study, clustering of *Phyllanthus* spp. in central zone based on biochemical parameters presented a different clustering pattern as compared to clustering based on morphological parameters (Table 17c, Table 11c). Here, based on biochemical characterization, *P. rheedei* and *P. virgatus* var. *virgatus* were grouped in a single cluster, while they existed in separate clusters during clustering based on morphological characters. From this study, it is clear that,

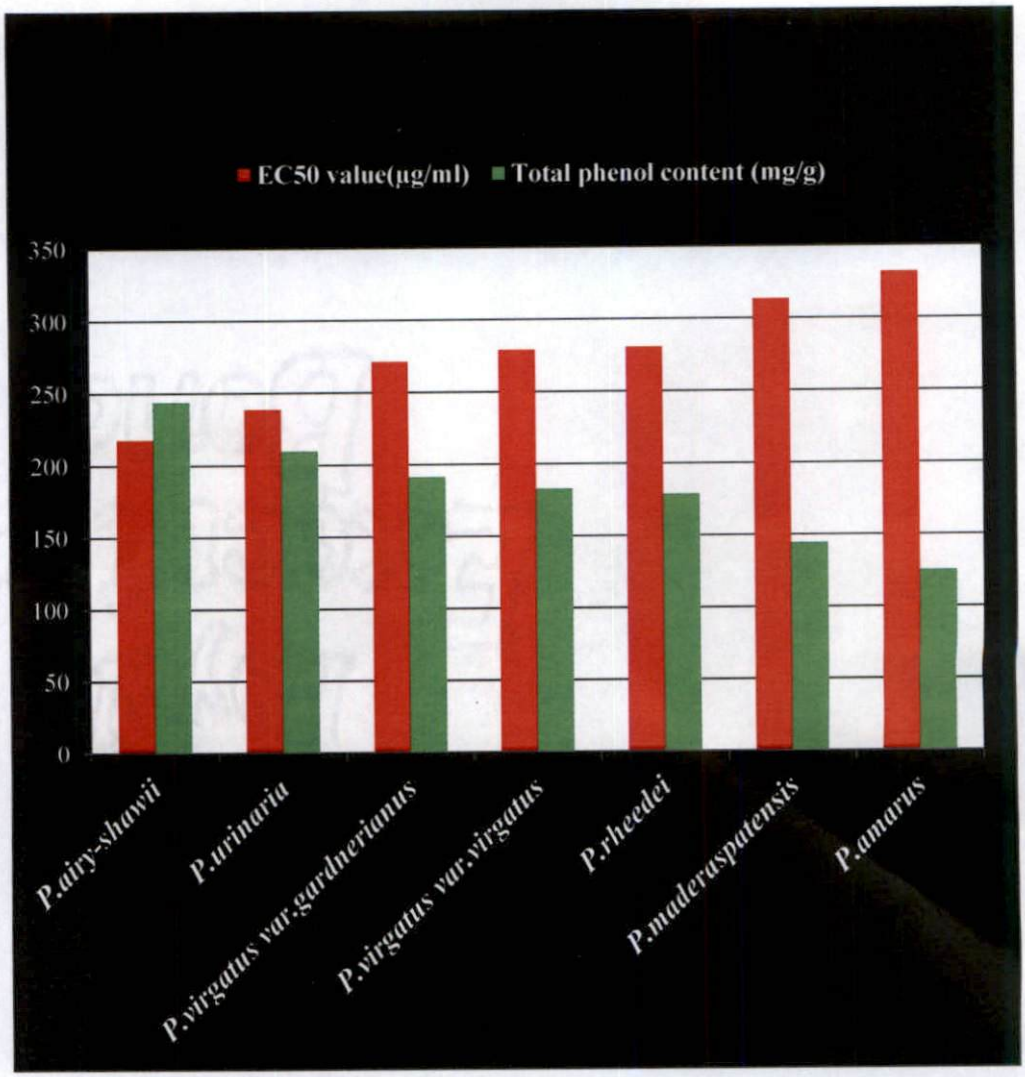


Fig 24. EC_{50} value Vs. phenol content in herbaceous *Phyllanthus* spp.

morphologically dissimilar *Phyllanthus* spp. may possess, comparable contents of active ingredients. Hence, from the therapeutic point of view, substitution is possible between various *Phyllanthus* spp. that are clustered together, based on similarity in contents of active ingredients. Thus *P. rheedei* and *P. virgatus* var. *virgatus* could be substituted for each other for therapeutic purpose and breeding programmes to evolve superior chemotypes.

Cluster III of central zone, being a representative cluster of *P. amarus,* recorded the highest cluster mean values for content of total extractives and phyllanthin. Cluster II, representing *P. airy-shawii* recorded the highest antioxidant capacity as indicated by lowest EC_{50} values (Tables 17c, 17d). Cluster mean values of major biochemical constituents of different medicinal species offer possibilities of bringing out novel types, combining the beneficial characteristics of constituent species of different clusters. Here, the high cluster mean value for phyllanthin content in cluster III representing *P. amarus* and low cluster mean value for EC_{50}, indicative of high antioxidant capacity of cluster II representing *P. airy-shawii,* offer the possibility of interspecific hybridization between the two, they being in different clusters, to combine the beneficial characteristics of the constituent species of the two clusters. In the present study, similar observations were made during clustering of accessions from southern and northern zones as well (Tables 11a, 11e, 17a and17e).

Clusters of *Phyllanthus* accessions derived altitudinal wise, as well as their cluster mean values are presented in Tables 17g, 17h, 17i, 17j, 17k, 17l, 17m and 17n. The accessions from coastal regions, plains, midlands and high ranges were grouped into four clusters each for coastal regions and plains and five clusters each for midlands and high ranges (Table 17g, 17i, 17k and 17m).

As is seen, during zone wise clustering, clustering based on biochemical parameters, altitudinal wise presented a different clustering pattern as compared to clustering based on morphological parameters (Fig. 14, Fig. 15, Fig. 16 and Fig. 17), which offers scope for substitution for therapeutic purpose and for initiating

breeding programmes between species in different clusters. Difference in clustering pattern during zone wise and altitudinal wise clustering was observed for the species, *P. rheedei*, *P. virgatus* and *P. virgatus* var. *gardnerianus*. In the altitudinal wise clustering, *P. rheedei* and *P. virgatus* var. *virgatus* occurred in different clusters for accessions from midlands (Table 17k) and high ranges (Table 17m) were grouped, whereas, in zone wise clustering the two species occurred together in central zone (Table 17c), where the two species were available. However, in plains, the two species were grouped in the same cluster (Table 17i), indicating the influence of altitude on contents of biochemical constituents. As revealed in the summary statistics for biochemical parameters of accessions from plains (Table 17j), from midlands (Table 17l) and from high ranges (Table 17n), the cluster representing *P. rheedei*, registered higher values for beneficial characters like content of phenol in plains (186.1mg g^{-1}) than that in midlands (178.8mg g^{-1}) and in high ranges (169.2 mg g^{-1}). This observation, points to the fact that, altitude can influence the content of certain active ingredients of *Phyllanthus* spp. and hence, raw drug collected from various altitudinal regions can differ in their therapeutic potential and the breeding strategies to be adopted should be different. In the present study, the behaviour of *Phyllanthus* accessions bears testimony to the above statement. Similarly, *P. virgatus* var. *gardnerianus*, which was grouped along with *P. rheedei* and *P. virgatus* var. *virgatus* (Table 17e) in zone wise clustering, was grouped in a different cluster, when clustered altitude wise (Table 17m). The above explanation holds good in this case as well.

5.2. EXPERIMENT II- DETECTION OF SPECIES ADMIXTURES IN TRADED CRUDE DRUG OF *Phyllanthus* spp.

5.2.1. Organoleptic evaluation of raw drug samples of *Phyllanthus*

Organoleptic evaluation of raw drug of *Phyllanthus* obtained from two user industries RD-1 and RD-2, revealed the superiority of samples from RD-1, when evaluated on a nine point hedonic scale (Table 18a). Raw drug sample from

RD-2, registered a low overall acceptability mode value of five, probably, due to the species admixture detected (*P. airy-shawii*)in the raw drug sample of *P. amarus*.

Species admixtures in crude drug samples of medicinal species, lower the efficacy of the drug prepared from that sample (Folashade *et al.*, 2012). Assessment of species admixtures in raw drug of *Phyllanthus* was conducted by Srirama *et al.* (2010), who detected species admixtures by morphiotaxonomic keys and DNA barcoding. Accordingly, analysis of traded samples revealed six species of *Phyllanthus*, in which *P. amarus* was the predominant species. In the present study also, analysis of traded crude drug samples, revealed the preponderance of *P.amarus*.

Based on the experimental results of the present study, presence of *P. airy-shawii*, in the crude drug sample of RD-2, does not appear to be a case of adulteration. As is evident from the assessment of biochemical parameters, *P .airy-shawii* registered the highest phenol content (Fig. 22) and greatest antioxidant capacity (Fig. 23). Antioxidant potential of medicinal species is considered to be beneficial in therapeutic regimes (Valko *et al.*, 2007). In spite of the low phyllanthin content (0.01 to 0.02 %) in *P. airy-shawii*, the high antioxidant capacity of the species, can be considered as a positive factor and hence, may contribute to the therapeutic potential of the drug sample. Srirama *et al.* (2010) had pointed out that phyllanthin and hypophyllanthin may not be the only compound responsible for the hepatoprotective activity of *Phyllanthus* spp. indicating that mere absence of phyllanthin is not an indication of the irrelevance of a *Phyllanthus* spp., with respect to its medicinal property. The samples from user industries did not register any appreciable difference with the reference sample with respect to all biochemical parameters studied.

After concluding the present investigation, certain inferences have emerged, which could be summerized as follows:

- Among the herbaceous *Phyllanthus* spp. maximum morphovariants were detected in *P. urinaria* and *P. airy-shawii* exhibited maximum morphological variation
- The commercially relevant species, *P. amarus,* had more or less equal distribution in the various zones and altitudinal regions surveyed
- The influence of altitude was not marked in the performance *Phyllanthus* spp. with respect to growth characters under domestication
- Altitude can influence the content of certain active ingredients of *Phyllanthus* spp.
- Morphologically dissimilar *Phyllanthus* spp. may possess, comparable contents of active ingredients and hence, substitution is possible between various *Phyllanthus* spp., based on similarity in contents of active ingredients
- *P. amarus* registered maximum content of the major active ingredient, phyllanthin and *P. airy-shawii,* the highest antioxidant capacity
- In the present study also, analysis of traded crude drug samples, revealed the preponderance of *P. amarus.*
- The samples from user industries did not register any appreciable difference with the reference sample with respect to all biochemical parameters studied.

Summary

6. SUMMARY

A total of fourty seven *Phyllanthus* accessions were collected from coastal regions, plains, midlands and high ranges of southern, central and northern zones of Kerala, wherein, seventeen accessions from southern, fourteen from central and sixteen from northern zones were collected.

Out of the fifteen qualitative characters observed, no notable variability was observed for eight qualitative characters viz., growth habit, branching pattern, stem shape, leaf margin, flower colour, capsule colour, capsule texture and capsule shape. *Phyllanthus* accessions collected were found to have erect growth habit with spreading branching pattern. Two types of stem shapes viz., terete and angular and capsule textures like rough and smooth types were found. *P. amarus*, *P.airy-shawii*, *P.rheedei* was observed to have terete stem shape, while, rest of the collected species had angular stem shape. Entire leaf margin, depressed globose capsule shape, pale green flower colour and yellowish green capsule colour were noticed in all the accessions.

Notable variation was noted in qualitative characters like stem colour, leaflet colour, rachis colour, leaflet shape, leaflet apex, leaflet base and peduncle colour. Except two accessions of *P.amarus* collected from high ranges, ten *Phyllanthus* accessions, were found to have oblong leaflet shape, obtuse apex and round base, while, those from high ranges had oblong leaflet shapes, round leaflet apices and round leaflet bases. Dark green, light green and purple green stem colour and faintly mucronate to mucronate leaf apices were observed for the accessions of *P.urinaria*. The lone accession of *P.maderaspatensis* had obcordate leaf apex.

P.virgatus var. *gardnerianus* and *P.virgatus* var. *virgatus* had the longest pedicel length (0.6 cm), while the rest of the *Phyllanthus* species were observed to record 0.1 cm for the same character. Except *P.amarus*, all other *Phyllanthus* species recorded six sepals each, while *P.amarus* recorded five sepals. Highest plant height, fresh weight and dry weight were observed for *P.virgatus* var.

gardnerianus, collected from high ranges. Broadest leaflets were observed in *P.rheedei*, while, longest leaflets were seen in *P.virgatus* var. *gardnerianus* and *P.virgatus* var. *virgatus*. The leaflets distance was higher in *P.virgatus* var. *gardnerianus* and *P. virgatus* var. *virgatus*. The accessions of *P.urinaria* recorded lowest plant height within and among the zones and altitude.

P. virgatus var. *gardnerianus* recorded maximum plant height (90.1 cm), number of branchlets plant (31.5), fresh weight (16.21 g) and dry weight (13.81 g). *P. virgatus* var. *virgatus* recorded maximum number of leaflets per compound leaf (36.4 – 40.9) and leaf length (2.01 – 2.21 cm). Minimum values for plant height (22.1 – 28.8 cm), number of branchlets per plant (9.5 – 10.3), number of leaflets per compound leaf (20.1 – 21.2), number of capsules per branch (10.6 – 22.3), fresh weight (3.62 – 5.18 g) and dry weight (1.42 – 3.08) were recorded for *P. urinaria. P.airyshawii* recorded least values for leaf length (0.42 – 1.21 cm) and leaf width (0.21 – 0.62 cm).

P. amarus was distributed equally in the three zones and *P. urinaria* was predominantly observed in southern and central zones. The northern zone of Kerala, had representations of all herbaceous species of *Phyllanthus* under study, except *P. maderaspatensis*. The only accession representing *P. maderaspatensis* was observed in southern zone. *P. virgatus* var. *gardnerianus* was represented only from northern zone. *P. urinaria* was the species with maximum distribution in the state

P.amarus was equally distributed in coastal regions, plains, midlands and in high ranges. *P.virgatus* var. *gardnerianus* had representation only in high ranges, while *P.maderaspatensis* was represented only in coastal region. Coastal region represented fewer species of *Phyllanthus* (four numbers), while, high ranges registered maximum representation of herbaceous *Phyllanthus* species.

In southern zone, clustering of *Phyllanthus* accessions based on morphological parameters, revealed that the species, *P. urinaria, P. airy-shawii,* and *P.amarus* occurred in more than one cluster. Similar trend wherein,

accessions of the same species occurred in different clusters, were observed in central and northern zone in the case of *P. amarus* and *P. urinaria* in central zone and *P. amarus* and *P. airy-shawii*, in northern zone. The other *Phyllanthus* spp., *P. maderapatensis, P. virgatus* var. *virgatus*, *P. virgatus* var. *gadnerianus* and *P. rheedei* formed single separate clusters.

In altitude wise clustering based on morphological parameters, clustering of accessions from coastal region, plains and high ranges resulted in *P. urinaria*, occurring in two different clusters in each of the above temporal sites and *P. amarus*, in two separate clusters in midlands.

In pot culture evaluation of *Phyllanthus* accessions, with respect to growth and yield parameters, accessions of *P.virgatus* var. *virgatus*, *P. virgatus* var. *gardnerianus* and *P. maderaspatensis* recorded significantly superior values, while those of *P. urinaria* proved to be inferior.

In species wise assessment of growth parameters of collected accessions, *P. amarus* and *P. urinaria* collected from various altitudes surveyed, recorded significant difference, only with respect to plant height wherein, accessions from coastal and midlands were rated superior in *P. amarus* and those from high ranges in *P. urinaria*. With respect to number of leaflets, *P.virgatus* var. *virgatus* from midlands was superior. For the rest of the growth characters, altitudinal difference did not exert a significant influence.

In species wise assessment of yield parameters of collected accessions, the accessions of *P. amarus* from plains, midlands and high ranges were rated superior, while, for *P. urinaria* those from high ranges proved to be the best. Accessions from plains rated superior with respect to yield parameters in *P. airy-shawii* and *P.virgatus var. virgatus*, and those from midlands in *P. rheedei*.

Biochemical characterization of *Phyllanthus* accessions revealed highest content of total extract in *P. amarus* (0.55 g to 0.61g), followed by *P.urinaria*. The total content of extractives ranged from 0.45 g to 0.50 g, 0.41 g to 0.49 g,

0.47 g, 0.40 g to 0.48 g and 0.39 g in, *P. airy-shawii, P. virgatus* var. *virgatus, P. virgatus* var. *gardnerianus, P. rheedei, P. maderaspatenis* respectively.

With respect to the major active ingredient, phyllanthin, *P. amarus* registered maximum content of phyllanthin within a range of 0.32 - 0.46 per cent. Other *Phyllanthus* species under study, registered low amounts of phyllanthin. Phyllanthin was absent in *P. urinaria.*

Maximum content of phenol was recorded in *P. airy-shawii* (232.1 -252.1 mg g^{-1}) followed by *P.urinaria* (196.2 -221.2 mg g^{-1}). *P. amarus* recorded the lowest content of phenol. *P.airy-shawii* recorded lowest EC_{50} value (211.3 to 222.3 μg ml^{-1}), indicating its high antioxidant capacity followed by *P.urinaria*, (227.1 to 251.2 μg ml^{-1}). Highest EC_{50} value was observed in the species *P. amarus* (320.2 – 338.4 μg ml^{-1}), indicative of its low antioxidant capacity. A positive correlation existed between total phenol content and antioxidant capacity, wherein, higher the phenol content, higher the antioxidant capacity, indicated by low EC_{50} values.

Clustering of *Phyllanthus* spp., based on biochemical parameters, presented different clustering pattern as compared to clustering based on morphological parameters.

The seventeen accessions collected from southern, central and northern zone, could be grouped into five and four clusters each respectively. Clusters of accessions in southern zone represented *P. rheedei, P. airy-shawii, P. urinaria, P. maderaspatensis* and *P. amarus,* those of central zone represented, *P. rheedei, P. virgatus var. virgatus, P. airy-shawii, P amarus* and *P.urinaria.* Clusters of accessions from northern zone represented all species excluding *P. maderaspatensis.*

Clustering of *Phyllanthus* species in central zone based on biochemical parameters grouped *P.rheedei* and *P.virgatus* var. *virgatus* in a single cluster indicating that morphologically dissimilar medicinal species may possess

comparable contents of active ingredients. However, they existed in separate clusters during clustering based on morphological parameters.

Altitudinal wise clustering of accessions, based on biochemical parameters, revealed that altitude can influence the content of certain active ingredients of medicinal species. Accessions from coastal region and plains could be grouped into four clusters and those from midlands and high ranges into five clusters. Accessions of *P. rheedei* and *P. virgatus* var. *virgatus* from midlands and high ranges occurred in different clusters. However, in plains, the two species were grouped in the same cluster. Similarly, *P. rheedei* which was grouped along with *P. virgatus* var. *virgatus* and *P. virgatus* var. *gardnerianus* in zone wise clustering, was grouped in a different cluster, when clustered altitudinal wise.

Clusters representing *P. amarus,* recorded the highest cluster mean values for content of total extractives and phyllanthin. Cluster representing *P. airy-shawii* recorded the highest phenol content and antioxidant capacity as indicated by lowest EC_{50} values. Phyllanthin was absent in clusters representing *P. urinaria.* Clusters with highest phenol content recorded lowest EC_{50} value, indicative of its high antioxidant capacity.

During organoleptic evaluation of raw drug samples RD-1 and RD-2, RD-1 proved to be superior, registering a mode value of 8, for overall acceptability and 9 for appearance and colour. The raw drug sample RD-2 registered only a mode value of 5 each for appearance and extent of species admixtures, lowering its overall acceptability level to a mode value of 5.

In the raw drug sample RD-2, though the predominant species was *P. amarus*, presence of *P. airy-shawii* was detected to the extent of seven per cent. The raw drug sample from RD-1 represented *P. amarus* alone, devoid of any species admixtures.

The biochemical parameters of raw sample RD-1 registered a total extractive content of 0.55 g, total phenol content 129 mg g^{-1}, phyllanthin content of 0.42 g, with an EC50 value of 327.1 µg ml^{-1}. The respective values for raw

drug sample RD-2 were 0.57 g, 123.3 mg g^{-1}, 0.38 per cent and 332.2 µg ml^{-1}. The samples from user industries did not register any appreciable difference with the reference sample with respect to all the biochemical parameters studied.

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Appendices

APPENDIX -I

Weather data during the crop period (Vellanikkara -2014-2015)

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Mean
	Mean maximum temperature (0 C)												
2014	32.9	34.7	36.7	35.3	33.2	30.9	29.5	29.5	31.3	31.9	31.6	31.9	32.5
2015	32.5	34.3	35.8	34.0	32.9								
	Mean minimum temperature (0C)												
2014	23.0	22.9	24.2	25.7	24.2	24.4	23.1	23.2	23.3	23.7	23.2	22.5	23.6
2015	22.1	23.0	24.9	24.6	24.7								
	Mean relative humidity morning (%)												
2014	66	75	76	89	90	95	95	97	95	93	84	78	86
2015	75	73	83	89	92								
	Mean relative humidity evening (%)												
2014	36	37	34	57	64	76	80	76	69	68	60	53	59
2015	41	37	44	64	68								
	Mean relative humidity (%)												
2014	51	56	55	73	77	85	87	87	82	81	72	65	73
2015	58	55	63	77	80								
	Rainfall (mm)												
2014	0.0	0.0	0.0	61.0	323.6	469.8	768	599.8	215.1	224.6	85.3	9.6	2756.8 (total)
2015	0.0	0.0	72	162.2	259.0								
	Rainy days												
2014	0	0	0	4	5	21	26	22	13	15	5	1	112 (total)
2015	0	0	2	8	12								
	Total evaporation (mm)												
2014	171.3	145.0	191.5	130.8	107.5	88.7	77.6	90.2	92.8	86.1	96.9	112.1	115.9
2015	135.4	157.4	151.6	106.9	95.8								
	Mean evapotranspiration (mm)												
2014	277.6	240.8	264.2	192.4	182.0	90.1	49.3	81.3	172.6	135.2	151.9	188.5	168.8
2015	271.5	246.7	248.6	203.8	156.4								
	Sunshine (hrs)												
2014	277.6	240.8	264.2	192.4	182.0	90.1	49.3	81.3	172.6	135.2	151.9	188.5	168.8
2015	271.5	246.7	248.6	203.8	156.4								
	Mean sunshine (hrs)												
2014	9.0	8.6	8.5	6.4	5.9	3.0	1.6	2.6	5.8	4.4	5.1	6.1	5.6
2015	8.8	8.8	8.0	6.8	5.0								
	Mean wind speed (km/hr)												
2014	6.9	4.5	3.9	2.3	2.5	2.2	2.1	1.9	2.2	2.2	3.7	5.4	3.3
2015	5.6	5.8	3.3	2.4	1.7								

SURVEY, COLLECTION AND CHARACTERIZATION OF '*Kizharnelli*' (*Phyllanthus* spp.) OF KERALA

By

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ABSTRACT OF THE THESIS

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ABSTRACT

An investigation on "Survey, collection and characterization of '*Kizharnelli*' (*Phyllanthus* spp.) of Kerala", was carried out at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara, and CARe KERALAM, Koratty, Thrissur during 2013-2014, with the objective of morphological and phytochemical characterization of *Phyllanthus* accessions collected after surveying select locations of Kerala and assessment of quality of traded crude drug of *Phyllanthus* by detecting species admixtures and estimating phytochemical constituents.

Genus *Phyllanthus* belonging to the family Phyllanthaceae, consists of about 833 species and is chiefly distributed in moist humid tropics. The most wide spread species of this genus, *P. amarus,* reputed for its hepatoprotective activity which contain phyllanthin and hypophyllanthin and is used in traditional medicine against jaundice. Other commonly ocuuring herbaceous *Phyllanthus* spp. of Kerala are *P. virgatus* var. *virgatus*, *P.virgatus* var. *gardnerianus, P. rheedei*, *P. airy-shawii*, *P. maderaspatensis* and *P. urinaria.* Taxomonic confusion exists in identification of these herbaceous *Phyllanthus* spp., mainly due to their similarity in gross morphology, close proximity in growth habitat as well as referring them with a common vernacular name, '*Kizharnelli*'. Preponderance of other *Phyllanthus* spp. often leads to ignorant as well as deliberate adulteration/substitution in its raw drugs, resulting in lowering the efficacy of the medication. Other *Phyllanthus* species have not been subjected to in-depth phytochemical and clinical investigations.

A total of fourty seven *Phyllanthus* accessions were collected from coastal regions, plains, midlands and high ranges of southern, central and northern zones of Kerala, of which, seventeen accessions were from southern zone, fourteen from central and sixteen from northern zones. The collected *Phyllanthus* accessions were decoded into respective species based on the key characters of herbaceous *Phyllanthus* spp. described in Flora of Madras Presidency. Out of the fifteen

qualitative characters observed, no notable variability was observed for six qualitative characters viz., growth habit, branching pattern, leaf margin, flower colour, capsule colour and capsule shape. Erect growth habit, spreading branching pattern, entire leaf margin, depressed globose capsule shape, pale green flower colour and yellowish green capsule colour were noticed in all the accessions. Stem colour, leaflet colour, rachis colour, leaflet shape, leaflet apex, leaflet base and peduncle colour were highly varying among the accessions. The lone accession of *P. maderaspatensis* had obcordate leaf apex.

P. virgatus var. *gardnerianus* and *P. virgatus* var. *virgatus* were observed to have longest pedicel length (1.0 cm), while rest of the *Phyllanthus* spp. had a pedicel length of 0.1 cm. *P.amarus* had five sepals and rest of the species, six. Highest plant height (90.1 cm), fresh weight (16.21 g) and dry weight (13.81 g) were observed for *P. virgatus* var. *gardnerianus*. Broadest leaves were observed in *P. rheedei*, and longest leaflets (2.01 – 2.21 cm) in *P. virgatus* var. *gardnerianus* and *P. virgatus* var. *virgatus*. The accessions of *P. urinaria* (22.1 – 28.8 cm) registered shortest stems length.

P. amarus was distributed equally in the three zones surveyed and *P. urinaria* was predominantly observed in southern and central zones. Lone accession of *P. maderaspatensis* was observed in southern zone. *P. virgatus* var. *virgatus* was not represented at all in southern zone. The northern zone had representations of all herbaceous species of *Phyllanthus* under study, except *P. maderaspatensis*. *P. amarus* was equally distributed in coastal regions, plains, midlands and high ranges. *P. virgatus* var. *gardnerianus* had representation only in high ranges, while *P. maderaspatensis* was represented only in coastal regions. Coastal regions represented fewer species of *Phyllanthus*, while, high ranges registered maximum representation of herbaceous *Phyllanthus* species.

Clustering of *Phyllanthus* accessions based on morphological parameters revealed that *P. urinaria, P. airy-shawii,* and *P. amarus* occurred in more than one cluster which indicates the presence of morphovariants in them.

P. maderapatensis and *P. rheedei* formed single separate clusters indicating their individual morphological identity. Altitude wise clustering based on morphological parameters also presented a similar clustering pattern. In species wise assessment of growth and yield parameters of collected accessions during pot culture, *P. amarus* and *P. urinaria*, recorded significant differences, only with respect to plant height, wherein, accessions from coastal and midlands were rated superior in *P. amarus*. With respect to number of leaflets, *P. virgatus* var. *virgatus* from midlands of central zone was significantly superior.

Biochemical characterization of *Phyllanthus* accessions revealed highest contents of total extractives (0.55 g to 0.61g) and phyllanthin (0.32 - 0.46 %) in *P. amarus*. Phyllanthin was absent in *P. urinaria*. Maximum content of phenol was recorded in *P. airy-shawii* (232.1 -252.1 mg g^{-1}) followed by *P. urinaria* (196.2 -221.2 mg g^{-1}). *P. airy-shawii* recorded lowest EC_{50} value (211.3 to 222.3 µg ml^{-1}), indicating highest antioxidant capacity. A positive correlation noticed between total phenol content and antioxidant capacity.

Clustering of *Phyllanthus* spp. in central zone based on biochemical parameters grouped, *P. rheedei* and *P. virgatus* var. *virgatus* in a single cluster while, they existed in separate clusters during clustering based on morphological parameters. Thus, morphologically dissimilar *Phyllanthus* spp. possess comparable contents of active ingredients. Hence, from the therapuetic point of view, substitution is possible between the species that are clustered together based on contents of active ingredients. Clustering based on temporal sites revealed that altitude can influence the content of certain active ingredients of *Phyllanthus* spp.

During organoleptic evaluation of raw drug samples RD-1 and RD-2, the raw drug sample RD-1, was superior, devoid of any species admixtures. In the raw drug sample RD-2, though the predominant species was *P. amarus*, presence of *P. airy-shawii* was detected. The biochemical parameters of raw samples of *Phyllanthus* from RD-1 and RD-2 did not register any appreciable difference with the reference sample, with respect to all biochemical parameters studied.