Modern Trends in Conservation, Utilization and Improvement of Plant Genetic Resources

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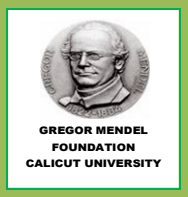

Gregor Mendel Foundation
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PREFACE

Gregor Mendel Foundation was established in 1991 and registered under the Societies' Registration Act of India with headquarters at Calicut University, Kerala, India. The major objectives of the foundation include local, national and international academic activities with special emphasis on genetics, breeding and biotechnology. The Foundation organizes Gregor Mendel Birthday Lectures, Seminars and Symposia and publishes Gregor Mendel Foundation Proceedings and Books. National Seminars are usually organized every alternate year in collaboration with research institutes or university departments. Gregor Mendel Foundation Seminar 2017 (**National Seminar on Modern Trends in Conservation, Utilisation and Improvement of Plant Genetic Resources)** was conducted on November 23 & 24, 2017 in collaboration with Department of Botany, University of Kerala, Thiruvananthapuram, Kerala, India at Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram. The present book embodies selected papers presented in the seminar.

Gregor Mendel Foundation Council and the Organizing Committee of the seminar use this occasion to thank the participants very sincerely for their effective participation and also for providing full texts of their presentations for publication.

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Modern Trends in Conservation, Utilization and Improvement of Plant Genetic Resources
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STUDY ON JUVENILE VARIABILITY OF TEAK (*TECTONA GRANDIS* L.F.) CLONES FROM DIFFERENT PARTS OF KERALA

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Abstract: Teak (*Tectona grandis* Linn. f.) belonging to the family Lamiaceae is one of the most well-known timbers of the world. In view of the importance of teak, attempts to raise teak plantations in Kerala were started in 1841. Evaluation of plus trees to prove their breeding value, could not be done so far. The present investigation was carried out to analyze the variability of selected teak clones. The variability of five plus trees of teak in Kerala was analyzed by keeping the clone developed from a standard control plant. Except leaf breadth, all the other growth characters showed statistically significant variation among the accessions. Among the biochemical characters studied, six characters showed statistically significant variation at 1% level. The statistical analysis showed significant difference in the case of most of the characters between the teak clones indicating significant levels of genotypic difference between them. Among the growth characters, highest GCV and PCV, highest heritability and highest genetic advance were shown by clear bole length followed by plant height. This shows that clear bole length is the most important growth character with the highest GCV, PCV, heritability and genetic advance. In all the characters that were statistically significant, PCV was found to be higher than GCV indicating polygenic control of characters and additive gene action. Most of the agronomic characters of the crop plants are polygenic and teak is no exemption. Polygenic characters show different levels of heritability based on their response to environmental factors. High heritability of characters indicates the limited influence of environment on these characters. Genetic advance of characters in percentage of mean is a very effective indicator of the characters that could be utilized in selection programmes. Characters with high genetic advance could be utilized for selection. Therefore the results of this research work mainly leads to the screening of the best clones with great potential for further afforestation programmes.

INTRODUCTION

Teak (*Tectona grandis* Linn. f.) belonging to the family Lamiaceae is one of the most well-known timbers of the world. Teak has been widely used in India for more than 2,000 years. In view of its importance, attempts to raise teak plantation in Kerala were started in 1841. It can be grown in almost every part of the country except the dry western zone, although the best teak forests develop in well drained deep alluvial soils. Mr. Chathu Menon of Malabar in Kerala is considered to be the father of Indian teak plantations (Venkatesh *et al.,* 1986).

Attempts to improve planting stock genetically were made as early as in 1961when Kedharnath and Mathews did the first selection of plus trees of teak. Fifty trees, outstanding in growth and stem form designated as 'plus trees' were selected in different teak growing areas in Kerala using check tree method (Kedharnath and Mathews,1962). With the advent of cloning technology for teak through rooted stem cuttings and micropropagation, it now because feasible to raise clones of the selected plus trees efficiently, without the above

mentioned problems that arise due to graft failure. Clonal hedge gardens (CHGs) and clonal seed orchards (CSOs) can thus be established and through the use of clones, it is expected to overcome the problem of graft failure that is the main reason for lack of success in CSOs in Kerala.

Evaluation of plus trees, to prove their breeding value, could not be done so far. The overall expression (phenotype) of an organism is the sum total of its genetic constitution and the environment. Through progeny trials it is possible to estimate the genetic diversity and heritability for each character and thereby help the breeders to select further breeding strategies. Through these trials, selection of best families also could be done. In this background the present investigation was carried out to study the variability of selected teak clones and their physicochemical characters.

MATERIALS AND METHOD

The present investigation was carried out in Vellikulagara ($10^0$25'05.9" N; $76^0$24'25.4" E) in Thrissur district to study the variability among the teak clones collected from different locations of Kerala state of India (Fig. 1). The clones were prepared from the epicormic buds from the branches of the plus trees on treatment with suitable growth regulating substance. The planting stock was transferred to the planting site and planting was carried out in June 2011. The locations from where the plants were collected experience humid tropical climate and the soils are forest loam with a pH of 5-6. The variability of five plus trees of teak in the traditional teak growing areas of Kerala is analyzed in the present study keeping the clone developed from a standard control plant. The plants were grown in randomized design with three replications and 15 plants per replication. Preliminary growth data were observed after five years in 2017 in relation to plant height, GBH, pruning height, leaf length and leaf breadth. Physiological and biochemical analysis was carried out using leaves of the selected teak clones. Fresh leaves were collected from each clone and biochemical changes were studied. The variations in Chlorophyll content (Arnon, 1949), Relative water content (RCW) (Barrs and Weatherly, 1962), Leaf proline content (Bates *et al*., 1973), Epicuticular wax, Reducing sugar and Total sugar content (Dubios *et al.*, 1956), Proline, Protein, Total free amino acids, Total soluble protein content, total insoluble protein content (Lowry *et al*., 1951) and Total soluble sugar content were studied using standard methods. Details on plus trees and control teak trees from which the clones were obtained is given in Table 1.

Table 1. The descriptions of the plus trees and control trees from which the clones were obtained

Sl. No	Plus tree No.	Forest Division	Tree height (m)	GBH (m)	Clear bole length (m)	Canopy type
1	T_1	Nilambur	46.6	2.68	25.6	One storey stand
2	T7	Konni	43.6	25	26.4	One storey stand
3	T_{36}	Aryankavu	42.7	2.47	22.6	One storey stand
4	T_{47}	Aryankavu	42.7	2.14	26.2	One storey stand

5	T_{16}	Konni	43.6	2.64	26.4	One storey stand
6	Control	Nilambur	38.1	1.87	20.1	One storey stand

Phenotypic and genotypic variances were analysed according to Singh and Choudhary (1985), coefficients of variation by Burton and Devane (1953), heritability (broad sense) by Jain (1982), genetic advance by Abraham (2000) and correlation by Rangaswamy (1995). Analysis of variance (ANOVA) was carried out to test the significance of variations between the accessions. Test of significance was done with reference to standard F table (Fisher and Yates, 1963).

RESULTS AND DISCUSSION

Teak is a very important commercial timber crop. Studies on its genetic improvement to develop genotypes suitable to specific teak areas will result in the development of varieties suitable for different agroclimatic regions. The present study has been designed so as to make a comparative analysis of clonal teak plants developed from five teak plus trees identified from the teak growing areas of Kerala State of India (Table 1) in comparison to the clone developed from a standard control plant. Observations on five growth characters, two physiological characters and ten biochemical characters were made for the purpose (Table 2). Out of the five growth characters studied presently, plant height, girth at breast height (GBH), clear bole height (CBH) and leaf length showed statistically significant variation among the accessions studied whereas leaf breadth showed non-significant variation (Table 3). Among the two physiological characters studied, relative water content showed statistically significant variation and epicuticular wax showed non-significant variation, and among the ten biochemical characters studied, six showed statistically significant variation at 1% level, two at 5% level and two characters were statistically non-significant. The biochemical characters such as reducing sugar, chlorophyll b, total chlorophyll, ash content, soluble protein content and insoluble protein content showed statically significant variation at one percent level and chlorophyll a content and amino acid content showed statically significant variation at five percent level. The biochemical characters such as Proline content and total soluble sugar content showed non-significant variation. (Table 2). The above statistical analysis showed significant difference in the case of most of the characters between the teak clones from different plus trees indicating significant levels of genotypic difference between them.

Table 2. Characters of the teak clones and control studied

Plant No.	T1	T7	T16	T36	T47	Control
Growth characters						
Plant height (cm)**	890.00 ±70.24	505.00 ±22.55	908.33 ±19.65	756.67 ±50.44	890.00 ±65.07	473.33 ±50.53
GBH (cm)**	32.00 ±4.62	19.83 ±0.83	39.00 ±1.73	25.60 ±1.31	33.13 ±0.64	26.30 ±1.46
Clear bole height (cm)**	3.27 ±0.07	1.37 ±0.07	3.60 ±0.29	3.17 ±0.03	3.83 ±0.17	1.19 0.10
Leaf length (cm)**	37.33 ±0.66	37.60 ±1.73	41.03 ±1.54	44.33 ±2.60	39.00 ±2.08	27.67 ±1.45
Leaf breadth (cm) ^{NS}	27.00 ±1.52	23.07 ±3.44	27.83 ±1.53	24.33 ±1.76	24.67 ±2.72	17.33 ±1.45

Physiological characters						
ECW (μ g/cm^2) ^{NS}	0.23 ±0.03	0.21 ±0.09	0.21 ±0.0003	0.20 ±0.04	0.25 ±0.001	0.35 ±0.03
RWC (%)- leaf **	38.19 ±4.14	55.98 ±0.49	27.83 ±3.45	40.78 ±3.56	42.72 ±3.71	37.38 ±1.05
Biochemical characters						
Reducing sugar (g 100 g^{-1} leaf flour)**	0.08 ±0.0030	0.01 ±0.0037	0.09 ±0.0014	0.02 ±0.0023	0.02 ±0.0023	0.06 ±0.0047
Proline(g 100 g^{-1} leaf flour) ^{NS}	0.25 ±0.0059	0.09 ±0.024	0.08 ±0.0015	0.09 ±0.044	0.13 ±0.079	0.07 ±0.060
Total soluble sugar(g 100 g^{-1} leaf flour) ^{NS}	0.06 ±0.0072	0.05 ±0.0049	0.06 ±0.0144	0.04 ±0	0.05 ±0.0093	0.07 ±0.0006
Chlorophyll a (mg g^{-1} fresh tissue)*	1.18 ±0.092	1.08 ±0.209	1.53 ±0.277	0.94 ±0.081	1.90 ±0.205	1.15 ±0.023
Chlorophyll b (mg g^{-1} fresh tissue)**	0.60 ±0.261	0.68 ±0.130	0.97 ±0.060	0.72 ±0.071	1.18 ±0.060	1.33 ±0.017
Chlorophyll total (mg g^{-1} fresh tissue)**	2.16 ±0.036	1.76 ±0.337	2.03 ±0.039	1.66 ±0.148	2.92 ±0.179	2.56 ±0.026
Amino acid (g 100 g^{-1} capsule flour)*	0.05 ±0.033	0.08 ±0.0312	0.26 ±0.094	0.04 ±0.012	0.03 ±0.0098	0.13 ±0.039
Ash content - (g 100 g^{-1} leaf flour)**	94.21 ±0.134	93.19 ±0.136	93.70 ±0.066	94.50 ±0.114	92.44 ±0.473	94.46 ±0.255
Protein soluble (g 100 g^{-1} fresh tissue)**	0.11 ±0.0081	0.13 ±0.031	0.96 ±0.096	0.14 ±0.026	0.45 ±0.315	0.11 ±0.0096
Protein insoluble (g 100 g^{-1} fresh tissue)**	0.14 ±0.046	0.23 ±0.015	0.15 ±0.034	0.17 ±0.020	0.21 ±0.043	0.08 ±0.026

*Significant at 5% ** significant at 1%

Among the growth characters, the highest GCV and PCV, the highest heritability and the highest genetic advance were shown by clear bole length followed by plant height. This shows that clear bole length followed by plant height is the most important growth character with the highest GCV, PCV, heritability and genetic advance (Table 3). This character shows 96.28% of heritability (broad sense). Among the physiological characters studied, the highest GCV, PCV, heritability and genetic advance were shown by epicuticular wax (Table 3).

Table 3. Genotypic variance, phenotypic variance, GCV, PCV, heritability (broad sense) and genetic advance of the characters studied in the case of the teak clones analyzed presently

Characters	GV	PV	GCV	PCV	H^2	GA
Plant height**	37454.34	45033.51	26.25	28.78	83.17	49.30
GBH**	40.79	55.42	21.79	25.40	73.60	38.51
Clear bole height**	1.31	1.36	41.92	42.72	96.28	84.74
Leaf length**	25.44	43.61	13.33	17.45	58.35	20.98
Leaf breadth ^{NS}	9.04	23.65	12.50	20.23	38.22	15.93
ECW ^{NS}	0.00090	0.0078	34.98	37.20	11.55	8.85
RWC**	74.55	103.04	21.33	25.077	72.34	37.37
Reducing sugar**	0.0011	0.0011	69.05	69.93	97.49	140.45
Proline^{NS}	0.0023	0.0086	40.98	78.20	27.46	4.25
Total soluble sugar ^{NS}	0.000078	0.00026	16.60	30.60	29.43	18.55
Chlorophyll a*	0.95	0.18	23.82	33.19	51.57	35.66
Chlorophyll b**	0.071	0.012	29.24	37.98	59.30	46.39

Chlorophyll total**	0.20	0.28	20.65	24.64	70.26	35.66
Amino acid*	0.005	0.012	76.36	111.88	46.58	107.36
Ash content**	0.60	0.78	0.94	1.08	76.46	0.020
Protein soluble**	0.09	0.15	99.41	124.24	64.02	163.85
Protein insoluble**	0.002	0.005	24.24	42.56	32.53	28.52

*Significant at 5% ** significant at 1%

Among the biochemical characters, soluble protein content showed the highest GCV, PCV and genetic advance. This character showed 64.02% of heritability (broad sense) also. The highest heritability was shown by reducing sugar of leaf. In all the characters that were statistically significant, PCV was found to be higher than GCV indicating polygenic control of characters and additive gene action. Differential variability of quantitative characters has been reported by earlier workers in cardamom (Radhakrishnan *et al.*, 2006), rice (Mini, 2006), coffee (Nikhila *et al.*, 2002; Raghu *et al.*, 2003), medicinal plants (Misra *et al.*, 1998; Jayasree *et al.*, 2006) and vanilla (Umamaheswari and Mohanan, 2004). Study of variability of the genetic resources of a crop is the first step towards the understanding of the genetic diversity of the genetic stock so as to use them in crop improvement programmes. Most of the agronomic characters of the crop plants are polygenic and teak is no exemption. Polygenic characters show different levels of heritability based on their response to environmental factors. High heritability of characters indicates the limited influence of environment on these characters. The reason for low heritability in the case of some characters is the influence of environment on them as suggested by earlier workers (Tripathy *et al.*, 2000; Radhakrishnan, 2003).

Genetic advance of characters in percentage of mean is a very effective indicator of the characters that could be utilized in selection programmes. It is a measure derived from heritability. Characters with high genetic advance could be utilized for selection programmes as reported by earlier workers in cardamom (George *et al*., 1981; Radhakrishnan *et al.*, 2006) and other crops (Jayasree *et al.* 2006).

Correlation analysis of the 17 characters under study showed significant positive correlation between several characters. Correlation between heritable characters is due to sharing of common genes between such quantitative characters (Tables 4 and 5). Among the characters under study, plant height showed significant positive correlation with the highest number of characters followed by GBH and clear bole height. Among the biochemical characters chlorophyll a content and soluble protein content showed significant positive correlation with the highest number of characters.It is observed that proline content was negatively correlated with Relative water content. Similar observations were recorded in cocoa also by Balasimba (1982). Characters that show significant positive correlation are interrelated due to sharing of common hereditary factors. This relationship could be exploited to find out the important characters to be considered during selection and other crop improvement programmes. Such approaches have already been utilized in different crops like cardamom, tea and medicinal plants (Radhakrishnan, 2003; Ramasubramanian, 2005 and Raghu, 2005).

CONCLUSION

In the present study, the variability of five plus trees of teak in the traditional teak growing areas of Kerala was analyzed by keeping the clone developed from a standard control plant. Seventeen characters including five growth characters, two physiological characters and ten biochemical characters were studied presently and the data were subjected to statistical analysis. Physiological and biochemical experiments were carried out using leaves of selected teak clones

Out of the five growth characters studied, except leaf breadth, all the other characters showed statistically significant variation among the accessions. Among the two physiological characters studied, relative water content showed statistically significant variation and epicuticular wax showed non-significant variation, and among the ten biochemical characters studied, six showed statistically significant variation at 1% level, two at 5% level and two characters were statistically non-significant. The above statistical analysis showed significant difference in the case of most of the characters between the teak clones from different plus trees indicating significant levels of genotypic difference between them. Among the growth characters, highest GCV and PCV, highest heritability and highest genetic advance were shown by clear bole length followed by plant height. This shows that clear bole length followed by plant height is the most important growth character with the highest GCV, PCV, heritability and genetic advance. Among the physiological characters studied, highest GCV, PCV, heritability and genetic advance were shown by epicuticular wax. Among the biochemical characters, soluble protein content showed the highest GCV, PCV and genetic advance. The highest heritability was shown by ash content of leaf. In all the characters that were statistically significant, PCV was found to be higher than GCV indicating polygenic control of characters and additive gene action. Study of variability of the genetic resources of a crop is the first step towards the understanding of the genetic diversity of the genetic stock so as to use them in crop improvement programmes.

Most of the agronomic characters of the crop plants are polygenic and teak is no exemption. Polygenic characters show different levels of heritability based on their response to environmental factors. Highest heritability of characters indicates the limited influence of environment on these characters. The reason for low heritability in the case of some characters is the influence of environment on them. Genetic advance of characters in percentage of mean is a very effective indicator of the characters that could be utilized in selection programmes. Characters with high genetic advance could be utilized for selection. Therefore the results of this research work mainly leads to the screening of the best clones with great potential for further afforestation programmes. Concurrent improvement in the aforementioned features cumulatively contributes to increased productivity of teak and improved quality of timber for market.

Table 4. Correlation of characters of teak clones studied

	Plant height	GBH	Clear bole height	Leaf length	Leaf breadth	ECW	RWC	Reducing sugar	Proline	Total soluble sugar	Chlorophyll a	Chlorophyll b	Chlorophyll total	Amino acid	Ash content	Protein soluble
Plant height	1															
GBH	0.825246*	1														
Clear bole height	0.978426*	0.768947*	1													
Leaf length	0.626017*	0.225771	0.696561*	1												
Leaf breadth	0.857787*	0.598541*	0.804938*	0.780607*	1											
ECW	-0.5053*	-0.10264	-0.51883	-0.92367*	-0.82503*	1										
RWC	-0.53963*	-0.83544*	-0.49083*	-0.02667	-0.2781	-0.14686	1									
Reducing sugar	0.36589	0.686079*	0.206386	-0.22926	0.290583	0.172004	-0.80013	1								
Proline	0.503847*	0.243046	0.386107	0.060666	0.46097	-0.16125	-0.02977	0.326793	1							
Total soluble sugar	0.030203	0.458295	-0.12354	-0.70557*	-0.14672	0.621423*	-0.51115*	0.801924*	0.297615	1						
Chlorophyll a	0.558137*	0.678431*	0.575391*	0.078361	0.310076	0.034891	-0.2713	0.093375	0.064121	0.250186	1					
Chlorophyll b	-0.19282	0.241938	-0.13674	-0.58071*	-0.57562*	0.779709*	-0.32781	0.071561	-0.46141	0.428923	0.505333*	1				
Chlorophyll total	0.151152	0.377341	0.158866	-0.49949*	-0.27105	0.642205*	-0.19623	0.094022	0.14819	0.525741*	0.737878*	0.772288*	1			
Amino acid	0.080302*	0.527337*	0.00449	-0.09565	0.149749	0.050026	-0.64587	0.671509*	-0.41019	0.46562	0.1669	0.275085	-0.09394	1		
Ash content	-0.22322	-0.1414	-0.26531	-0.18237	-0.24038	0.218621	-0.37462	0.402537	0.02089	0.153642	-0.76696	-0.17119	-0.43322	0.136586	1	
Protein soluble	0.564294*	0.798529*	0.550207*	0.345585	0.539383*	-0.28266	-0.61653	0.432827	-0.26088	0.183906	0.645092*	0.248845	0.116137	0.764993*	-0.34339	1
Protein insoluble	0.152444	-0.218	0.214792	0.584098*	0.41784	-0.68725*	0.656879*	-0.64894	0.007964	-0.65011	0.257166	-0.42308	-0.19897	-0.35738	-0.75886*	0.079902

*Significant at 5%

Table 5. Correlation of characters of teak clones studied- number of characters positively correlated

Sl No.	Characters	Number of characters positively correlated	Characters showing positive correlation
1	Plant height	8	GBH, Clear bole height, Leaf length, Leaf breadth, Proline, Chlorophyll a, Amino acid, Protein soluble.
2	GBH	7	Plant height, Clear bole height, Leaf breadth, Reducing sugar, Chlorophyll a, Amino acid, Protein soluble.
3	Clear bole height	6	Plant height, GBH, Leaf length, leaf breadth, Chlorophyll a, Protein soluble.
4	Leaf length	4	Plant height, Clear bole height, Leaf breadth, Protein insoluble.
5	Leaf breadth	5	Plant height, GBH, Clear bole height, Leaf length, Protein soluble.
6	ECW	3	Total soluble sugar, Chlorophyll b, Total chlorophyll.
7	RWC	1	Protein insoluble
8	Reducing sugar	3	GBH, Total soluble Sugar, Amino acid
9	Proline	1	Plant height
10	Total soluble sugar	3	ECW, Reducing sugar, Total chlorophyll
11	Chlorophyll a	6	Plant height, GBH, Clear bole height, Chlorophyll b, Total chlorophyll, Protein soluble.
12	Chlorophyll b	3	ECW, Chlorophyll a, Total chlorophyll.
13	Total chlorophyll	4	ECW, Total soluble sugar, Chlorophyll a, Chlorophyll b
14	Amino acid	4	Plant height, GBH, Reducing sugar, Protein soluble.
15	Ash content	0	Nil
16	Protein soluble	6	Plant height, GBH, Clear bole height, Leaf breadth, Chlorophyll a, Amino acid
17	Protein insoluble	3	Leaf length, RWC, Ash content

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SOME PROMISING NATIVE RICE CULTIVARS OF WAYANAD REGION OF INDIA

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Abstract: Wayanad district of Kerala state is situated in the southern tip of the Western Ghat region of India. The culture of Wayanad is very much tribal oriented and has the highest tribal population in the state of Kerala. The district is very rich in flora and fauna. More than 35% of the area comes under forest cover. Rice is the principal cereal that is cultivated and consumed by the inhabitants of this agricultural hamlet. The name of the district- Wayanad itself means 'the land of paddy fields'. The tribal people of the district cultivate several native cultivars of rice and they are phenotypically and genotypically diverse. They are adapted to the peninsular agro-ecological situations of the region. Rice cultivars collected from the tribal farmers of the Wayanad region are generally found to be suitable for both wetland and upland conditions. They have been characterized presently based on their morphological characters that are agronomically important. The main objective of the present paper is to study the promising rice cultivars of Wayanad in terms of their morphological and agronomical characters. Among the rice cultivars studied, characters like total number of tillers at the time of harvest and EBT number were found to be the highest in Karimbalan. Jeerakasala, Marathondi, Urunikaima and Vellimuthu equally showed the highest number of leaves per tiller. Thavalakkannan showed the highest number of grains per panicle as well as panicle density. Weight of 100 grains, plant height and panicle length were found to be the highest in Marathondi. Number of primary branches per panicle was found to be the highest in Mahamaya. Spikelets per panicle was found to be the highest in Thavalakkannan and sterility percentage was found to be the highest in Adukkan.

Keywords: Rice, Wayanad, Tribal rice cultivars

INTRODUCTION

Rice is the most widely consumed cereal food for more than 50% of the world's human population (Surekha *et al*., 2016) and it is widely cultivated throughout the world (Zhu *et al.*,2010). After wheat and corn, rice is considered the third most important grain crop in the world (Binod *et al.*, 2010). About 90% of the world's rice is produced and consumed by Asia and the remaining production is from Africa and Latin America (IRRI, 2006). Rice is largely cultivated in subtropical regions, having characteristically warm and humid conditions (Reiter *et al.*, 2010; Lai *et al.*, 2015). Now, it is acclimatized to and naturalized in most of the tropical, subtropical and Mediterranean regions (Abraham *et al.*, 2016). It is grown in a diverse range of environment conditions, such as irrigated uplands, rainfed lowlands and rainfed upland ecosystems (Kaur*etal.*, 2015) and therefore, divergent classifications of rice environments are mainly based on altitude (upland vs. lowland) and water source (irrigated vs.rainfed) (IRRI, 1970). Also, approximately 80% of the rice population is cultivated under flooded conditions (Towprayoon *et al.*, 2005).

Rice, scientifically known as *Oryza sativa* L., belongs to the genus *Oryza* L. of the grass family Graminae (Poaceae) classified under the tribe Oryzeae and subfamily Oryzoideae (Lu, 1999). *Oryza sativa* L. is known as Asian rice. There is one more cultivated species known as *Oryza glaberimma* Steud. and it is known as African rice (Linares, 2002; IRRI, 2006; Molina *et al.*, 2011). The top ten rice producing countries in the world are China, India, Indonesia, Bangladesh, Vietnam, Thailand, Myanmar, Philippines, Brazil and Japan where China and India alone contribute rice to nearly half of the world's human population (FAO, 2014). Normally grown as an annual crop, in tropical regions it can survive as perennial which can perennate through ratoon tiller production (Surekha *et al*., 2016).

India is the region of the greatest diversity of wild rice which might have given rise to very large number of forms of cultivated rice (Roschevicz,, 1931) and it is the second largest producer in the world (FAO, 2014). In Kerala, Wayanad district is known as the land of paddy fields and is situated in the Western Ghats on the eastern portion of North Kerala (Surekha *et al*., 2017). The region is enriched by typical monsoon climate and is located between north latitude $11^{o}27'$ and $15^{o}58'$ and east longitude $70^{o}27'$ and $75^{o}47'$. Wayanad has the highest tribal population in the state of Kerala where agriculture is the major occupation and rice is the major cereal that is cultivated and consumed by the inhabitants of this agricultural hamlet (Mohanan, 2009; Mohanan, 2011). More than 300 varieties of rice including local cultivars and improved varieties are grown in the state of Kerala (Leenakumari, 2004). Several native rice cultivars have been cultivated in Wayanad district of Kerala especially by the tribal inhabitants. The present study is envisaged as an effort to analyse some of the promising native rice cultivars of Wayanad district of Kerala.

MATERIALS AND METHODS

Rice seeds directly collected from tribal farmers of Wayanad were used for the present study. The study was carried out in the experimental net house of the Genetics and Plant Breeding Division, Department of Botany, University of Calicut, ($11^{o}25'$N latitude and $75^{o}45'$E longitude), India in the first crop season of 2016. Ten days old rice seedlings were planted in experimental pots of 20 cm diameter and 10 cm depth filled with soil and farmyard manure in 3:1 ratio used as planting medium, on one plant per pot basis. The plants were maintained under upland conditions to ensure maximum tillering. Sprinkler irrigation was provided once a day on all non rainy days and 1 g of NPK (18:18:18) was applied per plant at 15 day intervals starting from the 30^{th} day of planting till flowering. Observations were made both on the plant characters and yield characters shown in Table 1. The plants were photographed and the details presented in Fig. 1.

Table 1. Plant and yield characters studied

Plant characters	Yield characters
1. Age at tiller initiation (days)	1. Panicle length (cm)
2. Age at flowering (days)	2. Spikelets per panicle
3. Age at maturity (days)	3. Grains per panicle
4. Tiller number at harvest	4. Panicle density
5. Number of leaves per tiller	5. Sterility percentage
6. EBT number	6. Number of primary branches per panicle
7. Plant height (cm)	7. Grain length (cm)
	8. Weight of 100 grains (g)

RESULTS AND DISCUSSION

From Fig. 1, it could be seen that the earliest tiller initiation was in Urunikaima (34 days) followed byAdukkan and Karimbalan (35 days). The late tiller initiating rice cultivar was Kothandan (47 days). The earliest flowering cultivars among the 12 rice cultivars studied were Mahamaya and Thondi (72 days). The late flowering rice cultivar was Kaima (132 days). Total seed to seed duration was the minimum in Mahamaya (101 days) and maximum in Kaima (160 days). Hence the study of the above native rice cultivars of Wayanad showed that they included both medium and long duration types. Maximum number of tillers at the time of harvest was seen in Karimbalan (18) and minimum number of tillers at the time of harvest in Thondi (7). Ear bearing tillers ranged from 7 to 14 with the minimum ear bearing tiller number in Thondi and the maximum in Karimbalan. Number of leaves per tiller varied from 4 to 6. Thavalakkannan with 4 leaves per tiller on the average showed the minimum and Jeerakasala, Marathondi, Urunikaima and Vellimuthu with 6 leaves per tiller showed the maximum. Plant height was found to be the maximum in Marathondi (127.94 cm) among the cultivars studied and the minimum in Mahamaya (77.04 cm).

Eight yield characters were also studied in the twelve rice cultivars collected from the tribal farmers of Wayanad. Panicle length was seen to be the maximum in Marathondi (26.6 cm) and minimum in Kothandan (14.0 cm) respectively. Number of spikelets per panicle ranged from 68 to 150 and it was seen to be the minimum in Kothandan and maximum in Thavalakkannan.

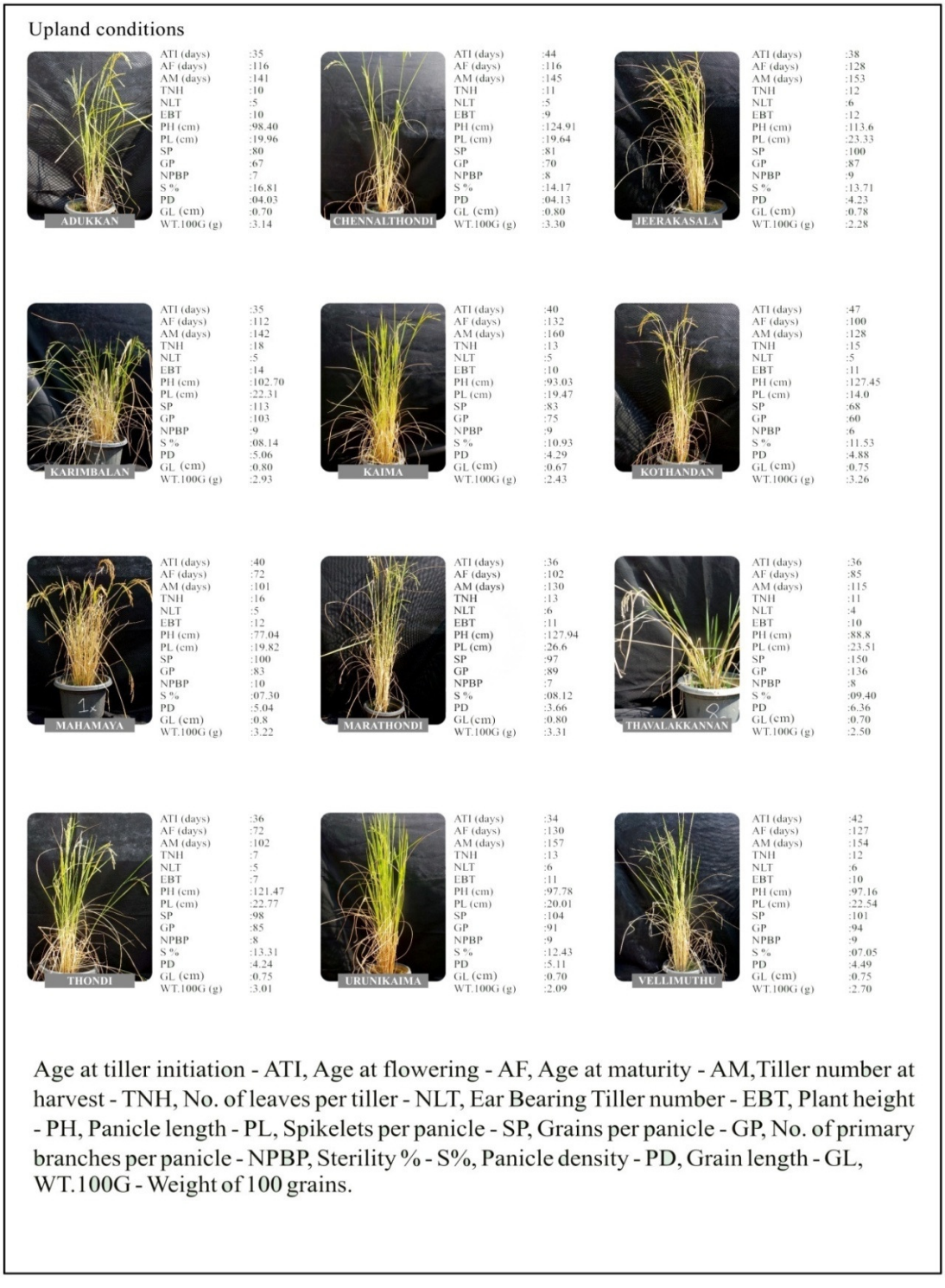
Grains per panicle was seen to be the minimum in Kothandan (60) and the maximum in Thavalakannan (136). Number of primary branches per panicle varied from 6 to 10 with the minimum in Kothandan and maximum in Mahamaya. Sterility percentage was found to be the lowest in Vellimuthu (7.05) and the highest in Adukkan (16.81). Panicle density was the lowest in Marathondi (3.66) and the highest in Thavalakannan (6.36). Minimum grain length was shown by Kaima (0.67 mm) and maximum by Mahamaya (0.85 mm). Weight of 100 grains was found to be the lowest in Urunikaima (2.09 g) and the highest in Marathondi (3.31g).

The present study showed that the native rice cultivars collected from the tribal farmers were good yielding and able to survive in different agroclimatic conditions. Several native rice cultivars are being cultivated in Wayanad especially by the tribal inhabitants and they are phenotypically and genotypically diverse (Surekha *et al*., 2017). The native land races and traditional rice varieties of Kerala are highly adapted to both human and environmental influences. Traditional rices of Kerala differ for a range of characters including crop duration and other growth and yield characters (Leenakumari, 2004).

CONCLUSION

The above study provides a bird's eye view to the diversity of growth and yield characters among the native rice cultivars of Wayanad region of Western Ghats of Kerala. It further indicates that this genetic stock can be conserved and exploited to sustain rice farming in the region and also to ensure the availability of the staple carbohydrate food to the tribal people of the area with minimum investments and technology.

Fig. 1. Plant and yield characters of the native rice cultivars studied.



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VARIATION IN RHIZOME YIELD IN DIFFERENT ACCESSIONS OF *CURCUMA AROMATICA* SALISB. OF KERALA STATE, INDIA

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Abstract: Crop yield is defined as the quantity of economic product of a crop harvested per unit land area. Estimation of the variability among the yield parameters of a crop will help us to identify promising genotypes having high yield. This subsequently leads to the development of high yielding varieties through various plant breeding programmes. In spite of its medicinal and cosmetic properties, *Curcuma aromatica* Salisb. still remains an underutilized species. It comes under the family Zingiberaceae and is popularly known as wild turmeric. There are no recorded studies regarding the yield parameters and crop improvement of this species so far. The present experiment was designed to assess the variability of yield among sixty two accessions of *Curcuma aromatica* collected from different locations of Kerala state of India. A crop of sixty two accessions was raised in randomized block design following standard agronomic practices during the first crop season of 2016-17. The plants were allowed to grow for eight months to reach maturity and harvested simultaneously. Observations on rhizome yield were recorded immediately after harvest. The data were analyzed statistically to assess the variability of yield. Rhizome yield varied from 25 g to 775 g in the case of different accessions. Analysis of variance showed that this variation was statistically significant. Among the different accessions studied, Accession No. CUK-4 showed the highest mean yield of 491.66 g followed by CUK-3, CUK-6, CUK-2 and CUK-1 in that order. This observation indicates the high level of variation in yield in the case of *Curcuma aromatica* populations available in Kerala state thus highlighting the existence of potential genetic variability that could be exploited for the production of superior planting material. Further analysis of this variation in association with other agronomic characters will lead to the selection of superior accessions and release of superior varieties subsequently.

Key words: *Curcuma aromatica*, Wild turmeric, Zingiberaceae, Genetic variability

INTRODUCTION

Curcuma aromatica Salisb. is an underutilized wild aromatic medicinal herb belonging to the family Zingiberaceae (Gamble, 2013). It is commonly called wild turmeric and in south India it is known as *kasturimanjal* or musk turmeric (Sikha *et al*., 2015). The aromatic rhizome is the economically important part and it has camphoraceous odour (Nadkarni, 1998). It is a perennial herb native to southern India. It is distributed across an altitude of 700-2500m amsl in India, Nepal, China and Sri Lanka (Sabu, 2006; Shamim *et al.,* 2006; Pant *et al.,* 2013). In India wild turmeric is cultivated in Kerala and West Bengal (Shamim *et al.,* 2011). When compared to *Curcuma longa, Curcuma aromatica* has a higher level of volatile oil content. The presence of camphene and camphor in the volatile oil is the characteristic feature of *Curcuma aromatica* (Pant *et al.,* 2013). The oil extracted from *Curcuma aromatica* rhizome is a bluish black dark liquid with woody, amber and spicy

characteristic odour (Vaze, 2003). Behura *et al.* (2002) revealed that *Curcuma aromatica* leaves are used as basic ingredients for making perfumes, because of the presence of the significant compounds 1, 8-cineol [28.01%] and linalool [7.67%].

Curcuma aromatica Salisb. constitutes an important drug in Ayurveda and other traditional systems of medicine. The rhizomes are used as tonic, carminative and it is applied extensively to cure bruises, sprains and snake bite injuries (Chopra *et al.,* 1956). The rhizome is used tremendously for scabies and eruption of small pox and is made into paste with benzoin and it is applied for relief from headache (Watt, 1972). Aqueous extracts of the rhizomes are used for curing indigestion, rheumatism and dysentery. The leaves are also used for healing wounds and fractured bones (Santhanam and Nagarajan, 1990; Kumar, 2002; Saleem *et al.,* 2011). Pant *et al.* (2013) has stated that *Curcuma aromatica* is considered as a potent anticancer herb. Early stages of cervical cancer can be treated using the oil extracted from the rhizome (Liu *et al*., 2014).

The rhizome is an esteemed drug for skin care. It is applied externally to the skin to get a peculiar lively tinge to the naturally dark complexion and a delicious fragrance (Watt, 1972). In India it is used as an aromatic medicinal cosmetic especially against pimples (Sikha *et al*., 2015; Anonymous, 2015). In some regions of India, *Curcuma aromatica* is replaced with *Curcuma zanthorrhiza* due to the non-availability of *Curcuma aromatica* (Anjusha and Gangaprasad, 2014). The genetic diversity of this species has not been assessed scientifically so far. Species density is declining due to various anthropogenic activities such as urbanization and industrialization. There are no improved high yielding varieties developed so far and scientific publications on its cultivation practices are limited. The release of high yielding varieties of *Curcuma aromatica* is necessary since it will be beneficial for the large scale production and conservation of this valuable medicinal plant.

MATERIALS AND METHODS

The present experiment was laid out in randomized block design (RBD) in the experimental plot of the Genetics and Plant Breeding Division of Department of Botany, University of Calicut, Kerala, India. Sixty two accessions of *Curcuma aromatica* collected from different locations of Kerala formed the experimental material (Table 1 & Fig. 1).

The experimental material

Curcuma aromatica is a perennial erect herb. The whole plant is about 70-100 cm tall with large greyish-yellow aromatic rhizome which is the economically important part. Root tubers are absent. Leaves are pale green, densely pubescent below, distichous and with lanceolate and acuminate lamina. Inflorescence is a spike 15-30×9 cm and lateral in position. Peduncle is 5-8 cm long and is covered by sheaths. Coma bracts are large, pink and spreading. Fertile bracts are upto 6 cm long, tips re-curved, slightly hairy on upper surface and pale greenish white in colour. Bracteoles are 2 cm long, white and sparsely pubescent. Calyx is short and cylindric. Corolla tubes are funnel shaped and just exceeding the calyx, lobes are unequal, white; dorsal lobes broadly ovate, arching over the anther and hooded where as lateral lobes are narrower and oblong. Labellam is obscurely 3 lobed, orbicular and deep yellow. Lateral staminodes are petalloid, oblong and obtuse and as long as the corolla lobes. Anthers with thecae parallel, each ending in a long sharp spur at the base. Style long and

filiform. Stigma two lobed with a perforation in the centre. Ovules many and axile. Fruit is a 3 valved capsule, tardially dehiscent and globose (Chopra *et al*., 1980; Sabu, 2006; Gamble, 2013; Anjusha and Gangaprasad, 2014; Shikha *et al.,* 2015).

Growth requirements

RBD with 3 replications was used for the experimental programme. A crop of 62 accessions was raised using fresh healthy rhizomes. Seed rhizome fingers each of approximately 3-5 cm length and 25-30 g weight were used as the planting material. The rhizomes were sown in 38 cm × 35 cm poly bags filled with garden soil, sand and cow dung in 3:1:1 ratio before the onset of south west monsoon during the last week of May 2016. Weeding was done as and when required. Plants were irrigated regularly and standard agronomic practices recommended for *Curcuma* (Sabu *et al*., 2011) were adopted. 2 g of N:P:K (18:18:18) was applied to each plant at the end of the first, second and third months.

Observations

The plants were allowed to grow for eight months and harvested simultaneously. Observations on rhizome yield were recorded immediately after harvest. The data were analyzed statistically to assess the variability in yield.

RESULTS AND DISCUSSION

The yield data were analyzed statistically so as to assess its variability and also to identify the high yielding genotypes. Crop yield / agricultural output is the measurement of the quantity of a crop harvested per unit land area (Anonymous, 2015). Rhizome yield per plant varied from 25 g to 775 g in the case of the different accessions of *Curcuma aromatica* studied presently. Analysis of variance showed that this variation was statistically significant (Table 1). Among the different accessions studied, Accession No. CUK 4 showed the highest yield followed by CUK 3, CUK 6, CUK 2 and CUK 1 in that order (Table 1 & Fig. 2). In the case of the best five accessions yield varied from 150g to 775g. CUK 58 showed the lowest yield among the different accessions. This observation indicates the high level of variation in yield in the case of the *Curcuma aromatica* populations available in Kerala, thus highlighting the existence of potential genetic variability which could be exploited effectively for the commercial exploitation of the same through selection, so that better planting material is made available for the organized farming of the species. Further analysis of this variation in association with other agronomic characters will lead to the selection of superior accessions and release of superior varieties subsequently.

Table 1. Variation of yield in the case of the *Curcuma aromatica* Salisb. accessions studied

Accession number	Location	Mean ± SE (g)	Range (g)	Rank	CD
CUK 1	Nellikunnu, Thrissur	380.55 ±63.6	225-575	5	71.35
CUK 2	Kottakkal, Malappuram	381.94 ±93.53	150-650	4	
CUK 3	Peruvannamuzhi, Kozhikode	422.22 ±31.34	250-675	2	
CUK 4	Puliyilapara, Thrissur	491.66 ±50.28	200-775	1	

CUK 5	Kumali, Idukki	311.10 ±39.23	150-525	9	
CUK 6	Pokalapara, Thrissur	405.55 ±5.55	325-500	3	
CUK 7	Muthanga, Wayanad	249.99 ±4.81	175-350	15	
CUK 8	Kurishupara, Idukki	183.33 ±9.63	125-250	26	
CUK 9	Villunniyal, Malappuram	272.21 ±53.05	75-375	11	
CUK 10	Audit 1, Idukki	302.77 ±32.78	200-400	10	
CUK 11	Kundalamtheru, Kozhikode	313.88 ±41.52	225-500	8	
CUK 12	Mukkam, Kozhikode	341.64 ±38.25	175-475	6	
CUK 13	Thoppipala, Idukki	169.44 ±10.02	100-250	30	
CUK 14	West Manasseri, Kozhikode	252.77 ±7.35	175-375	14	
CUK 15	Meenkunnam, Ernakulam	199.99 ±4.81	100-350	22	
CUK 16	Dhottapankulam, Wayanad	197.22 ±29.03	125-300	23	
CUK 17	Vetilapara, Thrissur	180.55 ±27.38	100-375	27	
CUK 18	Cheruthoni dam top, Idukki	147.22 ±21.71	100-325	36	
CUK 19	Maradi, Ernakulam	252.77 ±29.43	150-350	14	
CUK 20	Anakkayam, Thrissur	338.88 ±30.59	200-550	7	
CUK 21	Odakkali, Ernakulam	191.66 ±17.36	75-475	24	
CUK 22	Thankamani, Idukki	149.99 ±9.63	100-200	35	
CUK 23	Vazhara Waterfalls, Idukki	184.72 ±25.52	125-250	25	
CUK 24	Sholayar upper dam, Thrissur	202.77 ±15.47	150-250	21	
CUK 25	Vazhachal, Thrissur	241.66 ±12.73	175-300	16	
CUK 26	Narakakkanam, Idukki	266.66 ±16.68	200-325	12	
CUK 27	Mariyapuram, Idukki	127.77 ±2.77	100-175	43	
CUK 28	Kalkkandi, Palakkad	137.49 ±26.49	75-200	39	
CUK 29	Palakkal, Kozhikode	169.44 ±7.35	100-200	30	
CUK 30	Pambla, Idukki	136.11 ±7.35	75-225	40	
CUK 31	Mukkali, Palakkad	169.44 ±12.12	75-250	30	
CUK 32	3rd mile, Kumali, Idukki	183.33 ±16.68	125-250	26	
CUK 33	Vazhikadav, Malappuram	258.33 ±14.45	125-425	13	
CUK 34	Kunduthode, Malappuram	174.99 ±33.71	100-250	28	
CUK 35	Imangalam, Wayanad	211.10 ±15.47	150-375	20	
CUK 36	Kukupadi, Palakkad	123.60 ±9.11	100-200	45	

CUK 37	Nadukani churam, Malappuram	152.77 ±18.23	100-200	34	
CUK 38	Kuppadi, Wayanad	199.99 ±4.81	100-300	22	
CUK 39	Koravankandi, Palakkad	133.32 ±8.34	100-200	41	
CUK 40	Thavalam, Palakkad	216.66 ±12.73	125-300	19	
CUK 41	Mannuthi, Thrissur	233.33 ±12.73	125-325	18	
CUK 42	Kangazha, Kottayam	238.88 ±14.71	200-325	17	
CUK 43	Tholpetti, Wayanad	258.33 ±9.63	125-300	13	
CUK 44	Kalpeta, Wayanad	183.33 ±17.36	125-250	26	
CUK 45	Thookupara, Idukki	172.22 ±36.46	100-275	29	
CUK 46	Chambakkara, Kottayam	111.10 ±12.12	75-175	47	
CUK 47	Mailamood, Kollam	105.55 ±10.2	75-125	48	
CUK 48	Palaruvi, Kottayam	163.88±22.76	100-250	31	
CUK 49	Chippanchira, Thiruvananthapuram	130.55 ±18.23	75-225	42	
CUK 50	Kulathupuzha, Kollam	97.22 ±7.35	50-150	49	
CUK 51	Madakkathara, Thiruvananthapuram	144.44 ±22.76	50-250	38	
CUK 52	West Maradi, Ernakulam	147.21 ±5.56	100-225	37	
CUK 53	Mannur, Ernakulam	116.66 ±9.63	75-150	46	
CUK 54	Poothankuti, Ernakulam	159.72 ±21.85	50-200	32	
CUK 55	Manjikadu, Ernakulam	130.55 ±12.12	75-175	42	
CUK 56	Matnampadi, Kottayam	123.61 ±6.05	75-175	44	
CUK 57	Poovakulath, Kottayam	158.33 ±12.73	125-250	33	
CUK 58	Mangalasseri, Kannur	86.10 ±22.76	25-150	50	
CUK 59	Edathwa, Alappuzha	211.10 ±14.71	100-325	20	
CUK 60	Thuruthikkad, Pathanamthitta	105.55 ±19.46	50-150	48	
CUK 61	Vazhoor, Kottayam	105.55 ±16.91	50-175	48	
CUK 62	Pallikathod, Kottayam	158.33 ±26.81	100-225	33	
Overall range		25-775			

Similar works have been carried out in different crops by earlier workers. Jayasree and co-workers (Jayasree *et al*., 2012a; Jayasree *et al*., 2012b) have reported variability studies in *Curcuma amada* Roxb. and *Kaempferia galanga* L. and have stressed the importance of studies on yield variability in the crops. Radhakrishnan *et al*., (2004) and Radhakrishnan *et al*., (2005) have reported the variability and performance of cardamom genotypes and observed the importance of yield per plant and other yield related traits in

cardamom improvement. Such an approach can be adopted in the case of *Curcuma aromatica* also to conduct detailed studies that may lead to useful information on the genetic structure, variability and scope for improvement in the species.

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A STUDY ON THE SEGREGATION OF THE PROGENIES OF A DWARF CxR (*COFFEA CONGENESIS* X *COFFEA CANEPHORA*) COFFEE PLANT IN RELATION TO HYPOCOTYL LENGTH

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Abstract: Among the sibmated progenies of CxR coffee, a dwarf plant with desirable characters like small leaves, short internodes, and bold fruits was noticed. Selection for dwarfness is possible in coffee at the nursery stage since in the field, it is not recommended to carry out selection of dwarf and tall varieties in mixed stands because of unequal competition that results between tall and dwarf plants. The seedlings of the above dwarf CxR segregated as tall, dwarf and intermediate based on their size. Observations on the progenies of CxR dwarf had indicated that the performance of intermediate plants was better compared to dwarf plants. Hence, the present study was proposed to identify the intermediate type of plants that were desirable at the nursery level. The study was carried out in the nursery set up at RCRS, Chundale, Wayanad, Kerala.

Key words: Coffee, CxR, 3R, Sibmating, dwarf, Intermediate, Segregation.

INTRODUCTION

Coffee is a non-alcoholic stimulant drink with a bitter, acidic flavour prepared from the roasted seeds of coffee plant. Today, coffee is the third most popular drink in the world, behind water and tea (Pendergrast, 2009). The chemistry of coffee flavour and aroma is complex since a large number of chemicals are present in this stimulant crop. Chlorogenic acids and polyphenols are some of them (Wilson, 1999). Coffee breeding programmes are mainly aimed on high yield, good quality and pest/disease resistance. Other objectives are adaptation to local environment and inherent stamina. So as to get consistent yield, tree habit with short internodes for increased cropping area and ease of harvesting and suitable branching habit for the ease of pruning and harvesting, precocious bearing which is an advantage provided it does not lead to exhaustion, even ripening so as to reduce the number of pickings, high pulping and hulling out-turn with low proportion of pulp and parchment and high proportion of clean coffee per sample and resistance to pest and disease is desirable (Wrigley, 1988).

A major proportion of research that led to improvement of coffee plants has been carried out on the tetrapolid species (2n=44) *Coffea arabica,* whose product is of higher quality than that of the diploid species (2n=22) *Coffea canephora* and therefore commands a higher price (Willson, 1999). In India, improvement of coffee with regard to both these species is important, as both are extensively cultivated. *Coffea canephora* is popularly known as robusta coffee due to its robust growth with light green and large leaves compared to *Coffea arabica*. This species contains more caffeine, ranging from 1.5% to 3.8% but is more neutral in cup quality (Wellman, 1961).

Robusta coffee selection Sln. 3R (CxR) is a hybrid variety of coffee developed through interspecific hybridization of *robusta* and *congensis* type of coffee. *Coffea congensis* is a diploid species native to Congo in Africa, showing compact bush size, better quality and lower caffeine content than robusta. It is a type of tree coffee resembling *arabica* and it grows well in low lands that may flood every year. Fruits are red and sweet fleshed. It can hybridize readily with *Coffea canephora*.

CxR coffee was evolved in India with a view of improving the quality characteristics of *C.canephora* (robusta). Sibmating was effected in CxR for further improvement. Among the sibmated progenies a dwarf plant with desirable qualities like small leaves, short internodes, and bold fruits was noticed. Dwarf plants have the advantage of having more number of plants per unit area coupled with resistance to rust (Sreenivasan, 1989). The variations on weight of the fruits and germination percentage of dwarf and normal CxR were non-significant and the dwarf CxR plant had desirable characters like small leaves, short inter nodes and bold fruits (Sureshkumar *et al*., 1999).

CxR is self-sterile and cross-pollinates easily. Hence, these plants give more segregating progenies. The seedlings of dwarf CxR segregated as tall, dwarf and intermediate based on their size. Observations on the progenies of CxR dwarf have indicated that the performance of intermediate plants is better compared to dwarf plants. Dwarf plants are characterized by biennial bearing. Tall plants are normal plants with bigger bush size. Hence intermediate plants are preferred by growers as those show consistent yield and lesser bush size compared to robustoid plants. As the seedling progenies of dwarf CxR are segregated into dwarf, intermediate and robustoid types, it would be advantageous if these intermediate plants were identified at nursery level itself to get uniform plants in the population. Hence, the present study was carried out to identify the intermediate type of plants that were desirable at the nursery level.

MATERIALS AND METHODS

The present study mainly focused on the segregation showed by the seedling progeny of the dwarf CxR plant at the cotyledon stage and at one year of age. The work was carried out in the nursery of Regional Coffee Research Station, Chundale, Wayanad, Kerala. The seedlings of the CxR dwarf were collected from the bulk in the germination beds. They were then sorted into three groups of 100 progenies each, based on their hypocotyl length. The data on stem length and root length of each of these plants were collected as detailed in Table 1. Initial observation to sort the seedlings at the cotyledon stage based on the stem length was taken before transplanting to polybags. The stem length was recorded by measuring the length between the collar and the cotyledon. The root length was calculated by measuring the distance between the root tip and collar.

Table 1. Categorization of seedlings based on hypocotyl length

Category	Tall	Intermediate	Dwarf
Stem length	Above 60 mm	50-60 mm	Below 50 mm
Number of plants selected	100	100	100

After the seedlings were grown to 7-8 leaf pair stage, observations on stem height, stem girth, number of nodes, leaf length, leaf breadth and leaf area were recorded to study the correlation between the stem height at cotyledon stage and segregation pattern of seedlings into dwarf, tall and intermediate. Stem length was calculated by measuring the length from the ground level and the point of initiation of young leaves. Three pairs of mature leaves, *viz.* one middle and two from ends were selected. Length and breadth of the each leaves were taken and average was calculated. Leaf area was calculated using the following formula (Awathramani and Gopalakrishnan, 1965).

$$Y=K \times L \times B$$

(Where Y= Leaf area L= leaf length, B= maximum leaf width, K= 0.65, the conversion factor)

Stem girth was measured as diameter of the main stem in millimetre, taken about 1cm above ground level using vernier calipers and Number of nodes of each seedling were counted and recorded. The trial was laid out in completely randomized design considering the three types of plants *viz.*, dwarf type, intermediate type and tall type plants as treatments with six replications in each treatments. Data were subjected to statistical analysis using the method suggested by Fischer and Yates (1963).

RESULTS AND DISCUSSION

Open pollinated progeny of the CxR dwarf is found to be highly segregating to dwarf types, intermediate types and robustoid types. Dwarf types of plants are characterized by biennial bearing nature. Robustoid types have larger bushes. Intermediates are semidwarf plants with good yield. Intermediate plants were found to be desirable, but true to type of plants were not available from seedling progenies. Hence seeds of dwarf cannot be distributed to growers. In dwarf CxR progenies the toppy stage seedlings were classified based on stem height and planted separately to see the nature of seedlings. Toppy stage seedlings were sorted into 60 mm, 50-60 mm and less than 50 mm catagories and planted to see whether the plants segregated into dwarf, intermediate, normal proportionate to the height of cotyledon. When the seedlings attained around seven to eight leaf pair stage, observations on stem height, stem girth, number of nodes, leaf length, leaf breadth and leaf area in each treatment of 20 plants with six replications totaling to 120 plants were recorded separately to compare the nature of seedlings and to study the segregation pattern. Data were collected from around 120 plants of each, normal (tall), dwarf and intermediate progenies (Table 2). Mean data from each group were subjected to statistical analysis.

Table 2. Growth characters of different types of plants among seedling progenies of CxR dwarf

Character/ Plant type	R1	R2	R3	R4	R5	R6	Mean
1. Plant height (cm)							
Tall	34.67	36.43	35.44	36.23	35.03	34.01	**35.30**
Intermediate	26.58	31.38	31.83	31.79	33.66	33.95	**31.53**

Dwarf	27.85	33.29	30.80	32.86	28.30	30.83	**30.66**
2. Stem girth (cm)							
Tall	4.63	4.76	4.49	4.85	4.91	4.10	**4.62**
Intermediate	4.09	4.61	4.45	4.48	4.52	4.43	**4.43**
Dwarf	4.01	4.41	4.19	4.72	3.92	4.65	**4.32**
3. Number of nodes per plant							
Tall	7.63	7.53	8.11	7.53	7.63	7.99	**7.74**
Intermediate	6.67	7.33	7.72	7.44	7.30	7.80	**7.38**
Dwarf	7.09	7.55	7.36	7.27	7.73	7.20	**7.37**
4. Leaf length (cm)							
Tall	15.08	15.21	14.49	16.21	15.17	11.81	**14.66**
Intermediate	13.87	13.53	14.31	14.61	15.60	15.09	**14.50**
Dwarf	13.64	14.72	14.76	15.98	12.98	14.75	**14.47**
5. Leaf breadth							
Tall	6.67	6.63	6.36	7.17	6.72	5.08	**6.44**
Intermediate	5.86	6.00	6.28	6.31	6.68	6.64	**6.29**
Dwarf	5.68	6.24	6.68	6.63	5.28	6.19	**6.12**
6. Leaf area							
Tall	66.12	66.49	61.59	76.05	67.17	45.08	**63.75**
Intermediate	55.64	54.21	60.33	60.65	68.87	65.66	**60.90**
Dwarf	51.30	62.36	65.40	70.73	48.10	60.86	**59.79**

Data on growth parameters *viz.* stem height, stem girth, number of nodes, leaf length, leaf breadth and leaf area were recorded from the seedlings classified based on the hypocotyl length *viz.* 60 mm , 50-60 mm and less than 50 mm. Data were subjected to statistical analysis and results were presented in Table 2. Data indicated that height of the seedlings vary significantly. Height of the seedlings raised from smaller cotyledons (less than 50 mm) was 30.66 cm, in intermediate it was 31.53 cm and in normal seedlings it was 35.30 cm and it varied significantly. Stem girth varied from 4.32 to 4.62 mm and no significant variation was observed with regard to stem girth. Mean number of nodes varied from 7.37 to 7.74 and variation was not significant. Size of leaf was also recorded. Leaf length ranged from 14.47 cm to 14.66 cm, leaf breadth ranged from 6.12 cm to 6.44 cm. Leaf area was computed by multiplying leaf length x leaf breadth x 0.65 (conversion factor). Leaf area ranged from 59.79 cm^2 to 63.75 cm^2. No significant variation with regard to leaf length, leaf breadth or leaf area was recorded among dwarf/ intermediate/ normal type of plants.

Generally, dwarfness is expressed by shorter internodes and smaller leaf size. But in these progenies no significant variation with regard to number of nodes or leaf size were

recorded. This clearly indicates that progenies of dwarf CxR cannot be segregated based on cotyledon length. Hence it is proposed to select the intermediate plants at one year and propagate them by vegetative propagation or tissue culture methods for mass multiplication and distribution to the growers.

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EVALUATION OF GENETIC DIVERSITY OF *CINNAMOMUM VERUM* J. PRESL IN SOUTH INDIA

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Abstract: *Cinnamomum verum* J. Presl (Syn. *C. zeylanicum* Blume), belonging to the family Lauraceae, is one of the important spice crops widely used for culinary purposes world over. True cinnamon (Ceylon cinnamon) is the dried inner bark of *Cinnamomum verum* J. Presl (syn. *C. zeylanicum* Blume; Family: Lauraceae), native to Sri Lanka. However, there exists an ambiguity regarding the authenticity of true cinnamon in the market. The barks of *C. cassia* (Nees & T. Nees) J. Presl (Chinese cinnamon) from South East China, *C. loureiroi* Nees (Saigon cassia) from Vietnam, *C. burmannii* Nees & T. Nees (Indonesian cinnamon) from South East Asia, *C. tamala* (Dalchini) from North India and *C. malabathrum* (wild cinnamon) from south India are also traded as cinnamon in various parts of the world. Also wide variation has been reported among *Cinnamomum verum* accessions collected from different parts of the world. The present study evaluates the genetic diversity of *Cinnamomum verum* collected from different regions of south India. Genetic diversity study was carried out using four polymorphic ISSR markers. *C. verum* contains high amount of polyphenols and polysaccharides, and pure DNA from the leaf samples was obtained by the addition of PVP in extraction buffer of QIAGEN, DNAeasy plant mini kit, to obtain an optimum yield of average 84.7 ng/µl DNA having A260/A280 ratio 1.91. Four ISSR primers (primer nos. 46,816,824 and 856) that generated polymorphic bands were used for analyzing the genetic diversity. The selected primers produced good, reliable, repetitive and distinct bands which enabled effective scoring for genetic diversity study within the populations. Number of alleles (na), number of polymorphic loci and percentage of polymorphism (p%) were estimated using software POPGEN *ver* 1.31. Within the 12 studied accessions, the number of observed alleles (na) ranged from 1.25 in accession collected from JNTBGRI to 2.00 in KAU, Pechiparai and Yercaud accessions. Percentage polymorphism of 100% and high gene diversity (h) were observed in samples collected from KAU and Yercaud Horticulture Research Station. Nei's genetic distance was used to reveal the genetic differentiation between the individuals from the different regions analyzed in the study. The highest genetic identity value and genetic distance (0.990, 0.0101) were observed among *Sugandhini* and *Nithyasree* varieties, whereas the lowest values (0.6897, 0.3714) were observed between KAU, Thrissur sample and CoA,Vellayani sample. A dendrogram constructed based on the genetic distance also showed high divergence among all the collected samples, exhibiting high intra specific genetic diversity. The results suggest the existence of variation within the studied cinnamon accessions and these variations in the population's gene pool allow them to adapt to the changing environmental conditions and habitats and human interventions.

Key words: *Cinnamomum verum,* Genetic diversity, ISSR markers

INTRODUCTION

Many of the flavours used by humans to season food are yielded by spice crops. As a spice, cinnamon stands first as a commercially essential commodity and it is one of the finest sweet flavours indigenous to Sri Lanka. In India, this crop was introduced by the spice traders. Earlier studies on diversity of *Cinnamomum verum* in North and Central India have shown an extreme degree of variability with respect to growth habitat. Reports on wild distribution of cinnamon in South India are scarce and it is believed that the plant is naturalized in South India and no extensive study is so far available for this species in this region.

Genetic diversity is the basis for development of elite varieties with desirable characteristics (Govindaraj *et al*., 2015). In recent years, limitations of morphological and biochemical markers has been overcome by molecular markers. Molecular markers offer consistent results despite the prevailing environmental circumstances. During the last decade, several novel DNA markers (RAPD, RFLP, SSR, ISSR, etc.) have been rapidly integrated into the molecular tools available for genome analysis (Salimath *et al*., 1995). DNA bands are treated as unit characters and their presence or absence in the amplicon may be used to study genetic relationship (Sang and Soren, 2000) and inter and intra-specific genetic variations.

Some molecular studies have been conducted to evaluate genetic differences in *Cinnamomum* species (Joy and Maridass, 2008, Sandigawad and Patil, 2011; Kuo *et al.*, 2010). A work was carried out to identify *Cinnamomum* species using reliable approaches like RAPD and SRAP techniques. Some primers gave highly polymorphic banding patterns using these techniques. The study showed that using these molecular markers, it was possible to identify the *Cinnamomum* species (genus specific and species specific) and intra-species variations (Abeysinghe *et al*., 2014).

RAPD-PCR analysis involving 11 decamer random primers was used to assess the genetic variation within *C. zeylanicum* in Western Ghats of southern India. Some primers showed appreciable intra-species variation or molecular polymorphism at amplicon levels. Despite morphological similarity, a great deal of genetic polymorphism was observed among the accessions. Unweighed pair group method with arithmetic averages (UPGMA) analysis showed up to 89% genetic variation among accessions, which was further supported by principal co-ordinate analysis (Sandigawad and Patil, 2011).

A study suggests that, different species of *Cinnamomum* are rich in polysaccharides and secondary metabolites, which hinder the process of DNA extraction. High quality DNA is the prerequisite for any molecular biology study. A modified method for high quality and quantity of DNA extraction from both lyophilized and non-lyophilized leaf samples was reported from different woody species and 4 *Cinnamomum* species. The method differs from normal CTAB procedure by the addition of higher concentration of salt and activated charcoal to remove the polysaccharides and polyphenols. The extracted DNA showed perfect amplification when subjected to RAPD, restriction digestion and amplification with DNA barcoding primers. Therefore, this protocol has been validated in different species of plants containing high levels of polyphenols and polysaccharides and can be applied for any plant molecular biology study (Bhau *et al*., 2015).

Among the widely used markers, inter simple sequence repeat (ISSR) marker is a PCR based molecular marker in which a DNA region situated between two similar microsatellite motifs aligned in opposite directions get amplified (Mohammad *et al*., 2015). Knowledge on DNA sequence of the study organism is not needed for ISSR marker study and can be undertaken for any plant species (Prakashkumar *et al*., 2015). The present study reports the diversity study of *Cinnamomum verum* in South India through ISSR mediated molecular evaluation.

MATERIALS AND METHODS

Collection of the plant material

Tender leaves of *Cinnamomum verum* were collected from various locations of South India and JNTBGRI, Palode.

Isolation of genomic DNA from leaf samples of *Cinnamomum verum*

100 mg of tender leaves was disrupted in the presence of liquid nitrogen using a mortar and pestle. 20 mg of polyvinyl pyrrolidone (PVP) was added while grinding to reduce polyphenol contamination. After that 400 µL of AP1 buffer and 4 µL of RNase enzyme were added to it. The lysate was incubated for 10 minutes at 65^0C in a pre-set water bath. Added 130 µL of P3 buffer and incubated on ice for 5 minutes. The lysate was centrifuged for 5 minutes at 20000 g (14000 rpm) and transferred in to a QlA shredder spin column placed in a 2 ml collection tube and again centrifuged for 20000 g.The flow-through was pipetted in to a new tube without disturbing the pellet present. Using a pipette, 1.5 times the volumes of AW1 buffer was mixed to it. From that, 650 µl of mixture was transferred in to a DNeasy mini spin column placed in a 2 ml collection tube and centrifuged for 1 minute at 6000 g. Repeated the same step with the remaining sample and flow through was discarded. Again, 500µl of AW2 buffer was added to the spin column placed in a collection tube and centrifuged at 20000 g. Spin column was placed in a new 1.5 ml micro centrifuge tube and added 100 µl of AE for elution. Then it was incubated at room temperature for 5 minutes and centrifuged for 1 minute and the tubes were stored at -20^0C.

Gel electrophoresis

0.8% gel was used for electrophoresis. 0.8 g of agarose was weighed and dissolved in 100 ml 1X TBE buffer and casted in a gel tray after adding 5 µL EtBr. 5 µL of each sample was mixed with 2 µL of tracking dye and loaded in an electrophoresis unit. Voltage was set and kept undisturbed for 1 hour and DNA was visualized using Gel Documentation System (UVP).

PCR amplification

Amplification of genomic DNA was performed with ISSR primers (Table 1). Each reaction was performed in 25 µL reaction volume, which contained buffer, dNTps, distilled water, primer, DNA polymerase and DNA (Table 2). After mixing the PCR components, the reaction was carried out in an Applied Biosystems Thermal Cycler using the conditions mentioned in Table 3.

Table 1. Details of ISSR primers used to analyze genetic diversity in 12 Cinnamon accessions

Sl. No	ISSR Primer	Ta (^{0}C)
1	46	47.6
2	816	52.6
3	824	55.0
4	856	55.0

Table 2. Reaction mixture

Sl. No	Reagents	Volume (µL)
1	Distilled water	10.59
2	Buffer	1.25
3	dNTps	0.03
4	Primer	0.07
5	DNA Polymerase	0.05
6	DNA	0.50

Table 3. Reaction conditions

Sl. No	Stages	Temperature (^{o}C)	Time
1	Denaturation	94^{o}C	30 s
2	Annealing	52^{o}C	1 min
3	Elongation	72^{o}C	2 min

The PCR conditions above mentioned were repeated for 30 cycles to produce the amplified PCR products. The amplified fragments were electrophoresed in 1.4% agarose gel. Then the gel was documented using a gel documentation system (UVP).

Data analysis and dendrogram construction

Data from the ISSR marker analysis were scored for presence (1) and absence (0) of bands. Faint and unclear bands were not counted. The software POPGEN *ver* 1.31 was used to measure the following parameters: observed number of alleles (na), effective number of polymorphic loci and percentage of polymorphism (p%). A dendrogram was constructed based on the genetic distance.

RESULTS AND DISCUSSION

Pure DNA is required for genetical study. As *Cinnamomum verum* contains high amount of polyphenols and polysaccharides, it is hard to obtain pure DNA from leaf samples. Polyvinyl pyrrolidone (PVP) is an important agent to remove the polyphenols by forming complex hydrogen bonding with polyphenols and efficiently separating it from DNA (Kit and Chandran, 2010). In the extraction buffer of QIAGEN, DNAeasy plant mini kit, all the components were kept constant and PVP concentration was changed to see its effect on the extracted DNA. In this experiment, 8 mg, 16 mg and 20 mg PVP were used respectively. Different concentrations of PVP were used previously for plants having high content of secondary metabolites like polyphenols and polysaccharides (Khanuja *et al*. 1999). In the present investigation, addition of 20 mg PVP yielded the optimum quality and quantity of

DNA. Approximately 84.7 ng/µl DNA having A260/A280 ratio 1.91 was obtained when 20 mg PVP was used. Likewise 16mg PVP produced 55.7 ng/ µl and A260/A280 ratio 1.50 and very less when 8 mg was used. However the samples have shown poor banding pattern in 0.8% agarose gel. Four ISSR primers (primer no; 46,816,824 and 856) that generated polymorphic bands were used for genetic diversity analysis of the twelve accessions of cinnamon. The experimental results are presented in Figs. 1 to 5 and Table 4.

Fig 1. Genomic DNA isolated from plant leaves (CVL1 toCVL12) resolved under 0.8% agarose gel

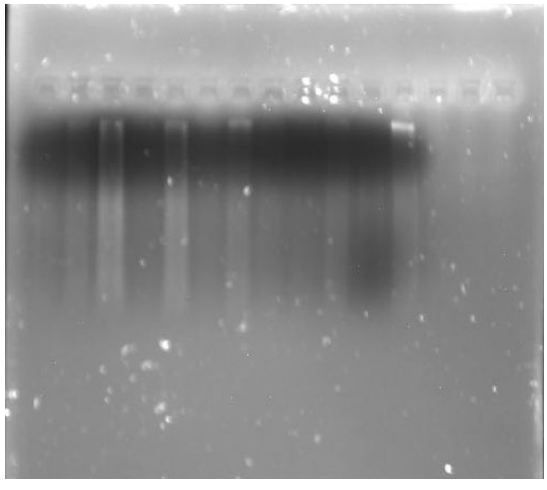


Fig 2. Amplified products of ISSR Primer No.46 resolved under 1.4% agarose gel

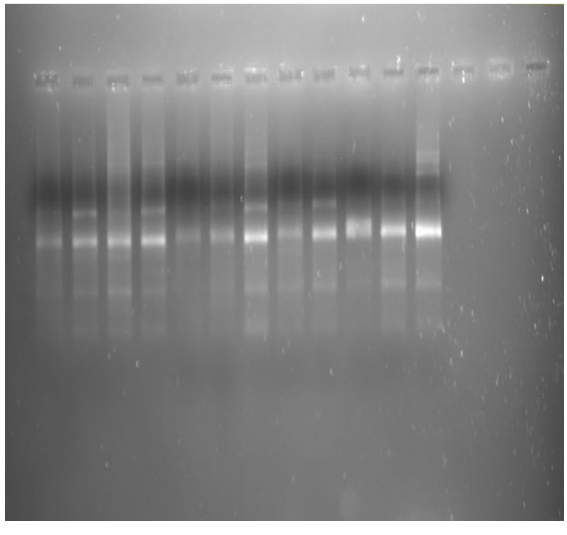


Fig.3. Amplified products of ISSR Primer No.816 resolved under1.4% agarose gel

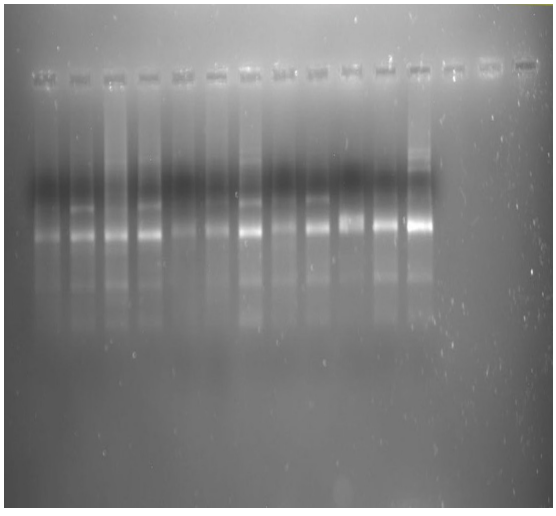
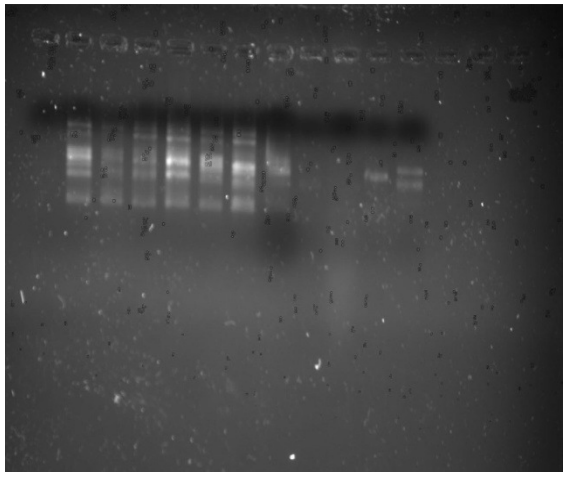


Fig. 4. Amplified products of ISSR Primer No.824 resolved under1.4% agarose gel



The selected primers showed good, reliable, repetitive and distinct bands, which enabled effective scoring for genetic diversity study within the populations. Four primers generated distinct bands and polymorphism was shown by all the samples. The results showed that within the 12 studied accessions, the number of observed alleles (na) ranged from 1.25 in CVL1 to 2.00 in CVL2, CVL7 and CVL16. Based on the (h) value, CVL1 showed a slightly lower genetic diversity, whereas results for the genetic diversity in CVL2 and CVL16 were convergent. A previous study conducted on the screening of the genetic relationships in *Cinnamomum zeylanicum* using RAPD marker showed polymorphism of 89% (Sandigawad *et al.*, 2011) in the selected individuals. In this study the highest percentage of polymorphism (100%) and gene diversity were observed in CVL2 and CVL16 collected from KAU and Yercaud Horticulture Research Station respectively. The lowest values were observed in

CVL1 collected from JNTBGRI. So, the samples CVL2 and CVL16 could be considered to possess higher genetic variation as compared to the other accessions.

Fig. 5. Amplified products of ISSR Primer No.856 resolved under1.4% agarose gel

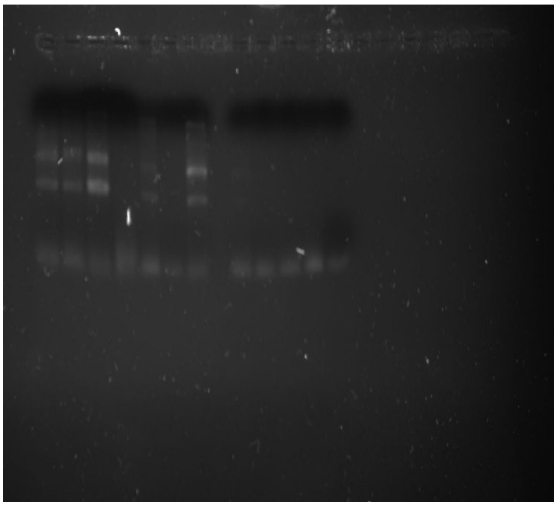


Fig. 6. Dendrogram showing the genetic relationship between twelve studied *C. verum* accessions

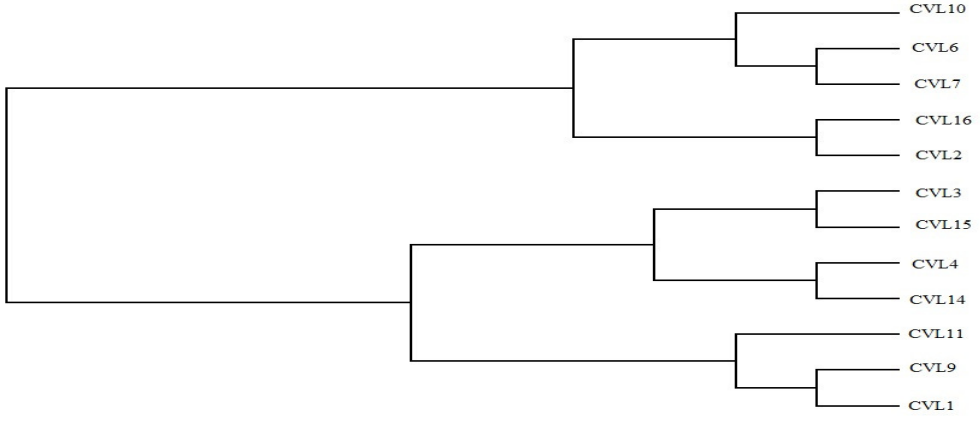


Table 4. Analysis of genetic polymorphism obtained with ISSR primers in different *C. verum* accessions

Sl. No.	Sample code	na	h	No. of polymorphic loci	*P* (%)
1	CVL1	1.25	0.06	1	25.00%
2	CVL2	2.00	0.41	4	100.00%
3	CVL3	1.50	0.22	2	50.00%
4	CVL4	1.75	0.27	3	75.00%
5	CVL6	1.75	0.24	3	75.00%
6	CVL7	2.00	0.34	4	100.00%

7	CVL9	1.50	0.11	2	50.00%
8	CVL10	1.75	0.21	3	75.00%
9	CVL11	1.50	0.18	2	50.00%
10	CVL14	1.75	0.35	3	75.00%
11	CVL15	1.50	0.18	2	50.00%
12	CVL16	2.00	0.43	4	100.00%

na=Observed no. of alleles, *h* = Nei's gene diversity, *P* (%) = percentage of polymorphic loci

Nei's genetic distance (Table 5) revealed the genetic differentiation between the individuals from the different regions analysed in the study. This matrix showed that the highest genetic identity value and genetic distance (0.990, 0.0101) were observed among CVL9 (Sugandhini) and CVL11 (Nithyasree), whereas the lowest values (0.6897, 0.3714) were observed between CVL2 (KAU, Thrissur) and CVL3 (CoA, Vellayani). This was also confirmed by dendrogram constructed based on the genetic distance. Dendrogram grouped all the accessions in to four main groups and seven sub groups. Accessions CVL10, CVL6 and CVL7 showed similarity as they belonged to same cluster. This can be correlated to their oil yield, morphological characters and chemical profile. Similarly CVL16 and CVL2, CVL3 and CVL15, CVL4 and CVL14, CVL11, CVL9 and CVL1 have shown convergence. High divergence was observed between samples, exhibiting high intraspecific genetic diversity. The above findings are in consonance with the study conducted by Alansi *et al*., 2016. Variation in the population's gene pool help them to adapt to the changing environmental conditions, habitat and human interventions that lead to diversity at various levels.

Table 5. Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

pop ID	CVL6	CVL16	CVL2	CVL3	CVL4	CVL15	CVL14	CVL7	CVL9	CVL1	CVL10	CVL 11
CVL6	****	0.8320	0.9037	0.7232	0.7975	0.7980	0.7865	0.9744	0.8784	0.8908	0.9809	0.8408
CVL16	0.1839	****	0.9378	0.8687	0.8420	0.8583	0.8254	0.9168	0.7681	0.7495	0.8588	0.7778
CVL2	0.1012	0.0642	****	0.6897	0.7262	0.7105	0.7269	0.9361	0.7050	0.6995	0.8928	0.6996
CVL3	0.3240	0.1408	0.3714	****	0.9315	0.9856	0.9303	0.8153	0.8983	0.8753	0.7783	0.9157
CVL4	0.2262	0.1720	0.3200	0.0709	****	0.9563	0.9592	0.8188	0.8974	0.8906	0.7824	0.8813
CVL15	0.2257	0.1527	0.3417	0.0145	0.0447	****	0.9339	0.8579	0.9435	0.9313	0.8374	0.9396
CVL14	0.2402	0.1919	0.3190	0.0722	0.0417	0.0684	****	0.8226	0.9230	0.9057	0.7821	0.9390
CVL7	0.0259	0.0869	0.0660	0.2042	0.1999	0.1533	0.1952	****	0.8914	0.8907	0.9906	0.8767
CVL9	0.1297	0.2639	0.3496	0.1073	0.1083	0.0582	0.0801	0.1149	****	0.9978	0.9004	0.9900
CVL1	0.1156	0.2883	0.3574	0.1332	0.1159	0.0712	0.0990	0.1157	0.0022	****	0.9061	0.9789
CV10	0.0193	0.1522	0.1133	0.2507	0.2454	0.1775	0.2458	0.0094	0.1049	0.0986	****	0.8754
CVL11	0.1734	0.2513	0.3572	0.0881	0.1263	0.0623	0.0630	0.1316	0.0101	0.0213	0.1330	****

CONCLUSION

Genetic variability study was carried out using four polymorphic ISSR markers. All the markers were reliable and showed considerable polymorphism. As *Cinnamomum verum* contains high amount of polyphenols and polysaccharides, pure DNA from leaf samples was obtained by adding 20 mg PVP in the extraction buffer of QIAGEN, DNAeasy plant mini kit.

PCR amplification of ISSR marker products resolved under1.4% agarose gel produced distinct bands. Each band was manually scored. The software POPGEN *ver* 1.31 was used to measure the following parameters: observed number of alleles (na), effective number of polymorphic loci and percentage of polymorphism (p%). A dendrogram was constructed based on the genetic distance. Dendrogram grouped accessions in to four. Gene diversity (h) was observed and found considerable genetic diversity in all the accessions. The samples CVL2 and CVL16 could be considered to possess a higher genetic variation as compared to other accessions.

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MORPHOLOGICAL VARIABILITY IN *AGERATUM CONYZOIDES* L. (ASTERACEAE)

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Abstract: The present study was undertaken to give a detailed account of the intraspecific diversity between different individuals of *Ageratum conyzoides* L. (*Asteraceae*) to explore pattern and ranges of variation regarding morphological characterization. Twenty *Ageratum conyzoides* populations collected from Thiruvananthapuram were examined and significant differences were observed in phenotypic characters. Features related to height of the plant and leaf size and colour were found to be important in separating the populations morphologically, but principal component analysis showed some stomatal characters (mean number of stomata and width of guard cells) also showed much variation in explaining the variation between the examined populations. In the present study trichome variation was very prominent. Glandular and non-glandular trichomes were seen in many populations where as some populations showed both the types. The anisocytic stomata were more distinct on both the upper and lower surfaces. Plants of some populations showed large stroma with comparatively large guard cells. Stomatal index showed not much variation among the populations. The morphological traits were numerically analyzed by cluster analysis and principal component analysis and found that some of the examined traits were important in delimiting the examined populations. These variables include height of the plant, width of the leaves, length of petiole, internode length, colour of stem, branching nature, petal length, bract length, corolla colour, pappus colour and stomatal length, width and frequency.

Key words: *Ageratum conyzoides,* Diversity, Population, Morphology.

INTRODUCTION

Ageratum conyzoides L. belongs to the family Asteraceae, tribe Eupatoriae. It is a native of tropical America. It has spread worldwide in the tropical and subtropical areas. In India, it was introduced in 1860 as an ornamental plant (National Focal Point for APFISN, India, 2005). Later it escaped as a weed in various habitats throughout India. In Kerala *A. conyzoides* has invaded predominantly in to grasslands,agricultural fields, forests, wastelands and pastures. It has a peculiar odour likened in Australia to that of a male goat and hence its name ‘goat weed’ or ‘billy goat weed’. *A. conyzoides* is used in various parts of India for curing various diseases in folk medicine. It is used as a purgative, febrifuge, against colic, against skin ulcers, as antienteralgic and antipyretic and for cuts as a wound dressing (Durodola, 1977). A wide range of chemical compounds including alkaloids, flavonoids, chromenes, benzofurans and terpenoids have been isolated from this species. Extracts, metabolites and essential oils from this plant have been found to possess pharmacological and insecticidal activities (Ayyanar and Ignacimuthu, 2005; Pattnaik *et al*., 1996). The insecticidal activity may in fact be the most important biological activity of this species. Some companies in Brazil are reported to be using *A. conyzoides* as raw material for phytochemicals (Ming 1999). The plant grows in a variety of soil types such as sandy, loamy and clayey, and tolerates a range of pH levels; however, it thrives best in rich and moist mineral soils and is

very common in waste places and on ruined sites. *A. conyzoides* normally occurs as a weed in frequently disturbed areas such as vegetable gardens, agricultural areas, pastures (especially when overgrazed), plantations, orchards and along roadsides. Hence it exhibits high morphological variation and easily adapts to different ecological conditions. There are a number of studies dealing with the economical importance of *Ageratum conyzoides*. But, no detailed morphological study had been carried out earlier for intraspecific variability analysis. Morphological characters, both quantitative and qualitative, have long been used to identify species and genera, to evaluate systematic relationships and to discriminate between varieties. In practice, distinctness is assessed by comparing the variety (or variety mean) of the candidate with the means of varieties of a reference collection. Knowledge of the genetic diversity is also a prerequisite for any *in situ* and *ex situ* conservation schemes (Hamrick *et al*., 1991). The environmental factors and its influence in plant variation (plant diversity) have been extensively studied in many economically important plants (Ghiselin, 1975). Intraspecific studies are limited in *Ageratum conyzoides*. Therefore, the present study was undertaken to give a detailed account of the intraspecific diversity between different individuals of *Ageratum conyzoides* in different stations from Thiruvananthapuram to explore patterns and ranges of variation regarding morphological characters.

MATERIALS AND METHODS

Ageratum conyzoides collected from various regions in Thiruvananthapuram were used for the study. Germplasm collection of *A. conyzoides* was organized as an assemblage of plants collected through explorations. While collecting plants from their natural habitats (natural populations) random sampling method was followed and directly used in various experiments. Twenty populations of *A. conyzoides* (separated by at least 5 km distance) were collected (Table1). Details of the morphology of leaves and flowers were collected from plants of uniform age.

Morphological variation

In order to analyze the morphological variations between the 20 populations, morphological characters (macroscopic and microscopic characters), were taken under consideration (Table 2).

Foliar epidermal studies

For foliar epidermal studies, mature leaves were collected and washed well in running water. Thin epidermal peelings were taken from the adaxial and abaxial surface of the leaves with a sharp razor blade. These peelings were stained in 1% aqueous saffranin. The stained peelings were washed well in distilled water and mounted on a clean slide in glycerine and observed under the microscope and photomicrographs were taken with the help of an Image Analyzer (Olympus BX 51, Japan). Frequency of stomata was calculated as the number of stomata per unit area by taking the mean from ten fields of an Olympus binocular research microscope. Five stomatal characters were studied and recorded. The length and breadth of stomatal complex and guard cell were measured with an ocular and stage micrometer using 40X magnification. Stomatal size was the product of the length and breadth of the stoma. Nature and distribution of trichomes were also analyzed.

Table 1. Details of collection of *Ageratum conyzoides* from various localities of morphological characters evaluated for the systematics of *A. conyzoides*

No:	Voucher code	Code	Places of collection
1	KUBH 6048	AG 1	Vamanapuram
2	KUBH 6049	AG 2	Kilimanoor
3	KUBH 6050	AG 3	Velavoor
4	KUBH 6051	AG 4	Chempazhanthy
5	KUBH 6052	AG 5	Neyyattinkara
6	KUBH 6053	AG 6	Nalanchira
7	KUBH 6054	AG 7	Pothencode
8	KUBH 6055	AG 8	Kaniyapuram
9	KUBH 6056	AG 9	Kattayikonam
10	KUBH 6057	AG 10	Mangalapuram
11	KUBH 6058	AG 11	Attingal
12	KUBH 6059	AG 12	Kovalam
13	KUBH 6060	AG 13	Kanjiramkulam
14	KUBH 6061	AG 14	Vattappara
15	KUBH 6062	AG 15	Nedumangadu
16	KUBH 6063	AG 16	Vellanadu
17	KUBH 6064	AG 17	Kariyavattom
18	KUBH 6065	AG 18	Palode
19	KUBH 6066	AG 19	Balaramapuram
20	KUBH 6067	AG 20	Kattakada

Table 2. Details of morphological characters of *Ageratum conyzoides* studied

Stem surface	1. Cylindrical; 2. Hairy
Stem colour	1. Green; 2. Green and reddish; 3. Pale green; 4. Reddish
Branching	1. Sparingly branched; 2. Profusely branched; 3. Unbranched
Petiole	1. Short; 2. Long
Leaf position	1. Opposite; 2. Alternate
Leaf Shape	1.Acute; 2.Ovate
Leaf tip	1.Acute; 2.Ovate
Leaf color	1. Dark green; 2. Yellow; 3. Green ; 4. Pale green with yellow spots
Pappus type	1.Capillary; 2. Bristles
Flower colour	1. White; 2. Purple
Plant height	cm
Number of branches	number
Internode length	cm
Petiole length	cm

Leaf length	cm
Leaf breadth	cm
Leaf area	cm^2
Pappus length	mm
Bract length	mm
Coralla length	mm
Stamen length	mm
Style length	mm

RESULTS

Morphological characters

During the present investigation, morphological characters (both quantitative and qualitative) were taken under consideration. Most of the populations showed variations in the case of quantitative characters (Table 3). They differed for plant height, branching, number of leaves and reproductive fitness. In general, population AG10 record maximum for plant height with a mean value of 60.88 cm and AG13, the lowest with a mean of 14.5 cm. The population showing maximum number of branches was AG10 (mean 8.8) while AG 13 showed the lowest (mean 1.2). Regarding qualitative attributes, yellow colour in leaves was characteristic of certain populations. For example, AG14 and AG15 showed yellow leaves towards their maturity. This became prominent when the plants were exposed directly to sunlight. Variation could be noticed in stem colour. Stem colour was green (AG5, AG11), reddish green (AG17), white (AG1, AG2, AG6, AG7, AG9, AG13, AG14), reddish (AG8, AG12, AG16, AG18) or reddish white (AG3, AG4, AG10, AG15, AG19, AG20). Variations could be noticed also in branching nature. Sparingly and profusely branched types were observed. AG1, AG2, AG6, AG10, AG13, AG14, AG16, AG17, AG18 and AG20 showed sparingly branched stem whereas AG9 and AG19 plants showed no branches. AG3, AG4, AG5, AG7, AG8, AG11, AG12 and AG15 showed profusely branched stem. AG3, AG4, AG8, AG14 and AG19 showed long petiole and others showed short petiole. Leaves were opposite and alternate above and near the apex. All populations showed ovate leaves with acute tip and serrate margin. Lamina showed symmetry. Variation could be noticed in leaf colour. Leaf colour was dark green, yellow spot with green, yellow and light green. AG1, AG5, AG8, AG9, AG15, AG16, AG17 and AG18 showed dark green leaves. AG2, AG4, AG6, AG7, AG10 and AG20 showed yellowish green leaves while AG3, AG11, AG12 and AG19 showed light green leaves. AG13 and AG14 showed yellow leaves. Inflorescences of all the populations consisted of numerous ray and disc florets in head (capitulum). Head was homogamous and axillary. Bract colour was green. Flower colour was white and purple. AG1, AG2, AG12, AG15, AG18, AG19 and AG20 populations showed purple flowers and others showed white flowers. Flowers of all the populations showed epipetalous stamens and bifid stigma.

Table 3. Variation in morphological (quantitative) characters of *Ageratum conyzoides*

Code	Plant height (cm)	Leaf length (cm)	Leaf breadth (cm)	Petiole length (cm)	No. of branches	Internode length (cm)	Leaf area (cm^2)	Corolla length (mm)	Bract length (mm)	Stamen length (mm)	Style length (mm)	Pappus scale length (mm)
AG1	32.6±11.1	8.2±3.3	3.5±1.1	3± 1.3	7.2±2.2	6.2±1.8	29.6±13.1	2±0	2.4±0.2	1.3±0.1	1.5±0.1	1±0.4
AG2	22.06±7.39	3.3±1.4	2.1±0.85	1.3±0.3	5.2±2.6	2.5±0.8	6.6±2.5	1.8±0.1	2.4±0.2	1.3±0.1	1.5±0.1	1.2±0.2
AG3	60.88±4.91	7.3±1.7	3.1±0.9	2.3±0.6	14.8±2.2	21.9±4.6	47.8±3.7	2 ± 0.2	2.6±0.2	1.4±0.1	1.6±0.1	1.1±0.2
AG4	18.62±5.8	3.1±1.2	2.6±0.5	2.7±0.6	4±2.4	3±0.9	9.3±3.1	1.6±0.2	2.4±0.2	1.4±0.1	1.6±0.1	1.3±0.1
AG5	36.18±9.2	8.1±1.7	3.3±0.6	3±0.8	8.8±3.3	11.7±2.6	54.2±7.2	2±0.2	2.5±0.2	1.4±0.2	1.3±0.1	1.3±0.1
AG6	20.72±4.3	3.3±1.3	1.5±0.3	1.1±0.5	0.4±0.5	3.1±1.1	4.2±2.3	2±0.1	2.3±0.2	1.4±0.1	1.5±0.1	1±0.1
AG7	46.1±15.1	8.3±1.0	3.4±0.6	2.7±0.5	4±2.0	9.1±0.8	38.7±8.1	2.8±1.7	2.6±0.2	1.3±0.1	1.3±0.1	1.3±0.4
AG8	19.48±8.3	9.5±2.2	3.6±1	3.2±0.6	6.8±2.4	9.6±1.4	6.3±1.5	2.8±1.7	2.5±0.1	1.3±0.3	1.3±0.1	1.3±0.1
AG9	16.64±3.7	6.3±1.2	3.6±0.4	2.8±0.5	0±0.0	7.5±1.1	3.7±1.2	2±0.1	2.2±0.2	1.5±0.1	1.2±0.1	1.3±0.6
AG10	62.06±5.6	12.9±1.9	5.3±0.6	3.1±0.7	6.8±1.0	12.5±0.5	54.9±1.7	2.1±0.1	2.6±0.2	1.2±0.1	1.3±0.1	1.3±0.2
AG11	32.1±9.3	9.8±1.7	4.4±1.2	3.9±0.8	10±2.0	5.5±2.7	19.6±1.2	1.8±0.2	2.6±0.2	1.4±0.2	1.3±0.1	1.9±0.2
AG12	27.4±1.8	9.9±1.9	4.1±0.7	4.7±0.9	8±2.0	3.9±1.1	8.8±2.2	2.1±0.2	2.5±0.2	1.2±0.1	1.3±0.1	0.9±0.4
AG13	14.5±2.7	5.6±0.9	1.9±0.5	1.6±0.4	0.8±1.0	15.8±0.7	4.3±1.1	1.7±0.3	2.5±0.1	1.4±0.1	1.3±0.1	1.6±0.3
AG14	27.4±7.2	6.3±1.2	2.9±0.7	3.1±0.2	2.4±1.0	9.5±3.8	10.9±2.1	2±0.2	2.5±0.4	1.3±0.1	1.3±0.1	0.8±0.5
AG15	37.9±7.2	4.3±1.9	5.2±0.6	6.6±0.6	9.2±1.0	8.8±1.3	36.5±8	1.9±0.2	2.6±0.2	1.3±0.1	1.2±0.1	1.4±0.6
AG16	30.66±10.4	9.9±1.8	5.6±0.7	5.4±0.8	6.8±3.3	6±1.7	48.6±9	2±0	2.7±0.2	1.3±0.1	1.6±0.1	1.3±0.3
AG17	47.76±12.2	7.1±2.3	2.7±0.9	4.5±0.9	2.8±1.6	6.9±0.6	34.9±8.3	2.2±0.5	2.4±0.2	1.3±0.1	1.4±0.1	1.2±0.5
AG18	20.82±4.7	11.4±2.9	5±0.9	5.7±1.1	14.4±1.6	2.7±0.7	33.4±3.8	1.9±0.2	2.5±0.2	1.3±0.1	1.4±0.1	1.2±0.6
AG19	27.56±6.2	3.7±1.8	2.5±0.5	1.7±0.5	0±0	5.1±0.8	19.3±3.8	1.7±0.3	2.7±0.3	1.2±0.1	1.4±0.1	1.2±0.3
AG20	30.08±4.7	7.3±1	2.7±1.2	2.8±0.8	7.2±1.9	4.2±1.4	15±3.4	2.1±0.2	2.3±0.2	1.2±0.1	1.4±0.1	1.2±0.1

Stomatal study

A comparative account on the stomatal and epidermal characters observed in 20 populations of *Ageratum conyzoides* is given in Table 4. In *Ageratum conyzoides*, stomata were seen on the lower and upper epidermis. Large number of stomata were seen on the lower surface of leaves. Each stoma was surrounded by two kidney shaped guard cells. Stomata were anisocytic, surrounded by three subsidiary cells. The number of stomata per unit area ranged from 7 to 11. The frequency of stomata per unit area (40X), length and breadth of stoma, length and breadth of guard cells and stomatal index are given in Table 4.

Table 4. Stomatal features in *Ageratum conyzoides*

Population Code	Frequency of Stomata per Unit area (40x)	Stoma length (µm)	Stoma width (µm)	Length of guard cell (µm)	length of guard cell (µm)	Stomatal index
AG1	10	31.613	19.159	43.25	39.71	0.27
AG2	9	29.98	18.916	39.71	37.95	0.21
AG3	7	30.032	16.242	42.36	35.30	0.24
AG4	5	27.95	16.585	35.3	32.47	0.23
AG5	11	26.07	18.069	41.34	36.54	0.26
AG6	8	23.284	15.69	43.79	38.84	0.22
AG7	9	24.765	13.485	42.56	37.94	0.21
AG8	10	29.909	16.039	40.84	34.5	0.23
AG9	9	26.448	17.528	39.74	32.74	0.25
AG10	11	35.421	19.834	43.952	40.213	0.29
AG11	7	19.410	11.377	32.91	29.54	0.23
AG12	10	27.905	15.886	44.5	37.89	0.24
AG13	7	20.9	11.012	37.2	39.9	0.28
AG14	11	29.561	18.091	38.59	30.56	0.2
AG15	7	21.883	14.900	41.56	37.8	0.21
AG16	8	29.539	17.741	43.41	39.79	0.22
AG17	11	34.756	19.195	39.86	33.47	0.27
AG18	10	22.121	16.532	37.56	31.43	0.29
AG19	7	27.562	18.341	43.54	38.91	0.24
AG20	8	26.342	14.153	42.01	37.67	0.21

Trichome study

Some of the epidermal cells get modified to form trichomes. Trichomes were present on both the surfaces, but frequency was high on the lower surface. Glandular and non-glandular trichomes were seen. Non-glandular trichomes were large and multicellular. Trichome variations are given in the Table 5.

Table 5. Trichome variations in *Ageratum conyzoides*

Population code	Nature of trichome
AG1	Glandular
AG2	Non-glandular
AG3	Glandular

AG4	Glandular and non-glandular
AG5	Non-glandular
AG6	Glandular
AG7	Non-glandular
AG8	Glandular
AG9	Non-glandular
AG10	Glandular
AG11	Glandular
AG12	Glandular
AG13	Glandular
AG14	Non-glandular
AG15	Glandular
AG16	Glandular
AG17	Non-glandular
AG18	Glandular and non-glandular
AG19	Non-glandular
AG20	Non-glandular

Phenetic analysis

In the PCA of morphological characters (qualitative and quantitative) and anatomical data (stomatal traits) of *Ageratum conyzoides*, 26.1% of phenetic variance was accounted for the first principal axis, 17.7% for the second, 13.2% for the third,11.8% for the fourth, 9.9% for the fifth and 7.03% for the sixth principal axis. Most of the selected morphological and anatomical traits were found principally influential in the PCA (Table 6).

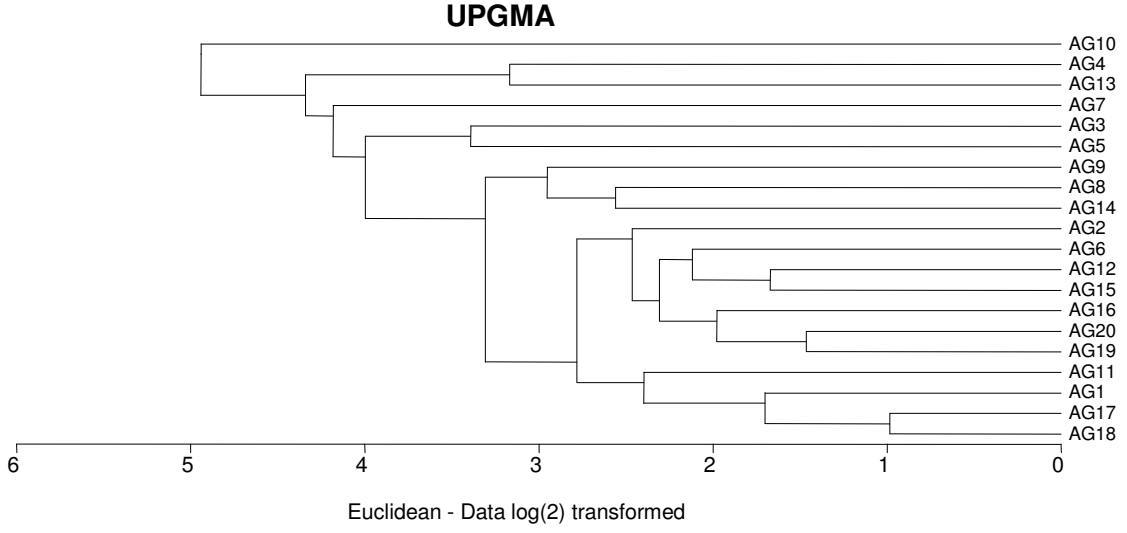
Table 6. PCA variable loading of morphological and anatomical traits in 20 populations of *Ageratum conyzoides*

CHARACTERS	AXIS-1	AXIS-2	AXIS-3	AXIS-4	AXIS-5	AXIS-6
Height	0.34	-0.14	-0.10	-0.05	-0.21	0.07
Leaf Length	-0.02	-0.11	-0.09	-0.26	-0.27	-0.01
Leaf Width	0.68	0.63	-0.08	0.32	0.23	-0.25
Petiole Length	-0.44	0.49	-0.59	0.45	-0.31	0.07
Internode	0.80	-0.04	-0.22	0.22	0.03	0.18
Branches	-0.08	0.13	0.12	-0.22	-0.08	-0.05
Leaf Area	-0.16	0.43	0.43	-0.02	0.31	0.69
Petal Length	-0.46	-0.46	-0.35	-0.16	0.17	0.05
Bract Length	-0.20	-0.13	0.68	0.27	0.46	0.08
Stamen Length	-0.24	-0.64	-0.14	0.73	-0.16	-0.2
Style Length	0.03	-0.34	0.19	-0.01	0.24	-0.07
Heair Length	0.20	0.07	-0.01	-0.3	0.21	0.04
Stem Colour	-0.79	0.33	0.04	0.03	-0.36	0.06
Branching	-0.43	-0.08	0.009	-0.1	0.29	-0.2
Petiole	0.04	-0.16	0.25	-0.07	0.28	0.01
CorolllaColour	-0.24	0.31	-0.15	-0.17	0.26	-0.06
HeairColour	0.24	-0.23	-0.15	-0.09	0.25	-0.19
Stoma Frequency	0.45	-0.37	0.23	-0.16	-0.26	-0.13

Stoma Length	0.42	0.08	0.106	-0.23	-0.11	0.13
Stoma Width	0.31	-0.15	0.12	-0.21	-0.14	0.11
Guard Cell Length	0.11	-0.02	0.19	-0.23	-0.11	-0.22
Guard Cell Width	0.2	-0.01	0.12	-0.15	-0.18	-0.005
Eigen Value	3.11	2.11	1.58	1.41	1.18	0.84
Cum.Frequency	26.1	17.71	13.26	11.86	9.92	7.36
Percentage	26.1	43.81	57.08	68.95	78.87	85.91

UPGMA phenogram of the qualitative data provided two clusters. The first cluster consists of AG10, AG4, AG13, AG7, AG3 and AG5. Here AG10 and AG7 are more distinct from others. The second cluster consists of three groups. First group consists of AG9, AG8 and AG14. Here AG9 is separated out from others. Second group consists of AG2, AG6, AG12, AG15, AG16, AG20 and AG19. Here AG2, AG6 and AG16 were distinct from others. The third group consists of AG11, AG1, AG17 and AG18. Here AG11 and AG1 are distinct from others (Fig.1).

Fig.1. UPGMA phenogram for 20 populations of *Ageratum conyzoides* based on morphological and anatomical characters



In the PCoA scatter plot for morphological characters of *Ageratum conyzoides* (Fig. 2) co-ordination was found on four regions. The first region group consisted of AG12, AG9, AG15, AG2, AG20 and AG11. The second group consisted of AG4, AG8, AG14 and AG10. Third region consisted of AG19, AG1, AG17, AG18 and AG16 and AG3, AG13 and AG5 constituted the fourth region.

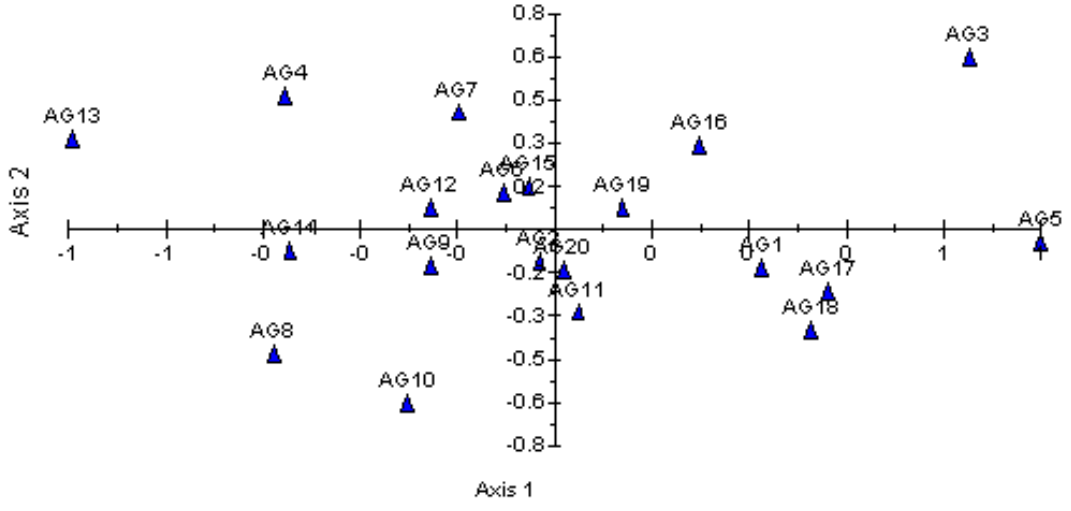
DISCUSSION

In this study, the characteristics of 20 populations of *Ageratum conyzoides* collected from Thiruvananthapuram were demonstrated by analyzing the results obtained from morphological and numerical investigations. It was determined that qualitative morphological

attributes, especially plant size varied greatly among the studied 20 populations. Generally phenotypic variations give valuable clue to the underlying genetic variations; however the two do not match always. It so happens because much of the variations that exist within populations or among progenies of the same individual may be selectively neutral (Baur and Schmid 1996). For the most cases, the species of *Ageratum* are very distinct morphologically (Johnson 1971) and lower taxa of *Ageratum* species are delimited primarily on morphological bases.

The existence of significant phenotypic differences in *A. conyzoides* gave reason to assume that the same genotype may show different phenotypes in diverse habitats. The plants growing in moist shady places in the plains had comparatively broader leaves with increased surface area. They also exhibited more branching than those with narrow leaves and leaf/stem colour. The plants collected from Kanjiramkulam (AG13) were very small, their height ranged from 12-16 cm with a few branches or no branching at all. The plants from population AG10 (collected from Mangalapuram) were tall, their height ranged from 56-68 cm with more branches. These differences may be related to the nature of the habitat supporting less growth of the plants due to non-availability of water and nutrients.

Fig. 2. PCoA scatter plot for 20 populations of *Ageratum conyzoides* based on morphological characters



The range of habitats in which the plants were collected in this study is indicative of the ability of the species to colonize newer areas. Besides, being a member of the family Asteraceae, the species is well specialized in profuse setting of seeds and dispersal. Wind is quite probably an important means of dispersal. The fruits are light and easily blown even by gentle air currents. Those fruits with a pappus of spreading scales presumably have additional

air buoyancy. Man has played a role in dispersal and introduction of *A. conyzoides*. Man's cultivation of crops in the tropics has aided in the spread of this weed as it readily establishes itself in open, worked soil. Observations in the field also revealed luxuriant growth and seed setting in wet regions and stunted growth in plants growing in dry areas.

The distinctive macroscopic features of the leaves revealed that the leaves were opposite, alternate in the upper region, petiolate, ovate, having an acute apex with serrate margins. The lamina is thin and more or less hairy on both sides. Venation was unicostate, reticulate and prominent on the lower side. The leaves were green or yellow, shriveled and twisted with aromatic odour and bitter taste. The stem was long, cylindrical, hairy, much branched and green, reddish and pale green when fresh. The dry young stem was pale green, pubescent, shrunken and flattened with fine longitudinal ridges on the surface with aromatic odour.

Microscopically the diagnostic features of the stem include single layered epidermis consisting of thin walled rectangular cells covered by cuticle. A few of the epidermal cells elongate to form glandular and non-glandular trichomes which may be uniserated and multicellular. In the present study trichome variation was very prominent. Glandular and non-glandular trichomes were seen in many populations where as populations AG4 and AG18 showed both types. AG10 which was phenotypically and anatomically distinct from others showed only glandular type of trichomes. Castro *et al*. (1997) presented a description of some types of trichomes occurring in some genera of Asteraceae and used them to elaborate an identification key. Besides its taxonomical importance, glandular trichomes are useful in attracting the pollinators and as defense against herbivorous insects and pathogens (Wagner 1991). At the specific level, studies on trichomes have been found to be of value by earlier workers (Faust and Jones 1973). The presence of a particular type of trichome can frequently delimit species, genera or even whole families. Isawumi (1989) in his study on the genus *Vernonia* found the trichomes to be more useful taxonomically in the discrimination of the species into sections than any other epidermal characters. The distribution of trichomes was amazingly complex in this study. Complexity results from, firstly the diversity in types of trichomes, secondly the marked differences in the density of trichome types taken individually and collectively and thirdly the differences in the distribution of trichome types on different parts of the plant. This diversity was observed in the present study.The non-glandular and glandular hairs as a micro character of leaves could be occasionally used in classification, especially at generic and specific level (Kallersjö 1986; Mukherjee and Sarkar 2007).

The peeled lamina showed that lower epidermal cells have greater waviness than the upper ones and smaller in size. Stomata are present in both the surfaces but are more in number on the lower surface. The anisocytic type of stomata were more distinct on both the upper and lower surface. Stomatal characters showed AG10 was more distinct from others. Plants of this population showed large stroma with comparatively large guard cells. Frequency of stomata per unit area of this population showed average value when compared to others. Epidermal cells irregular to rectangular in shape. Leaf surface amphistomatic, stomata anisocytic, elliptic to circular in shape; stomatal length ranged from 19 to 35 µm and stomatal width, 11 to 19 µm. Stomatal index showed no much variation among populations.

The results of PCA analysis of 20 populations taxa based on 22 variables is given in Table 6. The first, second and third components claim 26.1 %, 43.81 % and 57.08 %, respectively. While the fourth component accounts for 68.95 % of the variation, the fifth component accounts for 78.87 %, and the sixth component for 85.91 %. PCA analysis has also shown that some of the examined traits are important in delimiting the examined populations. These variables include the height of the plant, width of the leaves, length of petiole, internode length, colour of stem, branching nature, petal length, bract length, corolla colour, pappus colour and stomatal length, width and frequency.

All the morphological observations studied in the present study are similar to the analytical key proposed by Johnson (1971). According to his observations species can be divided into two subspecies based on the characters such as pappus scales 1.5-3 mm long, apically tapering to a scabrous seta at least in some heads and pappus scales 0.25-0.9 mm long, apically truncate to obscurely acuminate, variously laciniate but not setiferous. The first type is *Ageratum conyzoides* subsp. *conyzoides* and other type is *A. conyzoides* subsp. *latifolium*. All the investigated 20 populations even in short plants also showed pappus scales apically tapering to a scabrous seta revealed they are *A. conyzoides*sub species *conyzoides*.

In the monograph of *Ageratum conyzoides* Johnson (1971) pointed out that *Ageratum conyzoides* subsp. *conyzoides* exhibits considerable variation. Sub shrubs up to 100 cm tall as well as ephemeral annuals as small as 4.5 cm tall are known. These extremes indicate the plasticity of this subspecies to varying ecological conditions; tall and robust in the optimum tropics to very dwarf ones in more rigorous environments of ecological stresses. Variations in size of achenes and length of pappus, width of involucral bracts and length and width of leaves is also evident. In all cases, however, the plants possess the key features of *A. conyzoides* subsp. *conyzoides*, and it is desirable to treat this wide spread subspecies as one variable taxon.

From the present study, it could be concluded that significant differences were observed in phenotypic characters. All the investigated 20 populations even in the case of short plants showed the important characters to identify the sub species as *A. conyzoides*sub species *conyzoides*. Since genetic differences lead to variation among plants, these variations might be of genetic origin.

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RHIZOME YIELD VARIATION IN DIFFERENT ACCESSIONS OF *CURCUMA ZANTHORRHIZA* ROXB. OF CENTRAL KERALA, INDIA

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Abstract: Estimation of the variability among the yield parameters of a crop will help us to identify promising genotypes having high yield. This subsequently leads to the development and release of high yielding varieties through various plant breeding methods. *Curcuma zanthorrhiza* Roxb., popularly known as Java turmeric or false turmeric, belonging to the family Zingiberaceae is a rhizomatous medicinal herb with strong antibiotic properties. In spite of its immense medicinal and cosmetic importance, *Curcuma zanthorrhiza* still remains an underutilized species perhaps due to the non-availability of high yielding genotypes as planting material. Rhizome is the commercial produce; the demand for this underutilized crop is on the increase owing to the preparation of various products from it. So far, no systematic effort has been made to study the yield parameters and improvement of this species. The present experiment was designed to assess the variability of yield among eighty four accessions of *Curcuma zanthorrhiza* collected from different locations of central Kerala of India. A crop of eighty four accessions was raised in randomized block design (RBD) following standard package of practices during 2016–17. The plants were allowed to grow for eight months to reach full maturity and harvested simultaneously. Observations on rhizome yield were recorded immediately after harvest. The data were subjected to statistical analysis in order to assess the genetic variability. Rhizome yield varied from 40 g to 660 g in the case of different accessions. Analysis of variance showed that this variation was statistically significant. Among the different accessions studied, Accession No. CUZ-19 showed the highest yield followed by CUZ-3, CUZ-6, CUZ-8 and CUZ-20 in that order. This observation indicates the high level of variation in the yield of *Curcuma zanthorrhiza* populations available in central Kerala thus highlighting the existence of potential genetic variability that could be exploited for production of high yielding and genetically superior planting material. Further analysis of this variation in association with other agronomic characters will lead to the selection of superior accessions and release of superior varieties in due course.

Key words: *Curcuma zanthorrhiza*, False turmeric, Zingiberaceae, Genetic variability

INTROUCTION

Curcuma zanthorrhiza Roxb., commonly known as false turmeric and Java turmeric is a herbaceous member of Zingiberaceae with many medicinal properties. The plant is, mainly distributed and cultivated in Indonesia, Malaysia, Thailand, Philippines, China, Barbados, India, Sri Lanka, Korea, United States and also in some countries of Europe. It grows in the edges of secondary forests, in semi wild conditions and in teak plantations, coconut grooves, road sides and rarely in high altitude grasslands (Skornickova and Sabu, 2005). Economically the most useful part of this crop is rhizome, which has lots of medicinal, therapeutic, pharmacognostic and cosmetic properties. According to Skornickova and Sabu

(2005), the whole plant is about 70–185 cm tall. Rhizome is large, aromatic, 5–8 cm × 7–9 cm in size, yellow to deep yellow, camphoraceous and bitter in taste; it possess many roots and root tubers. Root tubers are yellow in colour. Pseudostem is 70 cm tall with green leaf sheaths and sheathed by 4–5 green leafy bracts. Leaves are distichous, petiolate, lamina 40–60 cm × 15–20 cm, oblong to oblong-lanceolate, glabrous. Leaves are characterized by a purple coloured patch on the upper side along the whole length of the midrib, fading on maturity. Inflorescence is lateral, peduncle 20–25 cm with large comma bracts, pink to violet, lower ones streaked green. Fertile bracts are 20–25 in number; each bract has a circinus of 8–10 flowers. Flowers are 5–6 cm long, as long as the bracts. Calyx is 3-lobed, greenish white and pubescent. Corolla tubes are white with pinkish tinge. Anther spurred, glandular hairs present on the side and back of the anther. Labellum shortly 3-lobed, middle lobe emarginate, pale yellow with a deep yellow band. Ovary is trilocular with many ovules. Fruit is ovoid, smooth, dehiscing irregularly.

Curcuma zanthorrhiza contain reducing sugars, proteins, saponins, anthraquinones, cardiac glycosides, alkaloid, phenol, quinine, curcuminoid, xanthorrhizol, flavanoids and volatile oil. The volatile oil contains phelandrene, camphor, borenol and sineol (Fatmawathy *et al.,* 2012, Mangunwardoyo *et al.,* 2012). In some regions of India, *Curcuma zanthorrhiza* Roxb. is used in place of *C. aromatica* Salib. To heal skin problems because of its easy availability. Rhizome powder of *C. zanthorrhiza* has been used for certain religious rituals, as face rub medicine and sometimes as a substitute for turmeric for culinary purposes. *C. zanthorrhiza* has the chromosome number 2n=63. (Skornickova *et al*., 2010). Xanthorrhizol is used against diarrhoea, dysentry, inflammation of the rectum, hemorrhoids, stomach disorders caused by cold, infected wounds, skin eruptions, acne, eczema, smallpox, anorexia, chronic cholecystitis and hypercholesterolemia (Hutapea *et al.,* 2000, Dalimartha, 2008). Curcuminoid has shown diuretic, anti-cancer, anti-inflammatory, anti-oxidant, anti-hypertensive, anti-rheumatic, anti-hepatotoxic, anti-dysmenorrheal, anti-spasmodic, anti-leucorrhoeal, anti-bacterial and antifungal effects. The compounds from the plant have anti-aging protective effects against UV irradiation (Park *et al*., 2014) and flower bract has anti-acne potency and whitening potency (Batubara *et al*., 2015).

Curcuma zanthorrhiza rhizome is used to treat abdominal complaints and liver disorders (jaundice and gall stones, promoting the flow of bile). Rhizome decoction is taken by women as a galactagogue and used as a remedy for fever and constipation and to lessen uterine inflammation after giving birth. In spite of its economic importance and medicinal properties*, C. zanthorrhiza* is a less studied crop with regard to its genetic improvement and varietal development. The demand for this crop increases because various products such as snack, soft drinks, mints, tooth paste and shampoo are prepared from it. It is general that growth yield and quality of a crop species vary with the soil type and the kind and balance of available nutrients.

Curcuma zanthorrhiza prefers slightly acidic well drained soil. It cannot withstand waterlogged soil. It flourishes up to 1500 m above sea level in tropical climate. Plant is typically grown from whole or split mother rhizomes, and fresh rhizomes are suitable for planting. During the growing season, the rhizome swells and forms cluster of fingers or secondary rhizomes. In terms of mannuring, compared with chicken manure and cow manure,

goat manure can increase the yield of *C. zanthorrhiza* on every variable except plant height. Mycorrhizal treatment at the dose of 10 g/plant can increase growth and yield (Samanhudi *et al.,* 2014). Productivity and quality of *C. zanthorrhiza* were influenced by nutrition availability and fertilizers applied. (Rahardjo and Ajijah, 2007). Pujiasmanto and Samanhudi (2011) analyzed the growth, yield and curcumin content of various clones of *Curcuma zanthorrhiza* grown with organic fertilizers, in which *Malang* clone had the best vegetative growth and maximum yield compared to other clones. The usage of good variety and the selection of suitable environment, proper soil preparation, planting technique and post harvest technology will result in high yield and qualityof the rhizomes of *C. zanthorrhiza.* Disease free rhizomes weighing 20-40 g with 2-3 shoots are good for planting. The need of inorganic fertilizers such as urea, SP-36 and KCl depends on soil fertility condition (Mono Rahardio, 2010).

Nowadays the species diversity of this crop is decreasing because of various anthropogenic activities such as urbanization, habitat destruction, industrialization, etc. The present study aims to analyse the variation in yield parameter of the species so as to identify superior accessions.

MATERIALS AND METHODS

The present experiment was laid out in RBD in the experimental plot of the Genetics and Plant Breeding Division of the Department of Botany, University of Calicut, Kerala, India. Eighty four accessions of *C. zanthorrhiza* collected from Central Kerala (Thrissur, Palakkad, Malappuram, Ernakulum and Idukki districts) formed the experimental material.

Fresh healthy rhizomes of *C. zanthorrhiza* collected from plants raised as mentioned above were used for growing the 84 accessions. Randomised block design (RBD) with 3 replications was used for the experimental programme. The plant is propagated vegetatively by rhizome. In the present experiment seed rhizome fingers of approximately 25-30 g were used as the planting material. The rhizomes were planted in polythene bags of size 38 cm × 35 cm. The polythene bags were filled with garden soil, cow dung and sand in 3:1:1 ratio. Planting was done before the start of southwest monsoon during the first week of May 2016. Weeding was carried out thrice, i.e., 60, 90 and 120 days after planting.

The plants were allowed to grow for eight months to reach full maturity and harvested simultaneously. After 8 months of growth the leaves turned yellow and began to dry up. The rhizomes were collected and cleaned by removing soil without damaging the rhizomes. Observations on rhizome yield were recorded immediately after the harvest. In order to assess the genetic variability of the character the data were analyzed statistically.

RESULTS AND DISCUSSION

Crop yield is always an important and effective economic index in crop development. Yield is the prime factor on which improvement programmes are to be envisaged. The yield data of *Curcuma zanthorrhiza* were analyzed statistically so as to assess its variability and also to identify the high yielding genotypes. Estimation of the variability among the yield parameters of a crop will help us to identify promising genotypes having high yield. Rhizome yield per plant varied from 40 g to 660 g in the case of different accessions of

C. zanthorrhiza studied presently. Analysis of variance showed that the variation was statistically significant (Table 1). Among the different accessions studied, Accession No. CUZ 19 showed the highest yield followed by CUZ-3, CUZ-6, CUZ-8 and CUZ-20 in that order. This observation indicates the high level of variation in yield in the case of *C. zanthorriza* in central Kerala, highlighting the existence of potential genetic variability which could be exploited effectively for the commercial exploitation of the same through selection so that better planting material is made available for the organized farming of the species. Further analysis of this variation in association with other agronomic characters will lead to the selection of superior accessions and release of superior varieties in due course. No such studies are hither to available in the case of *C. zanthorriza* while studies have been carried out in *Curcuma longa* (Jaganath and Ng, 2000). Mode of reproduction in *C. zanthorrhiza* is mainly by vegetative propagation. In the breeding of vegetatively propagated crops, the identification of one genotype with superior hybrid combination is sufficient because vegetative propagation fixes its heterozygosity and non- additive interaction and allows its multiplication, even of sterile genotypes.

Similar works have been carried out in different crops by earlier workers. Jayasree *et al.,* (2012a and 2012b) conducted variability studies in *Curcuma amada* Roxb. and *Kaempferia galanga* L. and observed that yield per plant showed very high coefficient of variation indicating high level of variability, They stressed the importance of yield and yield related characters. Radhakrishnan *et al*. (2004 and 2005) studied the variability and performance of cardamom genotypes and observed the importance of yield per plant and other yield related traits in cardamom improvement.

Table 1. Variation of yield in the case of the *Curcuma zanthorrhiza* Roxb. accessions studied

Accession Number	Place	Mean (g) ± SE	Range (g)	Rank	CD (5%)
CUZ 1	Kottekad, Thrissur	192.22±7.78	135–325	16	73.29
CUZ 2	Mapranam, Thrissur	286.11±39.54	85–480	6	
CUZ 3	Kuthiran, Thrissur	294.99±30.78	85–500	2	
CUZ 4	Chemmanda, Thrissur	229.44±68.39	75–395	11	
CUZ 5	Anandapuram, Thrissur	242.77±36.04	75–480	9	
CUZ 6	Perinjanam, Thrissur	291.66±109.30	55–660	3	
CUZ 7	Puthankurisu, Ernakulam	172.22±9.64	75–305	26	
CUZ 8	Koovapally, Ernakulam	289.99±63.47	125–610	4	
CUZ 9	Nellayi, Thrissur	209.99±44.23	75–535	13	
CUZ 10	Athirapally Thrissur,	242.22±18.38	100–365	10	
CUZ 11	Vaalkulambu, Palakkad	181.11±5.88	40–365	22	
CUZ 12	Chovoor, Thrissur	179.99±25.91	95–270	24	
CUZ 13	Perinthalmanna, Malappuram	180.55±27.49	85–255	23	
CUZ 14	Kumbalangi, Ernakulam	186.66±20.83	110–255	19	
CUZ 15	Thenjipalam, Malappuram	146.10±44.87	65–385	43	

CUZ 16	Thechery, Thrissur	248.88±66.64	60–480	7	
CUZ 17	Cherakkara, Thrissur	140.55±29.84	40–300	47	
CUZ 18	Karuvannoor, Thrissur	248.33±8.56	55–555	8	
CUZ 19	Kodali, Thrissur	361.10±55.94	150–625	1	
CUZ 20	Ponnani, Malappuram	286.66±21.30	90–490	5	
CUZ 21	Vangarapadi, Ernakulam	124.99±26.06	40–135	59	
CUZ 22	Valakam, Ernakulam	126.66±14.45	75–170	57	
CUZ 23	Chulliyod, Malappuaram	145.55±23.87	80–220	44	
CUZ 24	Elakkallu, Malappuram	143.3±13.59	100–200	46	
CUZ 25	Erumbanam, Ernakulam	138.33±20.99	80–335	50	
CUZ 26	Amalapuram, Ernakulam	130.55±20.71	40–195	55	
CUZ 27	Edappal, Malappuram	113.33±6.67	90–170	65	
CUZ 28	Kottanelloor, Thrissur	114.99±14.18	60–185	62	
CUZ 29	Karimpuzha bridge, Palakkad	153.33±4.19	100–170	38	
CUZ 30	Pattakarimbu, Palakkad	200±0	160–255	15	
CUZ 31	Panagad, Thrissur	152. 22±20.75	80–245	39	
CUZ 32	Kottamkulam, Thrissur	118.33±25.32	85–200	61	
CUZ 33	Vellimukku, Malappuram	133.33±20.58	80–280	65	
CUZ 34	Thattanthodi, Palakkad	153.33±26.69	80–205	38	
CUZ 35	Edakochi, Ernakulam	133.33±7.27	75–170	54	
CUZ 36	Edakunnam, Ernakulam	159.99±10.72	110–220	33	
CUZ 37	Valakkavu, Thrissur	217.21±17.90	125–355	12	
CUZ 38	Edathala north, Ernakulam	154.99±20.10	105–250	36	
CUZ 39	Thoikkavu, Thrissur	123.33±11.83	65–195	60	
CUZ 40	East koratty, Thrissur	113.88±11.48	60–165	64	
CUZ 41	Ezhumuttam, Idukki	154.44±16.61	90–180	37	
CUZ 42	Mulappuram, Idukki	158.33±19.90	50–265	34	
CUZ 43	Vaniyampara, Thrissur	161.66±17.04	110–250	31	
CUZ 44	Kizhuthani Thrissur	166.11±30.60	85–235	27	
CUZ 45	Thekkumoola Thrissur	137.22±15.89	85–190	51	
CUZ 46	Chendrapinni Thrissur	103.33±30.58	50–245	69	
CUZ 47	Thommankuthu, Idukki	162.77±39.54	60–270	30	
CUZ 48	Marottichal, Thrissur	147.22±14.71	60–205	41	
CUZ 49	Peramangalam, Thrissur	191.10±8.30	160–245	17	
CUZ 50	Kannambathoor, Thrissur	136.10±27.52	60–195	52	
CUZ 51	Moovattupuzha, Ernakulam	81.10±15.65	40–135	74	
CUZ 52	Nilambur, Malappuram	123.33±14.57	40–225	60	
CUZ 53	Paingothur, Ernakulam	105.55±13.15	40–160	68	
CUZ 54	Alathoor, Palakkad	113.88±26.45	65–195	64	
CUZ 55	Karumbil, Malappuram	148.33±10.19	100–215	40	
CUZ 56	Chittanda, Thrissur	163.88±22.92	75–285	29	
CUZ 57	Kalloor,Thrissur	114.44±25.31	45–215	63	

CUZ 58	Kallayi, Thrissur	126.11±13.49	55–230	58	
CUZ 59	Potharikkad, Ernakulam	129.99±5.36	80–160	56	
CUZ 60	Thuvanoor, Thrissur	144.99±11.10	60–260	45	
CUZ 61	Kalambur, Ernakulam	62.22±2.78	45–95	76	
CUZ 62	Changaramkulam, Malappuram	112.22±8.94	80–150	66	
CUZ 63	Maranchery, Malappuram	93.88±10.11	50–135	71	
CUZ 64	Neriyamangalam, Ernakulam	87.77±24.03	40–225	72	
CUZ 65	Chovannor, Thrissur	130.55±13.49	75–205	55	
CUZ 66	Kechery, Thrissur	98.88±7.47	60–125	70	
CUZ 67	Elappara, Idukki	93.88±8.63	80–115	71	
CUZ 68	Nandipulam, Thrissur	139.99±24.57	65–240	48	
CUZ 69	Kothamangalam, Ernakulam	153.33±0.95	110–220	38	
CUZ 70	Koratty, Thrissur	160.55±20.23	95–315	32	
CUZ 71	Edarikkodu, Malappuram	209.44±20.75	125–410	14	
CUZ 72	Chittissery, Thrissur	147.22±15.68	60–220	41	
CUZ 73	Kannikkal, Idukki	190.55±20.75	80–325	18	
CUZ 74	Puthanathani, Malappuram	157.77±13.07	50–230	35	
CUZ 75	Pullikkanam, Idukki	179.44±9.15	120–235	25	
CUZ 76	Puthukadu, Thrissur	186.10±14.03	100–275	21	
CUZ 77	Komaramchira, Thrissur	186.11±4.54	90–290	20	
CUZ 78	Muttom, Idukki	110.55±11.89	45–140	67	
CUZ 79	Aduparambu, Ernakulam	71.10±2.94	55–80	75	
CUZ 80	Kaduppasseri, Thrissur	133.88±8.94	95–195	53	
CUZ 81	Iruttukkanam, Idukki	138.88±13.49	95–230	49	
CUZ 82	Koprakalam, Thrissur	85±8.67	50–105	73	
CUZ 83	Kozhikkada, Thrissur	146.66±6.31	95–180	42	
CUZ 84	North Chalakudy, Thrissur	164.44±8.01	85–270	28	

CONCLUSION

The study on variation in *Curcuma zanthorrhiza* rhizome yield will lead to useful information on the genetic structure, variability and scope for the improvement of the species. This observation indicates the high level of variation in the yield of *Curcuma zanthorrhiza* populations available in Central Kerala thus highlighting the existence of potential genetic variability that could be exploited for production of high yielding and genetically superior planting material. Further analysis of this variation in association with other agronomic characters will lead to the selection of superior accessions leading to the development of new varieties.

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PRELIMINARY PHYTOCHEMICAL SCREENING AND STUDY OF ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF *CARDIOSPERMUM HALICACABUM* L., A MEMBER OF 'DASHAPUSHPA'

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Abstract: *Cardiospermum halicacabum* is a climbing tendril bearing herb with wiry stem found throughout the plains of India belonging to the family Sapindaceae. In the vernacular language Malayalam, this plant is known as *Uzhinja*. In India, its leaves are commonly consumed as leafy vegetable. It contains saponin, alkaloids, flavanoid, etc. The leaves and stem are used against common cold and angina. The leaf paste is applied on domestic animals to kill lice and other insects. Traditionally, it is used in the treatment of rheumatism, skeletal fractures and nervous diseases and is stomachic. The main objectives of the present study were screening of various phytochemicals and antioxidant activity of the methanolic whole plant extract of *C. halicacabum*. Phytochemical screening showed the presence of alkaloids, terpenoids, carotenoids, saponins and flavonoids. Antioxidant activity of the methanolic extract of *C. halicacabum* was 43.93% for 1mg/ml.The results obtained in this study confirms low antimicrobial and antioxidant potential of *C. halicacabum*.

Keywords: *Cardiospermum halicacabum*, DPPH, Spectrophotometric assay, Multi drug resistant strains, Well diffusion method.

INTRODUCTION

Plants have been the major sources of medicines since the beginning of human civilization. There is a growing demand for plant based medicines, health products, pharmaceuticals, food supplements and cosmetics in the recent days. India is well known for its plant diversity and is rich in medicinal plant wealth. It hosts three biodiversity hotspots: the Western Ghats, the Eastern Himalayas, and the hilly range that straddle the India-Myanmar border. These hotspots have numerous endemic species. Kerala is one of the lovely states in India famous for scenic beaches and serene backwaters. The Western Ghat region of Kerala is famous for its medicinal plant wealth and the tradition of the indigenous system of therapy, the Ayurveda.

In India, the Ayurvedic system of medicine has been in use for over three thousand years. Charaka and Susruta, two of the earliest Indian authors had sufficient knowledge of the properties of the Indian medicinal plants. This medical system is governed by the laws of nature, which suggest that life is a combination of senses, mind, body and soul. This holistic approach gained worldwide acceptance to Ayurvedic treatments. Phytochemistry or plant chemistry has developed in recent years as a distinct discipline, somewhere in between natural products, organic chemistry and plant biochemistry. It deals with the enormous varieties of organic substance that are synthesized and accumulated by plants and also deals with the chemical structures of these compounds, their biosynthesis, turnover and metabolism, their natural distribution and their biological function. Challenge of phytochemistry is to carry out

the methods which are needed for separation, purification and identification of many different constituents present in plants through operations on small amount of material. Phytochemical process has been aided enormously by the development of rapid and accurate methods of screening plants for particular chemicals through chromatographic techniques. It has been established that oxidative stress is among the major causative factors in induction of many chronic and degenerative diseases, ageing, diabetes mellitus, cancer, immune suppression, neurodegenerative diseases and others. A great number of aromatic, medicinal, spice and other plants contain chemical compounds exhibiting antioxidant properties. Oxidative process is one of the most important routes for producing free radicals in foods, drugs and even in living systems. The most effective path to eliminate and diminish the action of free radicals which cause oxidative stress is antioxidative defense mechanism. Antioxidants are those substances which posses free radical chain reaction breaking properties. Recently there has been an upsurge of interest in the therapeutic potential of medicinal plants as antioxidants in reducing oxidative stress-induced tissue injury.

Cardiospermum halicacabum L. is a plant of Sapindaceae family (Fig. 1). In Malayalam, this plant is known as *Uzhinja*. It is a climbing tendril bearing herb with wiry stem seen throughout the plains of India. In India, leaves are commonly consumed as leafy vegetable. It contains saponin, alkaloids, flavanoid, etc. The leaves and stem are used against common cold and angina. The leaf paste is applied on the body of domestic animals to kill lice and other insects. It is used in the treatment of rheumatism, skeletal fractures and nervous diseases and is stomachic (Arun Raj *et al.,* 2013).

The present study was designated to carry out the preliminary phytochemical analysis, and to evaluate the antimicrobial and antioxidant activities of the methanolic extract of the whole plant of *C. halicacabum*.

MATERIALS AND METHODS

The plant was collected from naturally growing populations of *C. halicacabum* from Kerala University Campus, Kariavattom, Thiruvananthapuram. The plant was properly identified with the help of authentic literature and documented with their characteristic features and a voucher specimen was deposited in the Botany Department Herbarium of University of Kerala (KUBH-9783). Phytochemical tests were carried out using standard procedures to identify the constituents.

Preparation of plant extract

The entire plant was used for the assay. The plant materials were washed and shade dried and chopped into small pieces for grinding. The plant material was powdered in an electric mixer. The powdered plant material was kept in air tight container with proper labeling for future use. The plant powder was extracted in a single solvent, methanol. 10 g of the plant powder was extracted in 200 ml of methanol for about 4-5 hours in Sohxlet apparatus at room temperature. The extract was collected and evaporated in an oven at a temperature of 55^0C. It was collected in a Petri dish, weighed and was stored in cold condition for further studies. The dried extract thus obtained was used for the phytochemical analysis and the analysis of antimicrobial and antioxidant properties using various standard procedures.

Fig 1. Habit of *Cardiospermum halicacabum* L.

Qualitative preliminary phytochemical screening

Chemical tests were carried out using standard procedures to identify the constituents present in the plant.

Detection of alkaloids

Solvent free extract (50 mg) was mixed with a few drops of dil.HCl and was then filtered. Test for alkaloids was carried out in this filtrate.

Test 1- Mayer's test: One or two drops of Mayer's reagent (mercuric chloride 1.36 g dissolved in 60 ml distilled water and mixed in a solution of 5 g of potassium iodide in 10 ml distilled water) was added to the filtrate through the side of the test tube; formation of white creamy precipitate indicates the presence of alkaloids.

Test 2- Dragendroff's test: The reagent (0.85 g Bismuth nitrate dissolved in 40 ml distilled water and 10 ml glacial acetic acid, followed by addition of 5 g potassium iodide dissolved in 20 ml distilled water) was added to filtrate. Formation of prominent yellow precipitate indicates the presence of alkaloids.

Test 3-Wagner's test: Reagent (1.27 g of Iodine and 2 g Potassium iodide dissolved in 5 ml distilled H_2O) was added to the filtrate. Formation of reddish brown precipitate indicates the presence of alkaloids.

Detection of glycosides

Molisch test: 2 ml of the prepared filtrate was mixed with 0.2 ml of alcoholic solution of α-naphthol 10% and 2 ml of sulphuric acid; a reddish violet zone is formed and this indicates the presence of carbohydrates or glycosides**.**

Detection of terpenoids

Salkowski test: 5 ml of the extract was mixed with 2 ml of chloroform and about 3 ml of con.H_2SO_4 was carefully added. At the separation level of the two liquids, a reddish-brown ring forms, which indicates the presence of terpenoids.

Detection of carotenoids

About 0.02 g of the plant extract was mixed with chloroform, mixed well and then the mixture was filtered. To the filtrate, conc.H_2SO_4 was added. Formation of a blue colour at the interface indicate the presence of carotenoids.

Detection of steroids

Libermann-Burchard test: 1 ml of the extract was treated with 0.5 ml of acetic anhydride and 1 ml of H_2SO_4 carefully. A colour change from violet to blue or green indicates the presence of steroids.

Detection of saponin

Foam test: About 0.5 g of the extract was mixed with 2 ml of distilled water and heated for a few minutes and filtered. The filtrate was vigorously shaken. Persistent froth was observed for 10 minutes; this indicates the presence of saponins.

Detection of flavanoids

The extract was shaken with 1 ml of dilute ammonia solution and con. H_2SO_4. Formation of yellow colour indicates the presence of flavanoids.

Detection of phenol

To the plant extract, a few drops of 1% aqueous or alcoholic ferric chloride were added. The formation of bluish-black colour indicates the presence of phenol.

Detection of quinone

1 ml of the plant extract was mixed with 5ml of con. HCl. The formation of yellow precipitate indicates the presence of quinone.

Detection of tannin

The sample was mixed with distilled water and boiled for 5 minutes and was filtered and used for the test. Two drops of 10% ferric chloride was added to 1 ml of the filtrate. Formation of bluish or greenish or brownish black colour indicates the presence of tannins.

Spectrophotometric assay for the evaluation of antioxidant activity of methanolic plant extract using DPPH

Free radical scavenging activity by DPPH and spectrophotometric assay

DPPH radical scavenging activity of methanolic extract of the plants was tested for antioxidant activity. The H-donor activity of the extract was estimated in this method.

Different concentrations of methanol extract ranging from 0.2 mg/ml, 0.4 mg/ml, 0.6 mg/ml, 0.8 mg/ml and 1 mg/ml were prepared. 100 ml of DPPH radical solution in methanol was freshly prepared. 1 ml extract of different concentrations was added to 2 ml of DPPH solution and the reaction mixture was incubated at 37ºC for 20 minutes. The absorbance was read at 517 nm against positive control which does not contain the extract. The assay was carried out in triplicate. A decrease in absorbance of DPPH solution indicates an increase in DPPH scavenging activity. The activity is given as percentage DPPH radical scavenging.

$$\% \text{ inhibition} = \left(\frac{Ab - As}{Ab}\right) X\ 100$$

Ab- absorbance of control
As- absorbance of sample

Determination of antibacterial activity

The following four samples of Multi Drug Resistant strains of clinical isolates were used for the study.

- ***Pseudomonas aeruginosa***
- ***Escherichia coli***
- ***Proteus mirabilis***
- ***Staphylococcus aureus***

All these samples were obtained from the Department of Microbiology, Pushpagiri Institute of Medical Sciences, Thiruvalla, Kerala, India. The cultures were grown on nutrient agar and stock cultures were maintained at 4^0C. In all the experiments, the antibacterial activity was screened with 18 hrs nutrient broth and was incubated at 37^0C for 18 hrs. Identification was done based on colony characteristics, gram staining, tube coagulase test, etc.

Well diffusion method

Agar diffusion method was employed to evaluate the antimicrobial activity. Wells of standard size (6 mm) were incised at specified distances in nutrient agar and 18 hrs old nutrient broth cultures adjusted to 0.5 McFarland of the selected strains were swabbed on separate agar plates. 100 µl each of the extracts prepared in 1 ml (100 mg extract mixed in 1 ml DMSO) were added to separate wells. 100% DMSO served as negative control and antibiotic of standard quality severed as positive control. After incubation at 37^0C for 24 hours, diameter of zone of inhibition was measured and consequently antimicrobial activity was assessed. Sensitivity of the strains used for the study against antibiotics was determined using Kirby-Bauer disc diffusion method as per CLSI guidelines.

RESULTS

Phytochemical evaluation

Preliminary phytochemical analysis of methanolic extracts of the plants was done using various preliminary analysis tests. Preliminary phytochemical analysis was done to identify the major groups of phytochemicals present in the plant samples. Preliminary analysis

results are shown in Table 1. The methanolic extract of the plant showed the presence of different phytochemicals like alkaloids, terpernoids, carotein, saponin and flavanoids.

Table 1. Preliminary screening of methanolic extract of whole plant of *C. halicacabum*.

Content	Presence (+) / absence (-)
Alkaloid	+
Glycosides	-
Terpenoid	+
Carotenoid	+
Steroid	-
Saponin	+++
Flavanoid	++
Phenol	-
Quinone	-
Tannin	-

'+' sign indicates presence and **'-'** sign indicates absence. Number of '+' signs indicate the intensity

Antioxidant activity

Free radical scavenging activity by DPPH spectrophotometric assay

The DPPH assay was used to measure the antioxidant activity of the plant extract as it offers a rapid technique to screen the antioxidant property. The antioxidant values (percentage of inhibition) of the crude methanolic extract were examined. The percentage of scavenging activity of DPPH radical was found to be concentration dependent, i.e., concentration of the extract between 0.2-1mg/ml increasing the inhibition activity. Methanolic extract of whole plant showed 10.6%, 23.15%, 31.93%, 37.51% and 43.93% of inhibition in 0.2, 0.4, 0.6, 0.8 and 1 mg/ml concentration of the extract respectively. From the result it is clear that the highest scavenging was 43.93% at 1mg/ml concentration (Table 2).

Table 2. Antioxidant property of methanolic extract of whole plant of *C. halicacabum.*

Concentration of sample (mg/ml)	DPPH	Sample	Absorbance at 517 nm	% of inhibition
Control	2 ml	1 ml methanol	0.717	0
Blank		3 ml methanol	0	0
0.2		1 ml sample	0.641	10.6
0.4			0.551	23.15
0.6			0.488	31.93
0.8			0.448	37.51
1			0.402	43.93

Antibacterial activity

The *in vitro* antimicrobial activities of the methanolic extracts of the plant were investigated separately using well diffusion method against four Multi Drug Resistant clinical isolates of bacterial strains including three gram negative (*Pseudomonas aeruginosa*, *Escherichia coli* and *Proteus mirabilis*) and one gram positive (*Staphylococcus aureus*) bacteria. The potency of the extract was assessed by the presence or absence of inhibition zone and zone diameter. The present study zone of inhibition was not obtained against any of the strain.

DISCUSSION

Herbal drugs play an important role in healthcare programmes especially in developing countries. There is a need for documentation of research work carried out on traditional medicine and also it becomes extremely important to make an effort towards standardization of plant materials to be used as medicine.

Secondary metabolites are molecules that are not necessary for the growth and reproduction of plants, but may serve some role in plant defence mechanism. They act as phytoalexin, killing bacteria that the plant recognizes on a threat. Successive isolates of botanical compounds from plant material is largely depending on the type of solvent used in the extraction procedure. In the present study, solvent system used for extraction was methanol. In Soxhlet extraction, the sample is continually exposed to fresh solvent that can withstand the temperature of the boiling solvent. The traditional healers use water as the solvent but later it was clear that plant extracts in methanol provide higher number of compounds (Jigna and Sumitra, 2007).

Phytochemical evaluation

Qualitative preliminary phytochemical screening

Phytochemical analysis revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities. Preliminary analysis is much helpful for the screening of secondary metabolites and other biochemicals present in the plant.

Alkaloids and flavanoides have been linked or suggested to be involved with antibacterial and antiviral activity while tannins and flavanoids are thought to be responsible for antidiarrheal acitivity (Enzo, 2007). This could explain the role of the plant material as an antimicrobial agent (Pithayanukul *et al*., 2007). The phytochemical analysis of some of the plants belonging to the group of *Dashapushpa* has been reported earlier also (Deepan *et al*., 2012; Majumder *et al*., 2012).

The result of the present study revealed that the plant material used for preliminary phytochemical screening possessed alkaloids, terpenoids, carotenoids, saponin and flavanoids. Singh and Bhat (2003) reported that flavonoids are responsible for the antimicrobial activity associated with some ethnomedicinal plants. According to the results obtained in this study, it is suggested that the identified phytochemical compounds may be the bioactive constituents.

Antioxidant evaluation

Several studies have been carried out to analyze the antioxidant properties possessed by different plants. Different classes of phytochemicals and several plant extracts have been found to have quite prominent antioxidant activity (Tripathi *et al*., 1996; Rao, 1997; Vani *et al*., 1997). Flavanoids are a group of naturally occurring compounds widely distributed as secondary metabolites in the plant kingdom. These flavanoids have also been reported to possess antioxidant and antiradical properties (Nakayoma and Yamada, 1995).

DPPH is one of the free radical widely used for testing preliminary radical scavenging activity of a compound or a plant extract. The DDPH test (Wagner, 1996) provides information as the reactivity of test compounds with stable free radical. Because of its odd electron, 2,2- diphenyl 1- picryl hydrazyl radical (DPPH) gives a strong absorption band at 517nm (Duh, 1999). DPPH radical is scavenged by antioxidants through the donation of a proton forming the reduced DPPH. The colour changes from purple to yellow after reduction which can be quantified by its decrease of absorbance.

As antioxidants have been reported to prevent the oxidative damage caused by free radicals, they can interfere with the extraction process by reacting with free radicals, chelating catalytic metals and also by acting as oxygen scavengers. Phenolic compounds and flavanoids are widely distributed in plants which have been reported to exert multiple biological effects, including antioxidant, anti-inflammatory and anticarcinogenic activities and free radical scavenging abilities (Miller, 1996).

The antioxidant activities of the methanolic extract of the plants were studied using DPPH (2,2- diphenyl 1- picryl hydrazyl). The antioxidant activity shown by the plant was below 50%. The phenolic compounds may contribute directly to antioxidant action (Duh *et al*., 1999). Hence the low antioxdant property may be due to the lack of phenolic compounds.

Antimicrobial evaluation

Antibacterial activities of the methanoic extract of the plant were assessed separately against one gram positive and three gram negative bacteria. The selection of the bacteria was based on the fact that majority of the bacteria that are pathogenic to human beings are gram negative in nature. The activity was assessed using Multi Drug Resistant strains.

Every time a patient takes an antibiotic for a bacterial infection, the antibiotic might kill most of the bacteria, but a few tenacious germs may survive by mutating or acquiring resistance genes from other bacteria. Such bacteria may multiply quickly creating antibiotic resistant strains and such strains get transmitted to others (Nordenberg, 1998). The indiscriminative use of antibiotics has resulted in the emergence of a number of resistant bacterial strains (Ramphile *et al*., 1991). The increasing antibiotic resistance is seen as an ecological problem (Kummerer, 2004).

Antimicrobial activities of methanolic extract of the plant were assessed by using four pathogenic bacterial species namely *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus mirabilis* and *Staphylococcus aureus.* Well diffusion method was used in assessing the

antibacterial studies. For the study DMSO was used as negative control and Gentamycine was used as positive control.

The present study showed the low activity of the plant extract, ie,, no inhibition zone was obtained against any of the strain used. This may be due to the absent of phenolic compounds in the plant.

CONCLUSION

Medicinal plants are now being used as model for antimicrobial agents and it is believed that plant based drug cause less or no side effects when compared with synthetic antibiotics. Phytochemical analysis helps to identify the presence of major phytochemicals like alkaloids, terpernoids, carotenoids, saponin and flavanoids. The antioxidant and antibacterial studies help to analyze the activity of the plant and helps in the development of new drugs for the treatment of various diseases. The results obtained from this study confirm low antioxidant activity of *Cardiospermum halicacabum*.

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PHYTOCHEMICAL SCREENING OF *PREMNA PAUCINERVIS* (C.B. CLARKE) GAMBLE (LAMIACEAE)

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Abstract: *Premna paucinervis*, an epiphytic woody climber of Lamiaceae is found in evergreen forests of Western Ghats. The present study was carried out to characterize the bioactive constituents present in the methanolic leaf extract of *P. paucinervis* using UV, FTIR, and GC-MS analysis. The preliminary anaysis showed the presence of different phytochemicals such as alkaloids, phenolics, flavonoids, terpenes and steroids. The UV- VS profile showed different peaks ranging from 400 nm to 700 nm with different absorption. The FTIR spectrum analysis confirmed the presence of various functional groups present in the extract. The presence of bioactive components was confirmed by Gas Chromatography- Mass Spectrometry (GC-MS) analysis. The mass spectrum of the unknown component was compared and interpreted with the spectrum of the known components stored in the National Institute of Standard and Technology (NIST) library. The analysis revealed the presence of 15 components having different bioactivity. The identification of these compounds in the plant serves as the basis in determining the possible health benefits leading to further pharmacological studies.

Key words: *Premna paucinervis,* bioactive constituents, FTIR, Gas Chromatography.

INTRODUCTION

Plants are the major sources of chemical compounds having biological and pharmacological importance. Over 80% of world population relies on plants for primary health care. The active principles of many drugs from plants are secondary metabolites. *Premna* is one among the age-old genera of medicinal plants of the family Lamiaceae and has been used as medicine and also in Vedic rituals (George *et al*., 2006). Traditionally many species of the genus such as *P. serratifolia*, *P. tomentosa, P. herbacea* and *P. mollissima* are being used in the treatment of various ailments. *P. serratifolia* is mainly used to treat various ailments like head ache, fever, cold and cough and as a remedy for liver and cardiac problems. The species *P. tomentosa* has been used to treat various stomach disorders by local people of tropical Asia and east Africa (Perry and Metzger, 1980). The investigation on the bioactive compounds present in plants is the primary step leading to further biological and pharmacological studies. According to Kabra *et al*. (2015) compounds present in different species of *Premna* exhibited remarkable biological activities. However no published literatures that determine the bioactive compounds present in *P. paucinervis* could be traced. The present study has aimed to investigate the bioactive compounds present in the leaf extracts of *P. paucinervis* (Fig. 1).

MATERIALS AND METHODS

Collection and processing of plant material

Fresh leaves were collected from the banks of river Pampa on the way to Sabarimala, Pathanamthitta District, Kerala, India in June 2017. The plant specimen was identified using

the available literature (Gamble, 1967; Rajendran and Daniel, 2002) . The samples were washed with sterile distilled water. The leaves were cut, shade dried at room temperature for 10 days, ground into fine powder and stored in air tight bottle until use. Dried and powdered samples were subjected to Soxhlet extraction using methanol as solvent. Then the extract was evaporated to dryness using rotary evaporator (Super fit rotovap).

Fig.1. *Premna paucinervis* habit

Phytochemical screening

Qualitative phytochemical analysis was carried out according to the methods suggested by Harborne (1984) and Evans (2009). The methanol extract was tested for alkaloids, flavonoids, steroids, tannins, terpenoids, saponins, coumarins anthroquinones and cardiac glycosides.

UV VIS and FTIR spectroscopic analysis

UV visible Spectroscopic analysis was conducted on *P.paucinervis* extract using UV visible spectrophotometer (Shimadzu UV-1700 Pharmaspec). The extract was examined under visible and UV light of wavelength ranging from 300 to 800 nm. For UV- VIS Spectroscopic analysis the extract was centrifuged at 3000 rpm for 10 minutes and filtered through Whatman No.1 filter paper. The sample was diluted to 1:10 with the same solvent. The peak values were recorded. FTIR spectroscopy was carried out to identify the characteristic functional group in the extract (Ashokkumar and Ramaswamy, 2014). The sample was scanned from 400 to 4000 cm^{-1}. The peak values were recorded.

GCMS Analysis

GCMS analysis of methanolic extract was performed using GCMS QP 2010, Shimadzu Tokyo Japan) equipped with a VF 5 mm fused silica capillary column diameter and 0.25 µm film thickness. The components were separated using helium gas as carrier at a constant rate of 1.2 m/min. The 2 µl sample extract was injected into the instrument. Injector and mass transfer line temperature were set at 200^0 C and 255 ^{0} C respectively. GC oven

temperature started at 70^0 C and was held at 300^0C and then for 10 minutes with program rate 4^0C/min for 9 minutes. The identification of components was based on the comparison of their mass spectra with those available in the database of National Institute of Standards and Technology (NIST) (Singh *et al*., 2011).

RESULTS AND DISCUSSION

Phytochemical analysis

Qualitative phytochemical analysis of methanolic leaf extract of *P. paucinervis* revealed the presence of alkaloids, flavonoids, terpenoids, steroids, and phenolic compounds. However, coumarins, cardiac glycosides, anthraquinones, tannins and saponins were absent (Table 1).

Table 1. Preliminary phytochemical analysis of the methanolic extract of *P. paucinervis*

Sl. No	Phytochemicals	Test	Result
1	Alkaloids	Dagendorff's test Mayer's test	+
2	Flavonoids	Shinoda test Lead acetate test	+ +
3	Terpenoids	Salkowski test Trichloro acetic acid test	+ -
4	Tannins	$FeCl_3$ test	-
5	Saponins	Chloroform test	-
6	Coumarins	Alkaline test Flourescence test	- -
7	Steroids	Liberman Burchard test	+
8	Cardiac glycosides	Raymond's test	-
9	Phenols	Ferric chloride test	+
10	Anthraquinones	Borntrager's reaction for free anthraquinones	-

UV-VIS and FTIR spectroscopy

The qualitative UV spectrum profile was selected at wave lengths ranging from 400 nm to 800 nm. The peaks obtained in the spectrum supports the presence of secondary metabolites such as phenols and flavonoids (Fig. 2).

FTIR spectroscopy was used to identify the functional group of the active components based on the peak value of infrared radiation (Soman and Ray, 2013). The results of FTIR peak values and functional groups are represented in Table 2. The FTIR spectrum confirmed the presence of alkenes, alkanes, amines, nitro compounds, alcohol and phenols (Fig. 3).

GCMS analysis

The spectrum profile of GCMS confirmed the presence of fifteen major components in *P. paucinervis*. Most of the identified compounds are pharmacologically important due to their unique properties. The compounds, molecular formulae, retention time and peak area are given in Table 3. Of these squalene is considered as an important compound having practical

and clinical uses with huge potential in neutraceutical and pharmaceutical industries as it has anticancer, anti oxidant, skin hydrating and hepatoprotective properties (Casuga *et al*., 2016). Caryophyllene, a sesquiterpene is another constituent with anti-inflammatory and anti-cancer properties (Legault and Pichette, 2007). Phytol is used widely as a food additive and an aromatic ingredient (Santos *et al*., 2013). It has been scientifically proved that phytol is effective in reducing the burden caused by schistosomiasis in human (de Moraes *et al*., 2014). It is also reported that phytol possesses inhibitory effect on cellular senescence and antimicrobial activity (Wei *et al*., 2011; Song *et al*., 2016). Thymol is a monoterpene compound having anti inflammatory, antimicrobial, anti oxidant, chemo preventive properties (Aeschbach *et al*., 1994). It is also used in the treatment of ringworm and hookworm infections (Bleakley, 2007). 1,2-Benzisothiazol-3-amine is used as preservative and also in emulsion paints and in laundry detergents (Correa *et al*., 2006). γ-Sitosterol can be used in the development of anti-diabetic drug as it can reduce levels of glucose in the blood by increasing insulin secretion and inhibiting glucogenesis and it has also been proved by molecular docking studies (Balamurugan *et al*., 2011).

Fig. 2. UV Visible Spectrum of methanolic extract of *P. paucinervis*

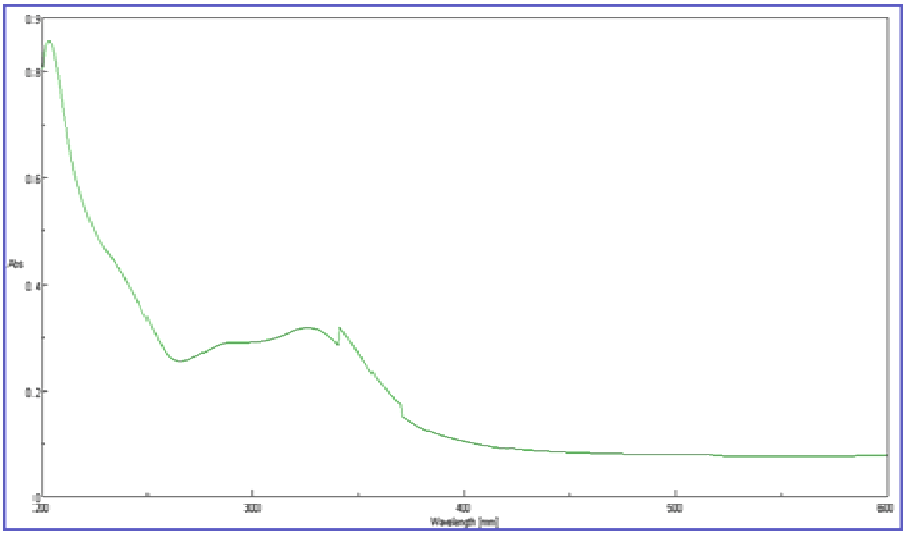


Table 2. FTIR peak values and functional groups of *P. paucinervis*

Wave number(cm^{-1})	Functional group
717	Alkenes
1054	Amines
1270	Carboxyl group
1378	Nitro group
1620	C-C linkage
2916	Alkanes
3290	Hydroxyl group

Fig. 3. FTIR Spectrum of methanolic extract of *P. paucinervis*

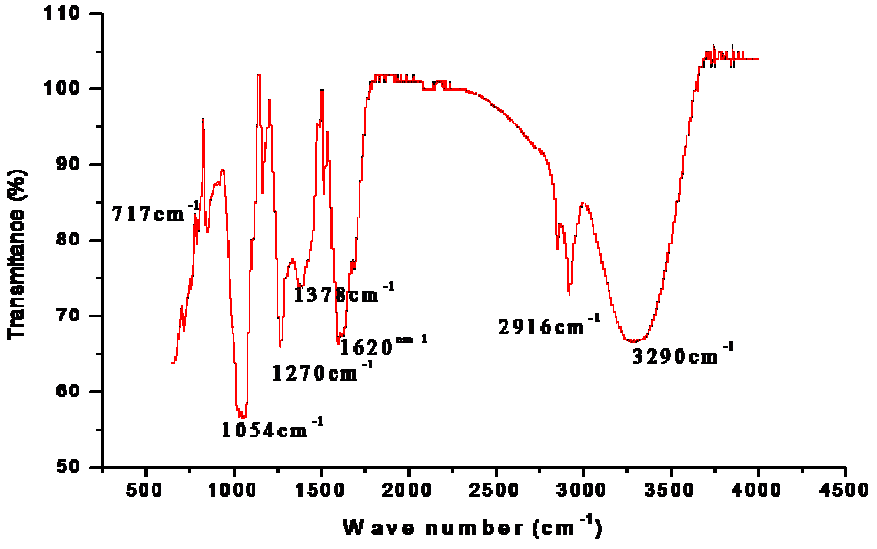
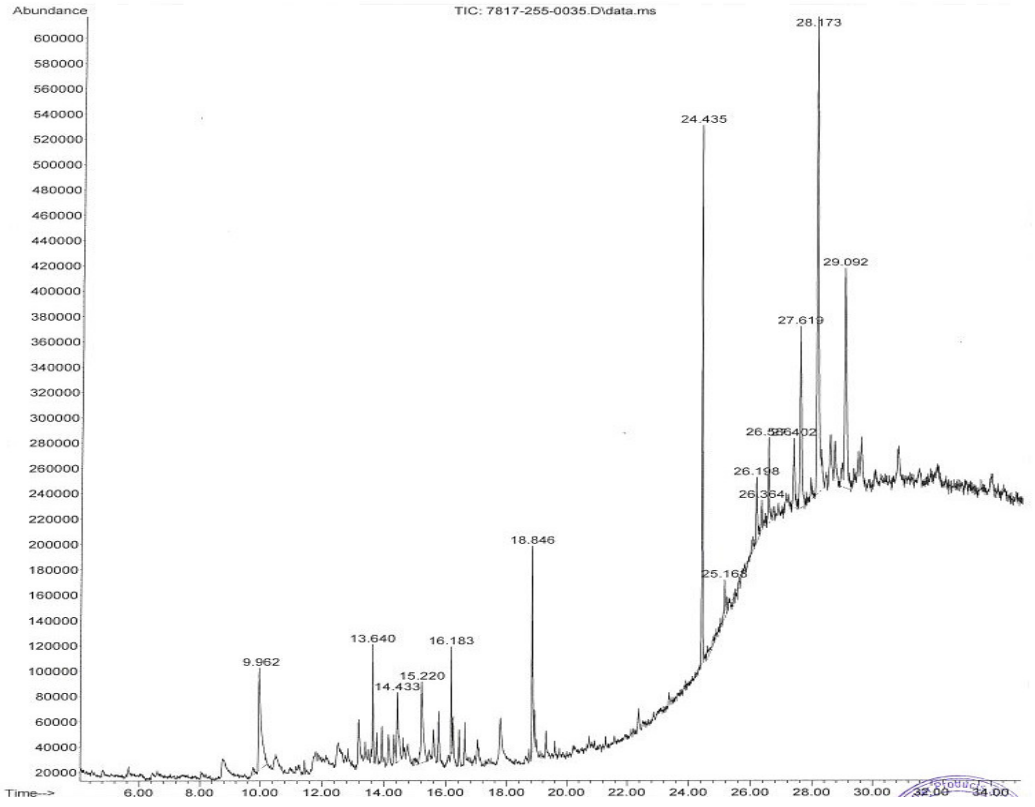


Table 3. Bioactive components in *P. paucinervis* identified by GCMS analysis

Sl. No.	Compound	Molecular formula	RT	Peak area (%)
1	Thymol	$C_{10}H_{14}O$	9.620	8.17
2	Caryophyllene	$C_{15}H_{24}$	13.640	2.86
3	3-Cyclopentylpropionic acid	$C_8H_{14}O_2$	14.433	3.23
4	3,7-Benzofurandiol	$C_4H_8O_3$	15.219	5.40
5	Bicyclo(3,1,1)heptane	C_7H_{12}	16.181	2.02
6	Phytol	$C_{20}H_{40}O$	18.845	5.16
7	Squalene	$C_{30}H_{50}$	24.435	11.50
8	Tris(tert-butyldimethylsilyloxy)arsine	$C_9H_{27}AsSi_3$	25.169	2.59
9	Tetrasiloxane decamethyl-	$C_{10}H_{30}O_3Si_4$	26.201	3.41
10	Cyclotrisiloxane,hexamethyl-	$C_6H_{18}O_3Si_3$	26.364	1.01
11	4-Methyl-2-trimethylsilyloxy-acetophenone	$C_{14}H_{24}O_3Si_4$	26.585	3.10
12	5-Methyl-2-phenylindolizine	$C_{15}H_{13}N$	27.401	3.09
13	1,1,1,3,5,5,5-Heptamethyltrisiloxane	$C_7H_{21}O_2Si_3$	27.617	8.27
14	1,2-Benzisothiazol-3-amine	$C_7H_6N_2S$	28.171	26.40
15	γ-Sitosterol	$C_{29}H_{50}O$	29.092	13.79

Fig. 4. GCMS chromatogram of methanolic extract of *P. paucinervis*



CONCLUSION

The phytochemical investigation by FTIR spectroscopy and GCMS analysis in *P. paucinervis* identified the potentiality of the species as new source of medicines due to the presence of active phytoconstituents like Caryophyllene, Squalene, Phytol, Thymol, γ-Sitosterol and Cyclotrisiloxane. However, scientific validation has to be carried out to determine the active principle behind various biological activities.

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KARYOTYPE VARIABILITY IN *SENNA SPECTABILIS* (DC.) H.S. IRWIN & BARNEBY- AN INVASIVE SPECIES IN KERALA

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Abstract: *Senna spectabilis* is an invasive species, coming under family Fabaceae (sub family Caesalpiniaceae). The species is extremely fast growing, flowers and sets seed profusely and re-sprouts readily when cut. In India, it was introduced as an ornamental plant in the botanical gardens. The present observation of this species in the area of Wayanad Wildlife Sanctuary shows that it has high potential to grow very rapidly and produce high number of viable seeds. Hence, it may become an aggressive invasive species in the forest area and it also may have an adverse impact on the survival of the indigenous species. A survey on the tree invasive species *S. spectabilis* was carried out in Kerala during 2016-2017. The collection points were geo-referenced with Google Earth website to obtain accuracy in coordinates for mapping. The distribution points of *S. spectabilis* were observed in an altitudinal range from 600 to 1800m MSL. The invasion was not recorded above 1800m MSL. In the present study 22 locations were identified with *S. spectabilis* populations distributed in Wayanad, Idukki and Palakkad districts. Wayanad district shows the highest rate of infestation with a potential distribution in both natural forests and road sides. Three populations such as Anakkatty, Muthanga and Vythiri were used in the present study for karyotype analysis. In mitotic metaphase cells of *S. spectabilis* all the three populations showed a stable chromosome count of 2n= 28, x=14. Statistical analysis showed that there was no significant difference between the characters studied. The non-significant difference in characters may be due to environment changes across the locations. When compared with the karyotype of native *S. spectabilis* specimen collected from Royal Botanical garden KEW the samples used in the present study showed the same chromosome number 2n=28. While comparing other characters, *S. spectabilis* populations collected from Kerala did not show much difference.

INTRODUCTION

Species that cross over their habitats of natural distribution and get introduced to new habitats are known as alien species or exotic species (Saxena, 1991). They colonize or invade their new habitats threatening biological diversity, ecosystems and habitats and human well being and are considered invasive alien species (IAS) and this process is biological invasion (Gaertner *et al.,* 2009). Biological invasions are regarded as one of the greatest current threats to global biodiversity (Sala *et al*., 2000). Invasive species may enter new environment through many routes. Some are transported to new places and established intentionally for variety of purposes like agriculture, horticulture (Reichard and Hamilton, 1997), forestry (Sankaran, 2002), aesthetic values (Cremer, 2003), fiber, habitat restoration, soil stabilizing and for food. According to Khuroo *et al.* (2012) more than 35% of plants introduced to India are native to South America. Worldwide 653 woody plant species belonging to 315 genera and 110 families, have been recorded as invasive (Binggeli,1998).

Senna spectabilis, a tree invasive species coming under family Fabaceae (sub family Caesalpiniaceae) is native of Tropical America and is listed in the global compendium of weeds as an 'environmental weed', 'garden thung' and 'naturalised weed' (Randalla, 2010). The species is extremely fast growing, flowers and sets seed profusely and re-sprouts readily when cut (Mungatana and Ahimbisibwe, 2010). *S. spectabilis* can tolerate a range of soil types and can reportedly adapt to alkaline soil (Gillman and Watson, 2011). The spread of the invasive alien plant *S. spectabilis* is posing a threat to wildlife and indigenous plants in the forest areas of the Nilgiri Biosphere Reserve, including the Wayanad Wildlife Sanctuary (WWS), a major habitat of Asiatic elephants in the country. Nearly 300 km^2 stretch of the region, including the Wayanad Wildlife Sanctuary, North and South Wayanad Forest Divisions and the adjacent Mudumalai, Bandipur and Nagarhole Tiger Reserves, have been infested by this invasive plant.

Karyotyping is the technique to identify and evaluate the size, shape and number of chromosomes in a sample. It often contributes to the genetic barriers to gene flow that exist between species and hence its role in species diversification has been heavily debated (White, 1978; King, 1993). Ploidy level studies and karyotype analysis are the first steps in genetic study of plants. Meiotic analysis, involving studies of the pairing and recombination of chromosomes, the passing of chromosomes to the next generation via gametes and sometimes revealing structural changes in the chromosomes, is an important tool in the understanding of the nature of genome organization in a species (Singh, 2004). The chromosome numbers of *Senna* species are 2n = 22, 24, 26 and 28 (Irwin and Barneby, 1982; 1983). In Minas Gerais state of Brazil, Katia *et al*., 2013) recorded the difference in chromosome number (n= 12, 13, 14 and 28). On the basis of centromeric index 4 types (Type A, B, C and D) of chromosomes are found in *S. spectabilis* (Suprava *et al*., 2006). The present study is an effort to find out the populations in Kerala and to analyse the karyotype of *S. spectabilis* in these regions.

MATERIALS AND METHOD

The locations were surveyed and samples for the study were collected from different areas of Kerala infected with *S. spectabilis*. The experiment was started during late summer in 2017.

Survey on the distribution of *Senna spectabilis* populations in Kerala

Intensive survey was started only after a preliminary herbarium survey of *S. spectabilis.* In the second stage, a comprehensive ecological survey was carried out in all the districts of Kerala during 2016-2017. The survey covered all the major and minor roads, natural forests, plantations, wetlands and vacant lands. GPS coordinates were collected and plotted in the map as per Suresh (2014). The places were geo-referenced with Google Earth website to obtain accuracy in coordinates for mapping.

Karyotype analysis of *Senna spectabilis*

Seedlings germinated from the seeds of *S. spectabilis* collected from different populations were planted in KFRI nursery. For the cytological observations, vigorously growing root tips were subjected to pretreatment and followed the methodology developed by Suprava *et al.* (2006) and Li *et al.* (1985). The details of the locations from which seeds were

collected for producing seedlings for karyotype analysis are given in Table 1. Observations were made on metaphase cells in which individual chromosomes were clearly distinguishable. A minimum of five mitotic cells were used to determine the chromosome number. Photographs of the 3 best individual cells were enlarged for karyotyping.

Table 1. Locations of *Senna spectabilis* population sampled for karyotype analysis

Population location	Latitude (N)	Longitude (E)	Altitude (Above MSL) (m)
Muthanga	11°39'59.41"	76°23'24.35"	875
Vythiri	11°34'23.46"	76°02'55.86"	789
Anakkatty	11°06'43.44"	76°44'45.12"	527

Root tips were collected before 10 AM, pre-treated in 0.2M of 8-hydroxyquinine for 3 hours and fixed in freshly prepared Carnoy's fluid (60% Ethanol, 30% Chloroform and 10% Glacial acetic acid) overnight. Root tips were cut without prior softening. Splitting of a piece of the sample with exterior wall often prevents extreme shrinking. The entire cross sections can often be obtained by dabbing the surface with absolute alcohol with fine brush prior to cutting (alcohol hardens the tissue immediately).Samples which are water saturated and very soft are placed for 24 hours in 30,60 and 100 % poly (ethylene glycol) 4000 and kept in an oven. Before cutting, we have glued a little strip of scotch tape on the surface. Doing so, the thin section remains complete.

Microtomy using Euromex microtome

Set the microtome knife to section thickness between 10 and 60µm. Placed a drop of absolute alcohol on the flat surface of the sample; slightly placed a wetted aquarelle brush over the sample and slided the knife across. The section can be placed on a wetted microscope and glycerol added as a mounting medium for temporary storage for hours.

The fixed samples were hydrolysed in 1N HCl for 8 minutes at room temperature after washing with distilled water 3 times, then stained with 1% Aceto-orcein for 15 minutes and squashed on a glass slide after being heated slightly. The squashed materials were observed under a microscope (Olympus BX 61 TRF motorized microscope with cytovision 3.92), and photographs were taken using cytovision 3.2. The experiment was repeated sufficient number of times. Total genomic chromosome length, total chromosome volume and form percentage (F%) of individual chromosomes of each karyotype were calculated. Mean values of total genomic chromosome length and total chromosome volume with standard error were calculated. A minimum of five mitotic cells were used to determine chromosome number. Characters such as somatic chromosome number, karyotype formula, genomic chromosome length, total form percentage, total chromosome length and genomic chromosome volume were calculated for the study.

Analysis of variance (ANOVA) was carried out to test the significance of variations between the accessions. Test of significance was done with reference to standard F Table (Fisher and Yates, 1963).

RESULTS AND DISCUSSION

Survey on the distribution of *Senna spectabilis* populations in Kerala

Populations of *S. spectabilis* were observed in an altitudinal range from 600 to 1800m above MSL. Invasion was not recorded above 1800m MSL. Twenty two locations were identified with *S. spectabilis* populations which were distributed in Wayanad, Idukki and Palakkad districts (Table 2 and Fig. 1). Among these, Wayanad district showed the highest rate of infestation with a potential distribution in both natural forests and road sides. The above survey showed that species the tree invasive species *S.spectabilis* is a big menace to different ecosystems at different spatial scales in the state of Kerala.

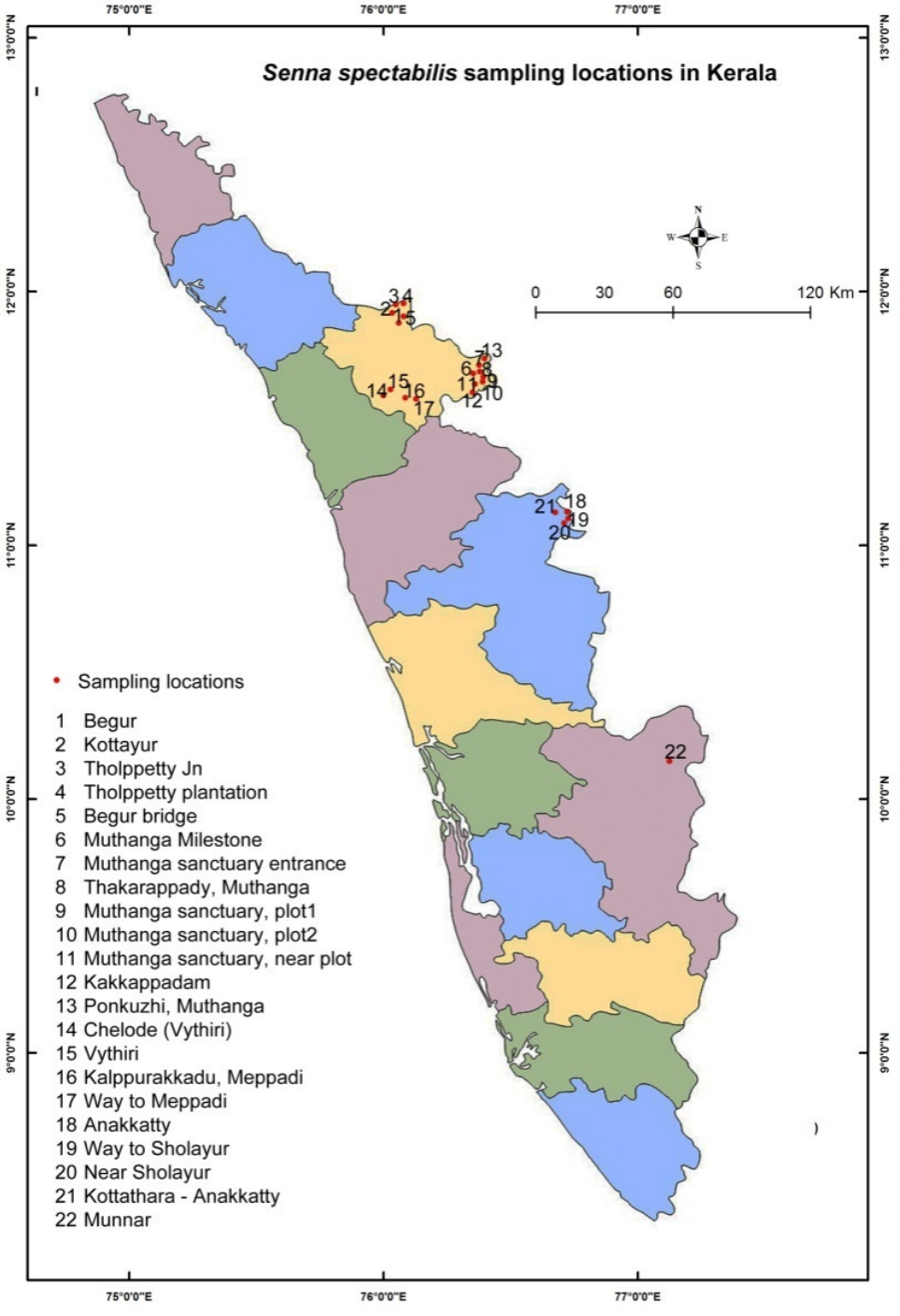
Table 2. *Senna spectabilis* population distribution in the state of Kerala.

Sl. No.	Place	Latitude	Longitude	Altitude (Msl)
1	Begur	11°52'29.36"	76°04'21.48"	763 m
2	Kottayur	11°54'18.21"	76°03'12.07"	804 m
3	Tholppetty Jn.	11°57'25.27"	76°03'46.74"	876 m
4	Tholppetty plantation	11°56'53.91"	76°03'59'02"	848 m
5	Begur bridge	11°52'58.75"	76°04'39.21"	712 m
6	Muthanga Milestone	11°40'12.11"	76°21'05.77"	871 m
7	Muthanga sanctuary entrance	11°39'59.41"	76°23'24.35"	875 m
8	Thakarappady	11^{0}40’36.34”	76^{0}22’14.34”	861 m
9	Kakkapadam	11^{0}39’55.64”	76^{0}23’28.36”	899 m
10	Muthanga sanctuary, plot2	11^{0}39’9.64”	76^{0}23’02.54”	885 m
11	Kallur	11^{0}39’57.09”	76^{0}22’58.80”	883 m
12	Kakkappadam	11°39'55.83"	76°23'21.63"	879 m
13	Ponkuzhi	11°41'37.44"	76°23'42.18"	848 m
14	Chelode (Vythiri)	11°34'23.46"	76°02'55.86"	789 m
15	Vythiri	11°34'25.97"	77°02'48.17"	771 m
16	Kalppurakkadu	11°56'44.38"	76°12'63.00"	739 m
17	Meppadi	11°56'46.66"	76°11'76.29"	571 m
18	Anakkatty	11°06'43.44"	76°44'45.12"	527 m
19	Sholayur	11°08'0.9.13"	76°41'39.31"	582 m
20	Moolagangal	11°06'25.46"	76°44'13.23"	604 m
21	Kottathara–Anakkatty	11°07'07.97"	76°44'45.89"	569 m
22	Munnar	10°06'09.55"	77°06'46.33"	1778 m

Karyotype analysis of *Senna spectabilis*

Three populations such as Anakkatty, Muthanga and Vythiri were used in the present study for karyotype analysis. Observations were made on metaphase cells in which individual chromosomes were clearly distinguishable. A minimum of five mitotic cells were used to determine chromosome number. Photographs of the three best individual cells were enlarged for karyotyping (Figs. 2 to 5).

Fig. 1. Map of *Senna spectabilis* population distribution in Kerala



In mitotic metaphase cells of *Senna spectabilis* all the three populations showed a stable chromosome count of 2n= 28, x=14 (Table 3). The statistical analysis shows that there is no significant difference between the characters studied (Tables 4-8). The characters studied such as chromosome length, total genomic chromosome length, total chromosome volume, total form percentage and arm ratio shows statistically non-significant variations. The non-significant difference in characters may be due to environment changes across the locations. The chromosome length of *Senna spectabilis* collected from the three locations ranged from 1.337 µm to 1.347 µm. Among the locations the sample collected from Muthanga shows the highest chromosome length (1.347 µm) and that from Vythiri shows the lowest chromosome length (1.330 µm) (Table 3). Comparison of relative length between or within species assumes that the total DNA content is similar within the group or a genus (Stebbins 1971). It is relatively common for chromosome arms to cross over, with small rearrangements conserving the DNA compliment.

Total genomic chromosome length of *Senna spectabilis* ranged from 25.87 µm to 28.41 µm. Samples from Anaikkatty show the highest total genomic chromosome length (28.410 µm) and from Muthanga show the lowest (25.847 µm) (Table 3). The total chromosome volume of *Senna spectabilis* varied from 8.773µm^3 to 7.957µm^3. Among these locations samples from Vythiri showed the highest total chromosome volume (8.773) and those from Anaikkatty showed the lowest (7.957) (Table 3). When compared the total form percentage of *S. spectabilis* collected from the three locations ranged from 37.527 to 36.533. The samples from Vythiri showed the highest total form percentage and the lowest was recorded in the specimens collected from Anaikkatty (Table 3).

Arm ratio is the relation of the length of the arm of mitotic chromosome to that of the short arm. In the case of arm ratio, it varied from 1.250 to 1.253. The samples from Anaikkatty showed 1.250 and that from Muthanga and Vythiri had the same arm ratio (1.253) (Table 3). The small difference in the length may be attributed to the differential condensation and spiralization of chromosome arm (Mohanty and Das, 2006).

Table 3. The karyotype details of the characters studied

Locations	Chromosome number (2n)	Chromosome length (µm)	Total genomic chromosome length (µm)	Total chromosome volume (µm^3)	Total form %	Arm ratio
Anakkatty	28	1.337	28.410	7.957	36.533	1.250
Muthanga	28	1.347	25.847	8.410	37.463	1.253
Vythiri	28	1.330	27.063	8.773	37.527	1.253

Table 4. ANOVA table showing the details of chromosome length

SUMMARY

Groups	Count	Sum	Average	Variance
Anakkatty	3	4.01	1.337	0.001733
Muthanga	3	4.04	1.347	0.000433
Vythiri	3	3.99	1.330	0.0001

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.000422	2	0.000211	0.279412	0.765554	5.143253
Within Groups	0.004533	6	0.000756			
Total	0.004956	8				

Table 5. ANOVA table showing the details of Total genomic chromosome length

SUMMARY

Groups	Count	Sum	Average	Variance
Anakkatty	3	85.23	28.41	28.2643
Muthanga	3	77.54	25.84667	4.472033
Vythiri	3	81.19	27.06333	2.643333

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	9.864467	2	4.932233	0.418226	0.676023	5.143253
Within Groups	70.75933	6	11.79322			
Total	80.6238	8				

Table 6. ANOVA table showing the details of Total chromosome volume

SUMMARY

Groups	Count	Sum	Average	Variance
Anakkatty	3	23.87	7.957	2.361633
Muthanga	3	25.23	8.410	1.0303
Vythiri	3	26.32	8.773	0.668133

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1.004467	2	0.502233	0.371102	0.704771	5.143253
Within Groups	8.120133	6	1.353356			
Total	9.1246	8				

Table 7. ANOVA table showing the details of Total form percentage

SUMMARY

Groups	Count	Sum	Average	Variance
Anakkatty	3	109.6	36.53333	12.10223

Muthanga	3	112.39	37.46333	3.892633
Vythiri	3	112.58	37.52667	8.082533

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1.855622	2	0.927811	0.115604	0.892765	5.143253
Within Groups	48.1548	6	8.0258			
Total	50.01042	8				

Table 8 . ANOVA table showing the details of Arm ratio

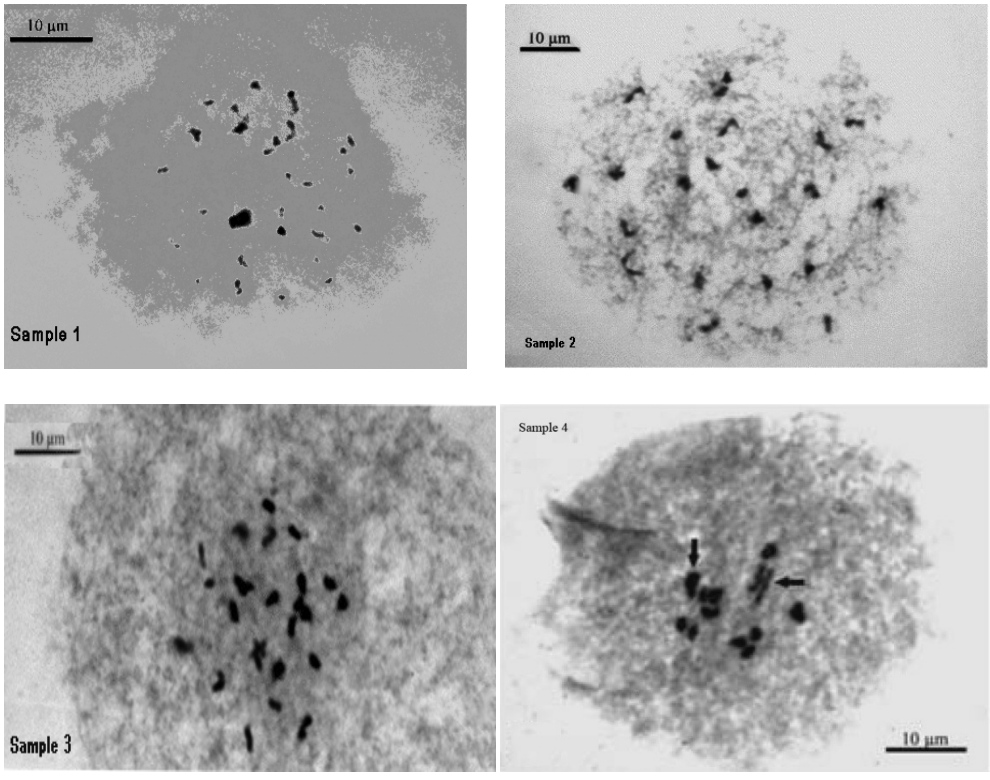
Groups	Count	Sum	Average	Variance
Anakkatty	3	3.75	1.25	0
Muthanga	3	3.76	1.253333	0.000233
Vythiri	3	3.76	1.253333	3.33E-05

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	2.22E-05	2	1.11E-05	0.125	0.884736	5.143253
Within Groups	0.000533	6	8.89E-05			
Total	0.000556	8				

When compared with the karyotype analysis of native *S. spectabilis* specimen collected from Royal Botanical garden KEW by Suprava *et al*. (2006) with the samples used in the present study, we found that the chromosome number remains same (2n =28) (Fig. 5). While comparing other characters, the chromosome length of native specimen was 1.33 µm and the *S. spectabilis* populations collected from Kerala did not show much difference. It ranged from 1.337 to 1.330 µm. In the case of total genomic chromosome length, it ranged from 25.847 µm to 28.410 µm, whereas *Senna* sp. from the native region showed 22.85 µm. When we compared the total chromosome volume of the native specimen having 9.5 μm^3 with that of the specimen collected from Kerala, it ranged from 7.957 μm^3 to 8.773 μm^3. Total form percentage of *S.spectabilis* in Kerala ranged from 37.527 to 36.533 and in the native specimen it was 38.55. In the case of arm ratio the native specimen showed 1.32 and the samples from Kerala showed 1.250 to 1.253. The small deviation in these data is not sufficient proof for chromosome variations. The results provide information on current genome structure characteristics for future investigations in the field.

Figs. 2-5. Regular somatic chromosomes of the samples collected (sample :1 Anakkatty, sample: 2 Muthanga, sample: 3 Vythiri, sample 4: Royal Botanical Garden KEWas per Suprava *et al*., 2006)



SUMMARY AND CONCLUSION

Senna spectabilis, an invasive species, coming under the family Fabaceae (sub family Caesalpiniaceae) is extremely fast growing and re-sprouts readily when cut. In India, it was introduced as an ornamental plant in the botanical gardens. The present observation of this species in the area of Wayanad wildlife sanctuary shows that it has high potential to grow very rapidly and produce more number of viable seeds. Hence, it may become an aggressive invasive species in the forest area and it also may have an adverse impact on the survival of the indigenous species. The present survey on the tree invasive species, *S. spectabilis* was carried out in Kerala during 2016-2017. The populations of *S. spectabilis* were observed in an altitudinal range from 600 to 1800 m above MSL. The invasion was not recorded above 1800m above MSL. In the present study 22 locations were identified with *S. spectabilis* populations distributed in Wayanad, Idukki and Palakkad districts. Among these, Wayanad district showed the highest rate of infestation with a potential distribution in both natural forests and road sides. Survey of the distribution of the tree invasive species *S. spectabilis* in Kerala shows that this tree invasive species is a big menace to different ecosystems at different spatial scales.

Plants from three populations collected from Anakkatty, Muthanga and Vythiri were used in the present study for karyotype analysis. In mitotic metaphase cells of *S. spectabilis* all the three populations showed a stable chromosome count of 2n= 28, x=14. The statistical analysis shows that there is no significant difference between the characters studied. The small difference in the characters may be attributed to differential condensation and spiralization of chromosome arm. When compared with the karyotype of native *S. spectabilis* specimen collected from Royal Botanical garden KEW with the samples used in the present study, it was found that the chromosome number remained the same 2n =28. While comparing other characters, *S. spectabilis* populations collected from Kerala did not show much difference. The information on cytological characteristics including numerical and structural changes is necessary to understand the taxonomic relationship and evolutionary changes in detail.

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A STUDY ON THE ALLELOPATHIC EFFECT OF *SENNA SPECTABILIS* (DC.) H.S. IRWIN & BARNEBY ON GERMINATION AND GROWTH OF NATIVE SPECIES

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Abstract: The present study focuses on the allelopathic effect of leaves of *Senna spectabilis*, an invasive species coming under the family Fabaceae (sub family Caesalpiniaceae) on seed germination and seedling growth of three native species *Bauhinia variegata, Syzigium cuminii* and *Shorea roxburghii.* The leaf samples were collected from Muthanga of Wayanad Wild Life Sanctuary, which was infected with *S. spectabilis* and the seeds of the three native species were collected from Seed Centre, KFRI and Wayanad. The seeds of the native species were germinated in root trainers filled with vermiculite. The leaf extracts from fresh and old leaves at concentrations of 0%, 25%, 50%, 75% and 100% were used to treat the seeds of the above three species and the germination parameters were recorded. The seedlings were irrigated with 5ml of aqueous solution of fresh and old leaf extracts at different concentrations and growth attributes were recorded at 15 and 30 days' intervals. Results of the study indicated that the leaf extracts from both old and fresh leaves can reduce the germination percentage of the seeds of the native species and can retard the seedling growth in later stages. Study on germination parameters indicated that there was drastic reduction in germination of the seeds when irrigated with *Senna spectabilis* leaf extract. For instance in *Bauhinia variegata, Syzigium cuminii* and *Shorea roxburghii,* the highest germination in control seeds was 24%, 32% and 5% respectively and it was reduced to 1%, 9% and 2% on application of 100 % concentration of leaf extract.. Similar to germination parameters, a drastic reduction in seedling growth characters was observed in the above three species. Further studies on the growth inhibiting activity of the leaf extracts of *Senna spectabilis* need to be undertaken at different stages of seedling growth and phytochemical profiling of the leaf extracts is to be carried out.

INTRODUCTION

Species that cross over their natural distribution and get introduced to new habitats are known as invasive species (Saxena, 1991). Biological invasion is one of the biggest problems affecting global biodiversity (Weber, 2003). The spread of invasive species is the second greatest threat to biodiversity, after habitat transformation Invasive alien species have devastating impacts on native biota, causing decline or even extinctions of native species, and negatively affecting ecosystems.

Several characteristics of the species help them to be invasive. Most important among them is the large quantity of seeds they produce which are mostly very small so as to be carried away to long distance by wind and water (Khare, 1980 and Enserink, 1999). In terrestrial ecosystems, exotic plants are very effective competitors against native species, decreasing their survival, growth and biomass (Levine *et al*., 2003).

One of the key mechanisms proposed to account for the invasive plant's success is allelopathy (Callaway and Aschehoug, 2000), where the released compounds by invaders suppress germination, growth and reproduction of native inhabitants of the invaded plant community (Hierro and Callaway, 2003). Allelopathy can be defined as the direct or indirect biotic interaction of a plant on another plant by the release of chemicals called allelochemicals from different parts of the plant into the environment (Rice,1984). The chemicals can be released from the plant through different mechanisms such as volatilization, leaching, root exudation and decomposition (Anaya, 1999). The release of these chemicals causes detrimental effects on plants sharing the same habitat as the allelopathic plant, therefore it can be said that allelopathy has an effect on plant ecology, such as growth, diversity, structure of community and productivity. The allelochemicals are mainly water soluble and may persist in soil or leachate out with water, affecting both neighboring plants as well as surrounding plants within the succession. The most common cited effects of allelopathy include reduction in seed germination and overall seedling growth; besides, some of these allelochemicals influence pollen germination, nutrition uptake, cell division, photosynthesis and also specific enzyme action.

Senna spectabilis is an invasive tree present in Wayanad Wildlife Sanctuary, Kerala. Biodiversity loss with regard to *Senna* occurs as general diversity loss, loss of endemic and threatened species, loss of habitat specific species, habitat loss of herbivores (feeding ground), etc. Invasive species can also alter the physical habitat. *S. spectabilis* is capable of outcompeting natives and taking over the habitat, and making the habitat less suitable for other species. It may decrease the suitability of soil for other species by acidifying the soil, or by releasing novel chemical compounds, as in allelopathy.

MATERIALS AND METHODS

Invasive alien species (IAS) are those that spread outside their normal distribution range and become invasive in the new locations. Several characteristics of the species help them to be invasive. Most important among them is the large quantity of seeds they produce which are mostly very small so as to be carried away to long distance by wind and water. The present study was an effort to analyse the allellopathic effect of the invasive species *S. spectabilis* on seed germination of native species. The samples for the study were collected from Muthanga of Wayanad Wildlife Sanctuary, which is infected with *S. spectabilis.* Wayanad Wildlife Sanctuary is divided in to three ranges such as Muthanga, Tholpetty and Kurichiyad. Among the three ranges Muthanga range and Tholpetty range are infected heavily with *S. spectabilis*. Muthanga region of the sanctuary was a natural moist deciduous forest converted into a Eucalyptus plantation. The plantation was harvested several years ago and the site was covered by invasive species such as *Senna spectabilis, Lantana camara* and *Chromolaena odorata* which disrupt regeneration of native species. Experiment was conducted to determine the allelopathic effect of *S. spectabilis* on the germination and seedling characters of native species. The experiment was started during late summer in 2017.

The seeds of native plants such as *Bauhinia variegata* and *Syzigium cuminii* were collected from the Seed Center of Kerala Forest Research Institute, Peechi and the seeds of *Shorea roxburghii* were collected from Muthanga of Wayanad Wildlife Sanctuary. All these

species grow naturally in the Muthanga region of the sanctuary. The laboratory experiments were conducted at KFRI.

Preparation of leaf extracts

Fresh leaves (green leaves) and senesced leaves of *Senna spectabilis* were collected to prepare the leaf extracts during May 2017. The leaf extracts were prepared according to the methodology described by Sofia (2013). 40 g of leaves were dried at 60ºC in a low heat oven and ground using mortar and pestle. This was done to extract the allelochemicals from the leaves because compounds in the leaves are not uniformly distributed. The leaves were placed in 1000 cm^3 beakers with 470 cm^3 of distilled water and placed onto a magnetic stirrer to mix well. Distilled water was used since it does not contain any ions that might decrease the level of allelochemicals (due to lack of stress). Clingfilm was placed on top of the beaker and the solution was heated to 50ºC for 10 minutes to exert stress on the plant to increase allelochemical production and release the allelochemicals into the water solution. 10 cm^3 of water was added due to any loss of water as water vapor due to evaporation. This saturated leaf solution was left overnight. The aqueous extract was obtained by filtering the mixture through a Whatman filter paper and diluting with distilled water to prepare different concentrations like 25%, 50% and 75%.

Seed germination and treatments with leaf extract

Seeds of the native species *Bauhinia variegata, Syzigium cuminii and Shorea roxburghii* were germinated in root trainers. Before sowing the seeds, the root trainers were washed in clean water as a precautionary measure against pathogens and pollutants. Physical purity of the seeds was ensured by cleaning manually.

The seedlings of all the three species were grown in randomized design with three replications and 24 plants per replication. The seeds were germinated in trainers filled with vermiculite. The extracts from two different categories such as fresh leaf extract and senesced leaf extract were used to treat the seedlings of all the three species. Four different concentrations such as 25%, 50%, 75% and 100% were used for the study. These treatments were compared with a control (without any treatment). The seedlings were treated with 5ml of the aqueous solutions of the fresh leaf extract and senesced leaf extract and the control was grown without any treatment. Treated root trainers were kept in mist chamber for germination.

The data on Germination percentage and Number of days taken for germination were observed daily. Preliminary growth parameters such as Height (cm), Girth (cm), Number of leaves, Leaf length (cm), Leaf width (cm), Number of roots and Length of root (cm) were observed 15^{th} and 30^{th} days after germination.

RESULTS AND DISCUSSION

Influence of leaf extracts of *Senna spectabilis* on seed germination

Bauhinia variegata

The influence of *Senna spectabilis* leaf extracts in different concentrations on germination, number of days taken for germination and the number of plants survived after 30

days interval was studied. Analysis of variance indicated significant difference in germination of the seeds due to fresh and dried leaf extracts (p=0.05) and concentration (p= 0.01) but the interaction effects of age and concentration was not significant. The germination of control seeds was 23.60 percent which decreased with increasing concentration of the leachate and the reduction was almost 94% at the highest concentration. At lower concentrations, decrease in germination was not drastic. Analysis of variance did not reveal any significant difference in number of days taken for germination. The effect of age of leaves and concentration was significant in the survival on 30th day. Similar to germination pattern, the number of seedlings survived also decreased with the increasing concentrations (Table 1).

Table 1. Influence of leaf extracts of *Senna spectabilis* on the seedling performance of *Bauhinia variegata* seeds

Type of leaf	Frequency of observation	Concentration (%)	Height (cm)	Girth (cm)	Number of leaves	Leaf length (cm)	Leaf width (cm)	Number of roots	Length of root (cm)
Fresh	15th day	25	5.0	0.2	1.9	2.0	1.7	7.5	3.3
		50	5.3	0.2	2.0	1.9	1.5	7.6	4.3
		75	9.0	0.4	3.1	3.2	2.7	11.9	8.3
		100	3.2	0.6	1.3	1.5	1.2	6.3	3.7
		Control	5.1	0.2	1.6	1.9	1.6	8.9	4.6
	30th day	25	4.2	0.2	1.6	1.5	1.4	5.9	3.9
		50	1.3	0.1	0.5	0.5	0.4	2.1	0.8
		75	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		100	1.4	0.1	0.4	0.6	0.5	1.3	1.2
		Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Dry	15th day	25	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		50	1.1	0.1	0.4	0.5	0.5	1.6	1.5
		75	9.2	0.3	2.9	2.9	2.4	7.8	7.8
		100	13.8	0.3	3.5	3.6	3.0	10.4	8.8
		Control	11.2	0.3	3.4	3.6	3.6	11.3	8.1
	30th day	25	8.2	0.2	3.1	2.6	1.7	7.5	3.3
		50	9.5	0.2	3.8	2.5	1.5	7.6	4.3
		75	14.7	0.4	5.9	3.9	2.7	11.9	8.3
		100	6.7	0.6	2.6	1.9	1.2	6.3	3.7
		Control	9.3	0.2	3.3	2.4	1.6	8.9	4.6

Shorea roxburghii

The influence of *Senna spectabilis* leaf extracts in different concentrations on germination, number of days taken for germination and the number of plants survived after 30 days interval in the case of *Shorea roxburghii* was studied. The analysis of variance indicated significant difference in germination of the seeds due to concentration of the extracts (p=0.01) and the interaction of age X concentration (p= 0.01)> but the individual effect of age was not

significant. The germination of control seeds was 5% which decreased with increasing concentrations of the leachate and the reduction was almost 53% at the highest concentration in the case of fresh leaf extract. There was decrease in the germination with increase in leachate concentration. The number of days taken for germination did not show much difference although the analysis of variance revealed significant difference in number of days taken for germination (p=0.01) due to interaction effect of age X concentration. The effect of concentration and concentration x age was significant in the survival on 30th day (p=0.01). Similar to germination pattern, the number of seedlings survived also decreased with increasing concentrations.

Table 2. Influence of leaf extracts of *Senna spectabilis* on the seedling performance of *Shorea roxburghii* seeds

Type of leaf	Frequency of observation	Concentration (%)	Height (cm)	Girth (cm)	Number of leaves	Leaf length (cm)	Leaf width (cm)	Number of roots	Length of root (cm)
Fresh	15th day	25	6.3	0.2	2.1	2.2	2.0	8.2	5.0
		50	3.5	0.2	1.2	1.4	1.3	5.1	3.2
		75	0.9	0.0	0.3	0.4	0.3	1.1	0.6
		100	0.4	0.0	0.1	0.2	0.2	0.5	0.5
		Control	11.4	0.3	3.3	3.4	3.0	9.8	8.2
	30th day	25	10.8	0.3	4.3	3.0	1.9	9.0	5.3
		50	7.3	0.3	2.8	1.9	1.3	6.7	3.8
		75	1.4	0.0	0.5	0.4	0.3	1.1	0.7
		100	0.6	0.0	0.1	0.2	0.2	0.5	0.5
		Control	19.4	0.3	5.6	3.6	3.0	9.8	5.5
Dry	15th day	25	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		50	1.4	0.0	0.4	0.4	0.3	1.3	1.1
		75	0.4	0.0	0.2	0.2	0.2	0.4	0.5
		100	5.1	0.3	3.3	3.4	3.0	9.8	7.3
		Control	11.4	0.1	1.5	1.5	1.4	4.4	3.4
	30th day	25	8.2	0.2	3.1	2.1	2.3	6.4	4.0
		50	4.6	0.1	1.7	1.1	1.2	3.2	2.3
		75	1.4	0.0	0.8	0.4	0.4	1.1	0.8
		100	0.6	0.0	0.3	0.2	0.2	0.4	0.5
		Control	19.4	0.3	5.6	3.6	3.0	9.8	5.5

Syzygium cuminii

The influence of *S. spectabilis* leaf extracts in different concentrations on germination, number of days taken for germination and the number of plants survived after 30 days interval in the case of *Syzygium cuminii* was studied. The analysis of variance revealed significant difference in germination of the seeds due to concentration of the extracts (p=0.01) only, The highest germination rate was obtained in control seeds (32.18%) which

decreased up to 9% at higher concentrations of leaf extract. Decreasing germination rate with increase in concentration was observed in this case also. The number of days taken for germination varied significantly among different treatments (p=0.01). The number of days for germination ranged from 12 to 19 days with increase in leachate concentration. The effect of concentration was significant in the case of survival on 7th, 15th and 30th day (p=0.01). Similar to germination pattern, the number of seedlings survived also decreased with the increasing concentrations.

Table 3. Influence of leaf extracts of *S. spectabilis* on the seedling performance of *Syzigium cuminii* seeds

Type of leaf	Frequency of observation	Concentration (%)	Height (cm)	Girth (cm)	Number of leaves	Leaf length (cm)	Leaf width (cm)	Number of roots	Length of root (cm)
Fresh	15th day	25	8.7	0.3	4.4	4.0	1.1	21.2	10.2
		50	5.5	0.2	3.0	2.4	0.7	13.7	6.8
		75	5.1	0.2	2.2	1.8	0.6	12.3	6.0
		100	4.6	0.2	1.8	1.6	0.6	12.7	5.1
		Control	11.2	0.4	6.0	4.3	1.4	28.8	14.0
	30th day	25	8.5	0.3	6.3	4.4	1.1	21.3	10.6
		50	5.9	0.2	4.7	2.6	0.7	13.8	6.5
		75	5.0	0.2	3.8	2.0	0.6	12.1	5.8
		100	3.5	0.1	1.9	1.3	0.4	9.0	3.6
		Control	13.9	0.4	10.1	5.2	1.4	28.8	14.0
Dry	15th day	25	8.5	0.3	4.0	5.0	1.8	23.0	15.0
		50	2.7	0.1	2.0	1.6	0.5	8.3	4.9
		75	3.1	0.1	1.8	1.4	0.4	8.3	2.7
		100	2.1	0.1	6.0	1.0	0.3	8.1	2.1
		Control	6.5	0.4	3.6	4.3	1.4	28.8	13.7
	30th day	25	4.9	0.1	3.3	2.0	0.5	8.6	4.1
		50	2.9	0.1	2.3	1.3	0.3	5.7	2.8
		75	2.1	0.1	1.8	0.9	0.2	4.3	2.4
		100	1.9	0.1	1.7	0.9	0.2	4.3	1.7
		Control	13.9	0.4	10.1	5.2	1.4	28.8	13.7

DISCUSSION

Invasive alien species (IAS) are those which spread outside their normal distribution range and become invasive in the new locations. *Senna spectabilis* is an invasive species, coming under family Fabaceae (sub family Caesalpiniaceae). This species is native of Tropical America. *Senna spectabilis* is listed in the global compendium of weeds as an 'environmental weed', 'garden thung' and 'naturalised weed' (Randalla, 2012). The species is extremely fast growing, flowers and sets seeds profusely and re-sprouts readily when cut. In

India, it was introduced as an ornamental plant in the botanical gardens. The present observation of this species in the area of Wayanad Wildlife Sanctuary, Sathyamangalam and suburban areas of Coimbatore of South India shows that it has high potential to grow very rapidly and produce large number of viable seeds. The present investigation was formulated to find out the allelopathic potential of this species on germination and growth of the native plant species like *Bauhinia variegata, Syzigium cuminii* and *Shorea roxburghii* and it indicated that the leaf extracts from both old and fresh leaves could reduce the germination of the seeds of the native species and can retard seedling growth in later stages. With regard to germination, there was drastic reduction in germination of the seeds when irrigated with leaf extract. For instance in *Bauhinia variegata, Syzigium cuminii* and *Shorea roxburghii,* the highest germination in control seeds was 24, 32 and 5 per cent respectively and it was reduced to 1, 9 and 2 per cent on application of 100% concentration of leaf extract. Similar to germination parameters, there was drastic reduction in seedling growth characters also in the case of the three tree species. Hence, there is need for further studies on the growth inhibiting activity of leaves of *Senna* at later stages of seedling growth. Similar studies are to be conducted on other native tree species to identify the severity of allelopathy from this species.

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LOW NaCl CONCENTRATION ENHANCES PRIME PHYSIOLOGICAL FEATURES IN *ORYZA SATIVA* CV. JYOTHI

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Abstract: NaCl stress is one of the major abiotic stresses negatively affecting rice growth and productivity, but lower concentrations were found to enhance the growth and related physiological features. The changes in morphological, physiological and biochemical parameters were analyzed in rice seedlings under various NaCl treatments (25, 50, 75, 100 and 125 mM NaCl). The parameters like germination percentage, seed vigour index, biomass, total chlorophyll and carotenoid content were decreased with increase in concentration (75, 100 and 125 mM) of NaCl, but lower concentrations like 25 and 50 mM NaCl enhanced germination percentage, biomass, chlorophyll and carotenoid contents when compared to control plants. Maximum chlorophyll and carotenoid content was observed in 50 mM. Lipid peroxidation rate was lowered in 25 and 50 Mm NaCl as compared with other NaCl concentrations and at par with the control. The content of various non-enzymatic antioxidants like total sugar, ascorbate, total phenolics and proline contents were elevated under increasing NaCl concentrations and reached maximum at 125 mM NaCl stress, while these paramets were reduced in lower concentrations of NaCl (25 and 50 mM). Low concentration of NaCl (25 and 50 mM) enhanced various physiological and biochemical features related to growth, productivity and stress tolerance in *Oryza sativa* cv. Jyothi, whereas only higher NaCl concentrations (75,100 and 125 mM) imparted stress.

Keywords: Abiotic stress, NaCl, Antioxidants

INTRODUCTION

The world population is increasing rapidly and there will be the need to produce 87% of more food crops than what we are producing today, especially in the case of crops such as rice, wheat and maize by 2050 (Kromdijk and Long, 2016). Abiotic stresses such as salinity, drought, heat and cold, critically affect crop production and causes significant yield reduction in large areas (Mantri *et al.*, 2012).

Rice is an important crop used as a staple food for over half of the world's population and the enhancement in rice grain production is the main goal for today. Due to soil salinity, rice production is declining every year (Ali *et al.*, 2014). While considering the various stresses affecting large areas of the world's cultivated land, soil salinity causes significant reductions in crop yield. In Asia, 21.5 million ha of land area is salt affected, with India having a share of 8.6 million ha (Sahi *et al.*, 2006). However, improvement in salt tolerance of crop plants remains elusive, because salinity affects physiology and biochemistry of plants at both whole plant and cellular levels (Rajakumar, 2013).

Salinity is damaging to the various processes of crops such as seed germination, seedling growth and vigor, vegetative growth, flowering and fruit set and ultimately it causes diminished economic yield and quality (Sairam and Tyagi, 2004). Based on the effect of salt

on plant growth, plants can be primarily divided into two groups: crop species sensitive to soil salinity are known as glycophytes, while plants which can generally tolerate high salt concentrations are known as halophytes (Tuteja *et al.*, 2011). Rice is sensitive to salinity during germination, young seedling and early developmental stages, especially for most of the commonly used rice varieties (Ologundudu *et al.*, 2014).Salinity influences stomatal closure which causes leaf temperature elevation and inhibition of shoot elongation (Sirault *et al.*, 2009). Salt stress affects seed germination, causes water deficit and ion imbalance of the cellular ions resulting in ion toxicity, which ultimately results in the production of reactive oxygen species (ROS) (Khan and Panda, 2008). Increased production of ROS causes an inhibition of growth and development and reduces photosynthesis, respiration and protein synthesis in sensitive species (Pal *et al.*, 2004).

Even though high level of NaCl imposes a stress effect on plant metabolism, the low concentration of NaCl has a priming effect. In low concentrations, germination percentage, seed vigour index, biomass and photosynthetic rate of the seedlings significantly get enhanced in *Capsicum annuum* (Khan *et al.*, 2009), maize (Bakht *et al.*, 2011; Agami, 2013). The present investigation was aimed to study the comparative effects of various concentrations of NaCl on seed germination, plant growth and various physiological features including free radical generation and scavenging mechanisms in the rice cv. Jyothi.

MATERIALS AND METHODS

Plant materials

Rice (*Oryza sativa* L.) belongs to the family poaceae. The seeds of rice cv.Jyothi was collected from Regional Agricultural Research Station, Pattambi, Kerala. The seeds were inoculated after surface sterilization with 0.1% $HgCl_2$ solution for 5min and further seeds were washed thoroughly with distilled water. After the washing, the seeds were germinated in plastic bottles (19 cm x 11 cm) containing absorbent cotton soaked with distilled water (control) and different concentrations of NaCl (25, 50, 75, 100 and 125 mM) solution. The bottles were kept under a 14/10 h light-dark cycles at 300 µmol $m^{-2}s^{-1}$, 25±3^{0}C and RH 55±5%. Various studies related to germination and physiology were carried out 9 d after germination.

Physiological, biochemical and germination related studies

For fresh and dry weight measurements, the seedlings were blotted dry and wrapped separately in pre-weighed and labeled aluminium foil. Fresh weights of the samples were determined by weighing them immediately after wrapping. For dry weight measurements, the samples were kept in an oven maintained at 80^{0}C. After 48 h, the samples were transferred to desiccators, allowed to cool and then weighed. The biomass was calculated from fresh and dry weights.

Estimation of total chlorophyll content was done according to the method of Arnon (1949). The malondialdehyde (MDA) content estimation was done according to Heath and Packer (1968). Total soluble sugar content was estimated according to the method proposed by Montgomery (1957). The estimation of ascorbate content was done according to the method of Chen and Wang (2002). Total phenolic was estimated using Folin-Denis reagent

according to the method of Folin and Denis (1915). Proline content in the plant parts was estimated according to the method of Bates *et al.* (1973).

RESULTS AND DISCUSSION

Seed germination

Parameters related to seed germination such as germination percentage and seed vigour index were analyzed in seeds incubated in different concentration of NaCl. Gradual increase of seed germination was observed with increase in NaCl concentration upto 50 mM and in higher concentrations seed germination decreased. The highest seed vigour index was recorded in seeds subjected to treatment of 50 mMNaCl as compared to control and other concentrations of NaCl (Fig. 1). According to Ologundudu *et al.* (2014), salinity affects the rice varieties during germination and in young seedling and developmental stages. Increased salt concentration negatively influences seed germination and seedling growth due to ion toxicity and osmotic stress in plants (Khan and Panda, 2008). Due to higher accumulation of proline and lower starch and protein content, germination percentage and biomass got reduced gradually with increase of salt stress in rice plant (Rajkumar 2013). Low concentration of NaCl act as a priming effect in rice seedlings. Due to the priming effect, germination percentage, vigour index, dry weight and chlorophyll content of the seedlings significantly get enhanced in *Capsicum annum* and maize (Khan *et al.*, 2009; Bakht *et al.*, 2011).

Physiological parameters

The biomass of rice seedlings subjected to different concentrations of NaCl treatments was found to be varying. A significant increase of biomass was observed in seedlings subjected to 25 and 50 mM NaCl concentrations but decrease in biomass was recorded in seedlings subjected to NaCl 75 mM and above as compared to control rice seedlings (Fig. 1). Gradual increase of total chlorophyll and carotenoids were observed in leaves of seedlings treated with 25 and 50 mM NaCl, but further higher concentrations of NaCl reduced the pigment content as compared to the control (Table 1). Under salinity stress conditions, total chlorophyll and carotenoid content were reduced in rice seedlings (Cha-um *et al.*, 2010; Chunthaburee *et al.*, 2016) and potato (Daneshmand *et al.*, 2010). Salinity increases the activity of the chlorophyll degrading enzyme chlorophyllase, which reduces the chlorophyll content in plants (Amirjani, 2012). Under low NaCl stress, sodium ions enhance the stacking of thylakoid membranes, which enhances the functional potential of the membranes and thus increases photosynthetic rate. While in high salinity, chloroplast thylakoid membranes swell up due to a change in the ionic composition of the stromal liquid due to sodium ions, which reduces the photosynthetic rate (Parida and Das, 2005). Moreover, the accumulation of NaCl in the leaf laminae reduces photosynthesis.

Table 1. Total chlorophyll and carotenoid content in rice seedlings subjected to various NaCl concentrations

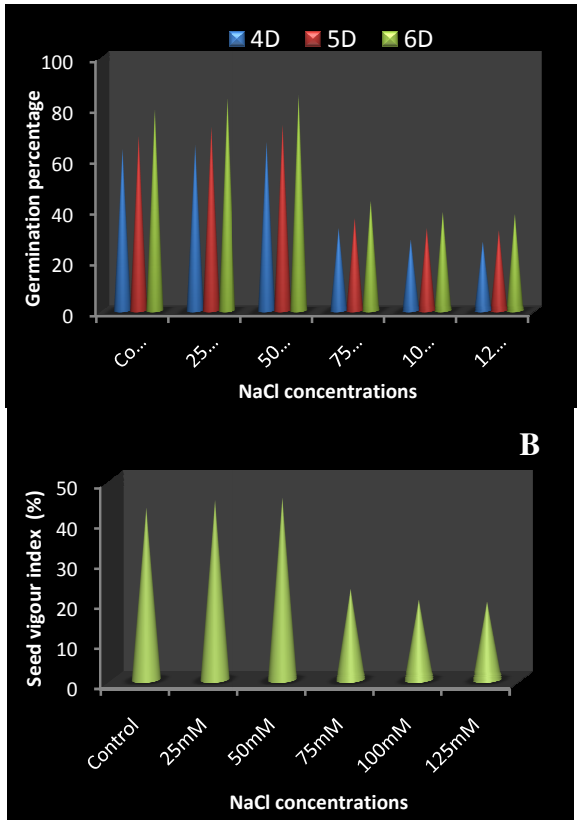
	Control	25 mM	50 mM	75 mM	100 mM	125 mM
Total Chlorophyll (mg/g DW)	4.53±0.07	4.78±0.08	5.43±0.08	3.77±0.02	3.51±0.12	3.21±0.12

Carotenoids (mg/g DW)	1.11±0.01	1.35±0.02	1.40±0.008	0.92±0.01	0.74±0.05	0.58±0.03

Lipid peroxidation

Significant increase in the rate of lipid peroxidation as assessed by MDA content was recorded in rice seedlings upon treatment with NaCl. In low concentration of NaCl (25 and 50 mM) treatment, the MDA content significantly declined as compared to other concentrations. Beyond 50 mM NaCl treatment, gradual increase in MDA content was observed (Fig. 2). In rice seedlings, the increased concentration of NaCl causes cellular membrane damages by the effect of lipid peroxidation and results in increased MDA level. Increased concentration of NaCl leads to the accumulation of free oxygen radicals that cause lipid peroxidation (Amirjani, 2012). Similar result was observed in earlier studies conducted in rice (Moradi and Ismail 2007; Amirjani 2012,) and maize (AbdElgawad *et al.*, 2016). The lower MDA content in rice seedlings subjected to 25 and 50 mM NaCl is because, these two concentrations turn to be optimal and does not induce any stressful condition.

Fig. 1. Germination percentage (A), seed vigour index (B) and biomas (C) in rice seedlings subjected to various NaCl concentrations. 4D, 5D and 6D represent 4th, 5th and 6th days of treatment



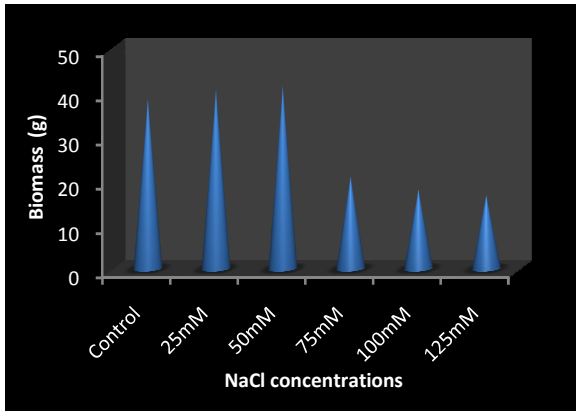
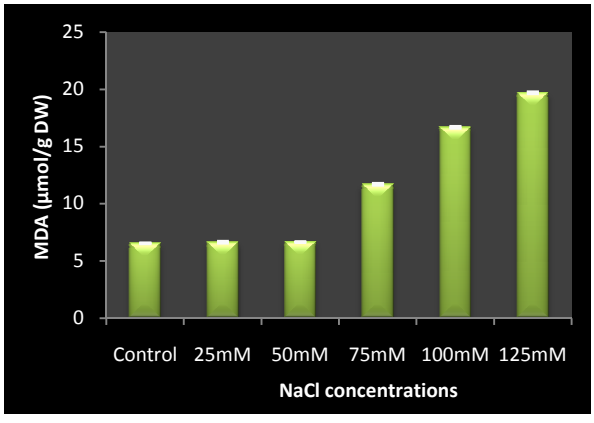


Fig. 2. MDA content in rice seedlings subjected to various NaCl concentrations



Free radical scavenging mechanism

Non- enzymatic antioxidants

Total sugar

Total sugar content was significantly increased in NaCl treated rice seedlings. Initially there was a gradual increase of sugar content in seedlings treated with 50 mM NaCl and beyond it, a rapid enhancement was recorded. Maximum sugar content was observed in 125 mM NaCl treated rice seedlings (Fig. 3). High salt concentration resulted in a condition that could not effectively use carbohydrate for plant growth, and therefore the soluble sugar content increased (Rajkumar 2013; Kibria *et al.*, 2017). According to Siringam *et al.* (2011) when plants are exposed to salt stress, the stress condition enhances sugar accumulation in plants imparting salt tolerance potential through build up of osmoticum, which is an important role in salt defence mechanism. Salt stress induced sugar accumulation was reported in many species, such as barley (Ahmad *et al.*, 2006), sorghum (Almodares *et al.*, 2008), tomato (Chookhampaeng *et al.*, 2008), rice (Pattanagul and Thitisaksakul, 2008; Thitisaksakul *et al.*, 2015), egg plant (Abbas *et al.*, 2010), etc.

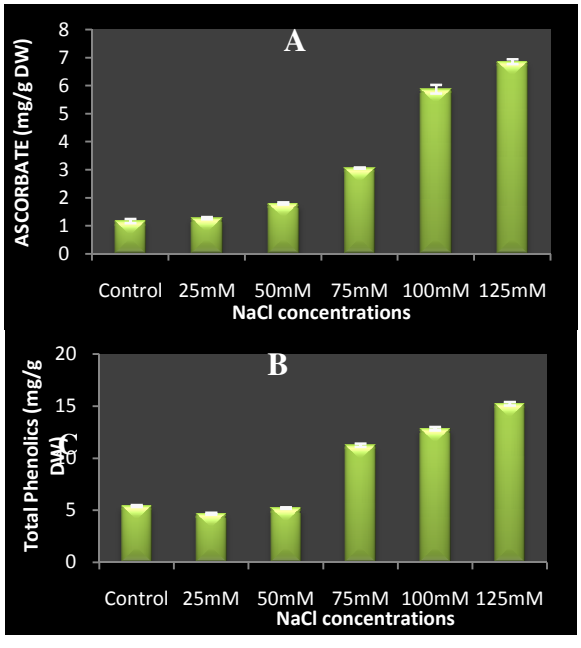
Ascorbate

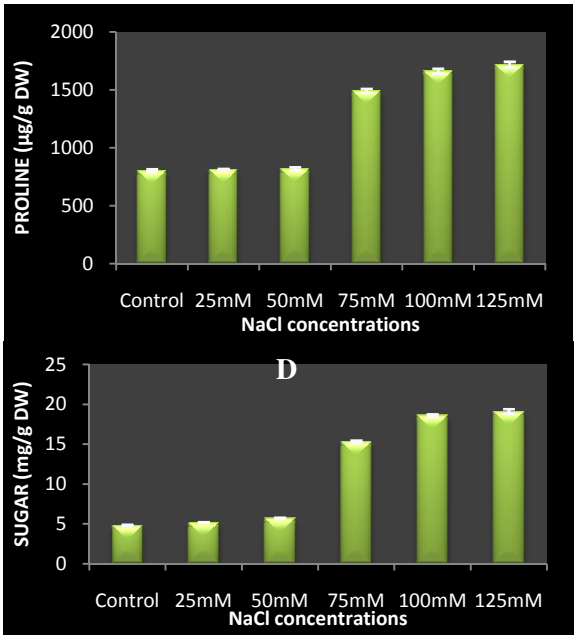
Increased accumulation of ascorbate content was recorded in NaCl treated rice seedlings. A continuous increase of ascorbate content was observed in rice seedlings subjected to 25 to 75 mM NaCl treatment. After 75 mM, ascorbate accumulation increased rapidly as compared to control (Fig. 3). Ascorbate is an ROS scavenger because of its ability to donate electrons in a number of enzymatic and non-enzymatic reactions. It can provide protection to membranes by directly scavenging the $O_2^{\bullet-}$ and $OH^{\bullet}$ and by regenerating α-tocopherol from tocopheroxyl radical (Gill and Tuteja, 2010). When the salt stress increases, the ascorbate content was found to be higher in rice seedlings (Moradi and Ismail, 2007), potato (Daneshmand *et al*., 2010) and maize (Abd Elgawad *et al.*, 2016).

Total phenolics

Significant elevation of phenol content was observed in rice seedlings with increase of NaCl concentration. Starting from treatment of 75 mM NaCl, there was steep increase in phenol content and it reached maximum at 125 mM NaCl treated rice seedlings (Fig. 3). The phenolics act as antioxidant scavenger of ROS in plants and thus inhibit lipid autoxidation (Daneshmand *et al.*, 2010). Increased concentration of NaCl enhances the higher accumulation of phenolics in rice (Chunthaburee *et al.*, 2014, 2015; Rajkumar 2013) wheat (Lim *et al*., 2012), strawberry (Jamalian *et al*., 2013), and black cumin (Bourgou *et al*., 2010).

Fig. 3. Ascorbate (A), total phenolics (B), proline (C) and sugar content (D) in rice seedlings subjected to various NaCl concentrations





Proline

The different concentrations of NaCl treatment enhanced the proline accumulation in rice seedlings. Gradual enhancement of proline accumulation was found in 25 and 50 mM NaCl treatments. However, above 50 mM NaCl, the proline accumulation rapidly increased upto 125 mM NaCl treatment (Fig. 3). Under salt stress, salt tolerant rice varieties exhibited several fold increase in proline content (Ghosh et al 2011). According to Rajakumar (2013), increased salt concentration enhances higher accumulation of proline in rice seedlings. Higher accumulation of proline content was reported in different rice varieties (Moradi and Ismail 2007; Amirjani, 2012; Kanawapee *et al.*, 2013; Rajkumar 2013; Chunthaburee *et al.*, 2016), and potato (Daneshmand *et al.*, 2010). Proline is an effective scavenger of $OH^{\bullet}$ and also an effective quencher of ROS formed under salt stress. Increased accumulation of proline content enhances the tolerance of plants against various abiotic stresses especially salt and drought (Gill and Tuteja 2010). It has been noted that salt stress increased the accumulation of proline in the leaves of two rice cultivars differing in salt tolerance (Demiral and Türkan, 2005). Proline has an important role in enhancing pentose phosphate pathway functions because this pathway is an important component of antioxidative defense mechanisms (Gill and Tuteja, 2010). The gradual increase of proline content in rice seedlings subjected to 25 and 50 mM NaCl could be to counter the slight osmotic changes in the external medium. Whereas, further rapid increase of this imino acid at treatments of higher NaCl concentration could be to counter the supra optimal stress situation by playing multiple roles of enhancing osmoticum, scavenging free radicals etc.

CONCLUSION

In this study, rice seeds were treated with different concentrations of NaCl. In rice seedlings priming effect of low concentrations of NaCl enhanced their physiological activities such as germination percentage, biomass, total chlorophyll content and carotenoid content.

Although low NaCl (25 and 50 mM NaCl) concentrations brought about optimal increases in metabolites such as total sugar, ascorbate, total phenolics and proline content, NaCl concentrations above 50 mM significantly enhanced the content of the above metabolites, which negatively affected the allocation of metabolites for growth related aspects. More studies on the priming effect of low NaCl concentration in rice can lead to exploiting this technique for enhanced growth and yield of this prominent crop.

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ISOLATION AND UTILIZATION OF STARCH FROM THE RHIZOMES OF SOME SELECTED *CURCUMA* SPECIES FROM KERALA

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Abstract: Plants have a major sustaining role in the lives of human beings, especially for their nutritional and medicinal value. Many wild edible plants including root tubers are under-utilized in spite of their higher nutritional value. Roots and tuber crops have a remarkable position in the food security of the developing world due to their high caloric value and carbohydrate content. About sixty percentage of the caloric requirement of human being is satisfied by starch, the predominant nutrient carbohydrate found especially in seeds or rhizomes of plants. Starch is a valued component of many foods and has an important role both as a macronutrient and structural component. The structure and physico-chemical properties of starch differ with its botanical origin. Starch is frequently isolated and is used in food industries to impart the desirable functional properties, and to modify food texture and consistency. The rhizomes of several species of *Curcuma* are being exploited by tribals and natives as source of edible starch. The genus *Curcuma* L. belongs to the family Zingiberaceae comprising over 1200 species of rhizomatous herbs and is endowed with widespread adaptation from sea level to altitude as high as 2000m. The genus *Curcuma* has over 120 species chiefly distributed in South East Asia. In India it is represented by around 29 species and in South India by 20 species. The acute toxicity studies proved the starch isolated from the *C. zanthorrhiza* Roxb. rhizome through traditional method as non-toxic. A preliminary characterization of starch from the rhizomes of six selected species of *Curcuma* is discussed in the present study. The yield of starch powder varies from species to species, season and the part used for isolation (mother rhizome or finger rhizome). Yield of starch showed variation in fresh, dormant and sprouted rhizomes. Seasonal rainfall variations also affected the starch yield in *Curcuma* species. The highest percentage of starch yield was recorded from *Curcuma aeruginosa* Roxb. (13.28%) which is comparable to the yield of 'arrowroot' *Maranta arundinacea* L., a popular source of edible starch.

Key words: *Curcuma*, Rhizome, Starch

INTRODUCTION

Most of the gingers are rhizomatous, and though some are sources of edible starch, very few species are exploited. Roots and tuber crops have a remarkable position in the food security of the developing world due to its high caloric value and carbohydrate content. Some of them are already cultivated, but others are grown wild as a neglected group of economic plants. Starch is the major storage carbohydrate in plants and is basically found in the seeds, fruits, tubers, roots and stems of various plants notably corn, potatoes, wheat and rice. Starch varies widely according to its source owing to its granular characteristics. Starches from different botanical sources also vary widely in structure and composition. All starches are made up of two polysaccharides, amylose and amylopectin typically in the ratio 20:80. But the ratio varies with the starch sources (Oxford *et al*., 1987). Starch has found immense

industrial use related to the manufacture of food, textiles, paper, adhesives, and pharmaceuticals. The industrial usage of starch is based on the adhesive, thickening, gelling, and film forming properties as well as its ready availability, low cost and controlled quality (Gebre-Mariam and Schmidt, 1996; Alabi *et al*., 2005).

Ethnic foods are savoured by people all over the world. In India large number of traditional foods are known to impart nutritional wellbeing to all age groups (Mala *et al*., 2015). Many wild plants form an important source of starchy food for the tribals inhabiting near to the forest tracts. Starch is a relatively cheap raw material with physical and chemical properties making it ideal in many food and non-food applications. Application of starch in food systems is preliminary governed by gelatinization, gelation, pasting, solubility, swelling and digestibility properties. The starch powders of *Curcuma* species have good potential in food application due to its high viscosity, gel strength and paste stability (Asem and Laitonjam, 2014). The swelling power of the starches from most of the *Curcuma* species was found to be comparable to the commercially important starches from *Dioscorea* and *Cassava.* Considerably high solubility of *Curcuma* starches indicates its potential as edible starch (Dan and Thaha, 2015).

Starch is a macro constituent of many foods, and its properties and interactions with other constituents are interests to the food industry. Starch is easily digestible and fine textured. It is used in many of the confectionary items and also consumed along with milk. Native people use different *Curcuma* species for starch extraction because of their medicinal properties (Vimala and Nambisan, 2005). The starch extracted from the rhizomes of *Curcuma aeruginosa* has been proved edible since long back (Sabu and Skornickova, 2003). The starch of *tikhur* (*C. angustifolia*) is used in the preparation of sweet meals and herbal dishes like halwa, barfi, jalebi, etc. It is highly nutritious and easily digestible and therefore, it is recommended for infants, weak children and invalids. This starch can be consumed by individuals during fast (*vrata*, *upwas*), as it is rich in energy. Farmers prepare the herbal drink "*sarbat*" using *tikhur* starch during summer due to its cooling effect (Singh and Palta, 2004). *Curcuma angustifolia* starch could be used as a promising pharmaceutical excipient in tablet technology as it showed adequate binding and disintegrating properties (Rajeevkumar *et al*., 2010). Limited species of C*urcuma* are still used by the natives and tribals in different parts of the country as a source of starch (Nedunchezhiyan *et al*., 2005). In the present study an attempt has been made to evaluate the yield and explore the potential of selected *Curcuma* species from Kerala as a source of edible starch.

MATERIALS AND METHODS

Sample collection

Mature fresh rhizomes of five species of the genus *Curcuma* were collected during its dormant season November to January from the established plants grown in the Medicinal Plant Germplasm of JNTBGRI, and one species from its natural habitat. To analyze seasonal variation, different accessions were collected in two different seasons, November to January and May to June.

Method of extraction

The method of extraction of starch from the rhizomes of *Curcuma* species is reported by Kokate (1994) but improved method was adopted here as ddtailed below.

Fresh *Curcuma* rhizomes were collected and washed thoroughly with water, scrapped off the outer layer and chopped into fine pieces, followed by grinding to get smooth paste. The paste was stirred well in water, decanted and the residue was washed repeatedly until the colour of the residue becomes pure white. The residual water was filtered off completely using a fine cotton cloth. The residue kept in a wide mouthed earthen pot was allowed to sediment overnight and later the residual water was decanted. The sedimented starch was well dried in sunlight. The starch powder was stored in airtight containers.

Starch recovery was calculated based on following formula:

$$\text{Starch recovery (\%)} = \text{Weight of extracted starch} \times 100 / \text{Weight of rhizomes taken}$$

Starch uilization

The utilization of starch as food and for cloth starching were carried out as per reviewed literature. Food items like '*kurukku*' and '*ilayappam*' were prepared using starch powder isolated from *Curcuma aeruginosa*, *Curcuma aromatica* and *Curcuma zanthorrhiza* along with suitable ingredients (jaggery, coconut, ghee, cardamom etc).

For cloth starching experiments, 10, 20, 30 and 40 g of *Curcuma aeruginosa* starch powder was boiled with 250 ml of water, cooled and diluted with half litre of water. Cotton cloth strips of uniform size were dipped, soaked for 5 minutes, squeezed and spread for drying.

RESULTS AND DISCUSSION

The percentage yield of starch in the genus *Curcuma* varied with respect to species, season and parts used for starch isolation. The effect of variation in monsoon on starch production was also detected. There is a wide variation in the percentage of starch yield in different *Curcuma* species collected during the normal season of maturity when the rhizomes become dormant devoid of aerial parts. i.e., November-January. The maximum starch yield was observed in *C. aeruginosa* as 13.28%, followed by *C. zanthorrhizha* as 10.40% and *C. zedoaria* as 9.14%. The lowest percentage of starch yield was observed as 2.00% in *C. longa* (Table 1). Percentage yield of starch production is lowered by 0.3% and 0.24% in the case of *C. aeruginosa* and *C. zanthorrhizha*, when the rhizomes were collected in off season when the rhizomes produced fresh aerial parts (Table 2). In the case of *C. zanthorrhizha*, the percentage starch yield for mother rhizome alone was detected as 10.41% and that of finger rhizome alone as 8.81%. Mother rhizomes showed better yield than finger rhizomes, when they were used separately for starch isolation (Table 3). Starch yield observed in *C. aeruginosa* and *C. zanthorrhizha* was reduced considerably in unexpected, prolonged monsoon in 2017 (Tables 4 & 5).

From the food preparation practices conducted using starch powder isolated from *C. aeruginosa*, *C. aromatica* and *C. zanthorrhizha* it was observed that, '*kurukku*' (a type of gruel) and 'ilyappam'(a steam cooked dish in folded banana leaves) of good quality can be

effectively prepared using these starch powders along with suitable ingredients. From the cloth starching trial conducted using *C. aeruginosa* it was observed that, 10g starch powder boiled with 250ml water, cooled and diluted with half litre of water was good enough for starching 1m cotton cloth piece nicely.

Table 1. Percentage yield of Starch from fresh rhizomes of *Curcuma* species collected during Nov.-Jan.

Sl. No.	*Curcuma* species	% of starch yield
1.	*C. aeruginosa* Roxb.	13.28%
2.	*C. amada* Roxb.	5.05%
3.	*C. aromatica* Salisb.	7.45%
4.	*C. longa* L.	2.00%
5.	*C. zedoaria* (Christm.) Roscoe	9.14%
6.	*C. zanthorrhizha* Roxb.	10.40%

Table 2. Percentage yield of starch of *Curcuma* species collected in different seasons

Sl. No.	*Curcuma* species	% of starch yield	
		Nov.-Jan.	May-Jun.
1.	*C. aeruginosa* Roxb.	13.28%	0.3%
2.	*C. zanthorrhizha* Roxb.	10.40%	0.24%

Table 3. Percentage yield of Starch of *C. zanthorrhizha* Roxb. from different parts

Sl. No.	*Curcuma* species	Nature of rhizome	% of starch yield
1.	*C. zanthorrhizha* Roxb.	Fresh mother rhizome	10.41%
		Fresh finger rhizome	8.81%

Table 4. Details of rai fall during seven months in consecutive years

Year	Rainfall parameters	May	Jun	Jul	Aug	Sep	Oct	Nov	TOTAL
2016	Rainfall (mm)	181.07	369.35	152.10	51.35	33.48	16	56.10	859.45
	Rainy days	29	30	29	23	25	7	22	165
2017	Rainfall (mm)	74.90	238.10	92.80	221	315	163	324.20	1447
	Rainy days	28	26	22	24	24	22	27	173

Table 5. Effect of unexpected monsoon in starch production in 2017

Sl. No.	*Curcuma* species	% of starch yield	
		Nov.-Jan. 2016	Nov.-Jan. 2017
1.	*C. aeruginosa* Roxb.	13.28%	5.63%
2.	*C. zanthorrhizha* Roxb.	10.41%	5.24%

Among the selected *Curcuma* species *Curcuma aeruginosa* and *Curcuma zanthorrhizha* showed above 10% of starch yield with respect to their fresh weight, which is comparable to the yield of 'arrowroot' (*Maranta arundinacea* L.), the most popular source of today's edible starch. It supported the study conducted by Vimala and Nambisan (2005). They observed 10-15% of starch yield in *Curcuma aeruginosa.* November-January is the best season for starch isolation when the rhizomes are fully mature and plant starts withering off its leaves indicating its dormant period. All the other seasons showed variation in starch yield. Negligible amount of starch was observed from the rhizomes of *Curcma aeruginosa* and *Curcuma zanthorrhiza* collected during May-June since the plant initiates the growth and establishment of leafy shoots. The results agree with the observations of Patel *et al*. (2015) that *Curcuma* sps. rhizomes are harvested normally during the months of November to January depending on weather and soil moisture conditions. The harvesting stage or the maturity of the rhizomes well indicated by the yellow coloured partially dried leaves was also indicated in their studies. In *Curcuma zanthorrhizha*, around 2% starch yield difference was observed, when mother rhizomes and finger rhizomes were used separately, where the mother rhizomes showed better yield than finger rhizomes, in agreement with the experiment conducted by Patel *et al*. (2015). But there was no appreciable difference in the quality of starch particularly the colour and fineness of the powder.

Usually in Kerala region the south west monsoon begins by May and usually lasts up to July-August which will be followed by north east monsoon during October. But in 2017, the initial monsoon diminished and by August it got strengthened and prolonged up to mid December (Table 4). This unexpected and extended rainfall disturbed the life cycle of *Curcuma* species. It adversely affected the starch yield in the case of *Curcuma aeruginosa*, and *Curcuma zanthorrhiza*, because off season monsoon triggered the sprouting of rhizomes soon after the yellowing of leaves, breaking the normal dormancy of the rhizomes. Though the rhizomes of both the species were collected during the same season and from same place in consecutive years, the yield of starch was found to be considerably low in 2017, due to prolonged off season monsoon extended till the end of November, and thus the plant continued its production of new shoots without undergoing usual dormant stage. The starch stored in the rhizome was probably used to generate large amount of energy required for sprouting, which would be the reason for reduced starch yield.

Starch powders of *Curcuma aeruginosa*, *Ccurcuma aromatica* and *Curcuma zanthorrhizha* along with suitable ingredients can be utilized as primary component for various healthy food items. The study by Sabu and Skornikova (2003) by interviewing several Ayurvedic practitioners found out that *Chavanaprash*, widely used as a rejuvenating tonic in

the form of paste contained nearly 16% of *Curcuma* starch. It is specially used to improve immunity, memory and general health. The study conducted by Nedunchezhiyan (2005) also reported that *Curcuma* starch was easily digestible and fine textured and was used in many of the confectionary items and also consumed along with milk. For children and patients, *Curcuma* starch is given along with milk during fever or other ailments for easy digestion and instant energy. The study conducted on *Curcuma angustifolia* by Abha *et al*. (2010) reported that the starch powder from some *Curcuma* sps. was used by tribals of India for the preparation of milk puddings. Observations from present study proved the utilization of starch isolated from *Curcuma aeruginosa*, *Curcuma aromatica* and *Curcuma zanthorrhizha* in the form of '*kurukku*' and '*ilayappam*', good healthy dishes.

The genus *Curcuma* act as an important source of starch. Except for cassava and to a smaller extent for sweet potato, starch form other tuber, root and rhizome crops has not been explored for industrial applications partly because of difficulty in extraction of pure starches and partly because of non-availability of information about properties of these lesser known starches (Deo *et al*., 2014). Modified starch could be used in textile industry for textile fabric finishing (*www.idealmanufacturing.com*). Our study strongly recommends *Curcuma aeruginosa* starch powder for starching cotton cloths.

CONCLUSION

Curcuma starch could offer valuable contributions to human diet regionally. The evaluation of the yield of starch from *Curcuma* showed that some of the species have optimum yield in specific season and can act as a carbohydrate reserve to support the food security of the country. Understanding the yield of different species, collecting season, seasonal variations and parts used for starch isolation, will lead to explore these under-utilized sources for large-scale production of starch and to recommend the cultivation and utilization of these by rural and urban society. Ample scope exists for these minor tuber crops for filling the probable lacuna arising in food shortage as well as for evolving as source of alternate food supplements.

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PRIMARY SEED CHARACTERIZATION AND EXTRACTION OF RUBBER (*HEVEA BRASILIENSIS*) SEED OIL

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Abstract: At present, India is ranked 5th in global rubber production behind Thailand, Indonesia, Malaysia and Vietnam. India is currently holding 8.49 lakh hectares of rubber cultivation, to which Kerala contributes a major share (80%). Rubber seeds are available in abundance and oil from the seeds is a secondary product and possesses several potential industrial applications in cosmetic, oil and oleochemical sectors. The main goals of this study were to provide preliminary characterization of the rubber seed and assessment of oil extraction from rubber seed oil (RSO). The present investigation was carried out using the seeds of RRII 105 clone of rubber collected from a rubber plantation maintained in University of Calicut. In this study, soxhlet extraction was used to extract RSO, which contains high content of Free Fatty Acid (FFA). Four different solvents were used for the extraction of RSO such as n-hexane, petroleum ether, ethyl acetate and methanol to identify the best solvent to extract RSO, so that maximum oil yield can be obtained. Temperature was set depending on the boiling point of the solvent. Oil recovery was done using rotary evaporator and the oil stored at 4°C for use in further analysis. RSO is golden yellowish in color and has a pleasant nut like odour. In the case of extraction using non-polar solvents, hexane gave higher oil yield of 39.5% at a time of 3h (30 cycles). To determine the acid value (45.32 mg KOH/g oil) and FFA content (22.66), RSO was compared with different vegetable oils such as rice bran oil, palm oil, sunflower oil, olive oil and groundnut oil. The present study provides good background information for the use of RSO as a raw material for biodiesel production.

Key words: Rubber seed oil, Soxhlet extraction, Acid value, FFA

INTRODUCTION

India produces abundant quantities of vegetable oils like coconut oil, sunflower oil, palm seed oil, cotton seed oil and groundnut oil which have been identified as edible oils. Major percentages of the versatile crops produced in the country include *Hevea brasiliensis* Müll.Arg.or para-rubber among others. This economically cultivated perennial crop belongs to the family *Euphorbiaceae* and is native to Brazil. As per the reports of India Rubber Meet 2016, Goa, India is the sixth largest producer and fourth largest consumer of natural rubber in the world. In India, rubber plantations are mainly concentrated to the southern region especially in the state of Kerala (approximately 80%). It is primarily used for the production of latex as a source of natural rubber for the production of various rubber products in use globally, while rubber seeds are also of importance. The oil obtained from the underutilized seeds of the plant is the secondary product after the latex (Onoji et *al*., 2016; Takas *et al*., 2015).

A rubber plantation is capable to produce about 2 tonnes of rubber seeds per hectare per year (Ikwuagwu *et al*., 2000). Several methods are employed for rubber seed oil extraction

such as mechanical pressing, solvent extraction, soxhlet extraction and their combinations. Mechanical pressing is widely used domestically with high running costs; but oil yield is very less (Subroto *et al*., 2015). In the oil extraction process using mechanical methods, around 18% yield is obtained while the oil content in the rubber seed is around 40-50% (Kyari, 2008). Soxhlet extraction method is practiced at industrial level which gives higher oil yield at low operating cost and shorter time (Reshad *et al*., 2015). Recent studies show that soxhlet extraction using n-hexane as solvent is practiced now because of its inherent advantages such as higher oil yield, low operating cost, shorter time, reduced volume usage and lower turbidity (Aigbodion and Pillai , 2000; Li *et al.*, 2013; Dos Santos *et al.*, 2008; Kostić *et al*., 2013). Rubber seed oil (RSO) has several industrial applications and is mainly used as lubricant in industrial motors and paints and coating formulations. Other uses include synthesis of biodiesel, cosmetics and pharmaceutical products (Iyayi *et al*., 2007). Hence, this study mainly involves the extraction and characterization of rubber seed oil from rubber seed.

MATERIALS AND METHODS

Materials

Analytical and bacteriological grade chemicals purchased from Himedia (India) and Merck India Ltd. were used for this study. Rubber seeds of RRII 105 (Rubber Research Institute of India 105) clone were procured from a plantation in University of Calicut, Kerala (11.1340° N, 75.8952° E) for oil processing.

Primary seed properties and extraction of oil

Collected rubber seeds were decorticated manually using a hammer to free the kernel from the shells. Shell and kernel were weighted separately to find out the seed to kernel ratio. The kernels were dried in hot air oven at 70°C for 4h. Dried kernels were kept in air tight plastic containers to avoid contamination. The kernels were milled using a laboratory blender.

Moisture content determination

Ten gram of the milled kernel was weighed in triplicate using an analytical balance and then placed in a thermostatically controlled oven at 105^{o}C for 24h. The samples were weighted after every 3h interval and proceeded until obtaining constant weight. Loss in weight of the sample was recorded and moisture percentage was calculated by the following equation.

$$\% \text{ Moisture content} = \frac{\text{Loss in weight (g)}}{\text{Weight of sample (g)}} \times 100$$

Extraction of oil

Forty gram of the milled sample was bagged in a cellulose thimble and fixed in a Soxhlet apparatus (Fig. 1). 150ml of the solvent was poured into clean dry round bottom flask equipped with heating mantle. Heating mantle was adjusted to 68^{o}C. Extraction was continued for 3h. The oil plus solvent mixture was collected and separated using rotary evaporator and refluxing at 70^{o}C for 30 min. Collected the solvent and oil separately and the

oil was stored in cold room (4^{o}C) for further analysis.

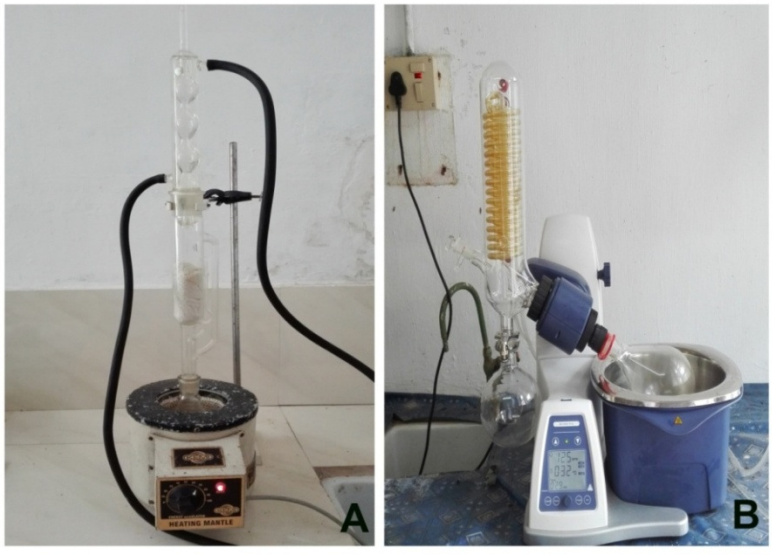
Choice of solvent for oil extraction

Polar (ethyl acetate and methanol) and non-polar (*n*-hexane & petroleum ether) solvents were used for this study. 150ml of the solvent was poured into 250ml RB flask and temperature was adjusted on the basis of boiling point of each solvent. Extraction was continued for 3h/50 cycles. At every 10 cycles extraction mixture was collected and removed. Subsequently the oil was separated using a rotary evaporator and further kept in oven at 110^{o}C for 30 min. Collected oil lots were purified using 0.2µm microfilter. Oil yield was calculated using the following equation (Ebewlel *et al*., 2010).

Calculation for Oil yield

$$\text{Oil yield (\%)} = \frac{\text{Oil Collected (g)}}{\text{Mass of Rubber seed Wt. (g)}} \times 100$$

Fig. 1. Extraction of oil: (A) Soxhlet apparatus; (B) Rotary evaporator.



Characterization of RSO

Determination of acid value and free fatty acid

One gram of the oil was weighed into a clean dry conical flask. Added 20 ml isopropyl alcohol and stirred until it completely dissolved. Phenolphthalein (2 drops) was added as indicator and titrated against 0.1N KOH solution. The volume for the end point appeared with pink colour and was recorded. Acid value was calculated using the following equation (Asuquo *et al*., 2012). RSO was compared with different vegetable oils such as rice bran oil, palm oil, sunflower oil, olive oil and groundnut oil.

$$\text{Acid value} = \frac{\text{N. KOH} \times \text{Eq. wt. of KOH} \times \text{V. KOH}}{\text{Weight of RSO used}}$$

Fourier transform infrared spectroscopy (FTIR)

RSO sample (1g) was mixed with spectral grade of anhydrous potassium bromide (KBr) and fixed on a sample holder for analysis. FTIR spectroscopic analysis of the sample was carried out at mid infrared region of 400-4000 cm^{-1} (Jasco FTIR 4100 series, Japan).

Determination of fatty acid profile

The high FFA containing aliquot (1µL) was diluted with commercial grade n-hexane (9 µL) and then injected (220°C) in split less mode to a gas chromatograph (Agilent, 7697A, USA) equipped with a DB-WAX capillary column (Agilent, 30m × 250µm, 0.25µm film thickness) and mass spectrophotometer (Agilent, 5973N). High purity grade Helium was used as carrier gas (1mL/min). Separation was performed on the basis of temperature programme. The programme started from 50°C (5min), rising to 65°C at a rate of 2°C/ min and then to 200°C (5°C/min, 5 min) and 250°C (10°C/min) and that was held for 10min. The retention time and mass spectra of the compounds were identified using Wiley 7n.1 database. The area percentage method was used to estimate the fatty acid compositions of the oil samples (Sanjel *et al*., 2014).

RESULTS AND DISCUSSION

Primary seed characterization

From Table 1 it is evident that the rubber seeds are brown colored and weighed from 3.68g to 5.039g and enclosed kernels with 2.43g to 3.41g of weight which is 66.03–67.67% of the total seed weight. However, average weight percentage of the kernel is 66.67% of seeds. In this study, rubber seeds showed 3.45% water content. Moisture content is the amount of water contained in a sample, such as in fruit, seeds or wood. It was determined by measuring the mass of rubber seeds before and after the water was removed by evaporation (Abdul Shokib *et al*., 2010). Low moisture content is an indication of good shelf life for the oil. Low moisture content of oil might be as a result of effectiveness of the rotary evaporator apparatus used for recovering the oil.

Table 1. Primary rubber seed properties of cultivar RRII 105

Properties	Observation
Colour	Mottled brown
Seed size (cm)	2.5-3.5
Seed weight (g)	3.68-5.03
Kernel weight (g)	2.43-3.41
Seed-kernel ratio (%)	66.03-67.67
Moisture content of kernel (%)	3.45

Oil yield

The extracted oil was transferred into a clean dry beaker, which was further heated with hot air oven (for 30min at 70°C) to ensure complete removal of the solvent and volume of the oil was noted and expressed as percentage of oil content (Fig. 2). Optimum yield was recorded by the usage of n- hexane (39.45 %). n-hexane and petroleum (34.05%) ether gave

higher oil yields than the polar solvents like ethyl acetate (30.52%) and methanol (26.85%). Moreover, non-polar oil of rubber seed is easier to be extracted by n-hexane. Hence, the present study shows that usage of n-hexane is more effective and the solvent is cheap, with high solubility and with low evaporation rate. After every 10th cycle, the oil was collected and percentage of yield was calculated. The maximum yield was obtained from the extraction by soxhletation with n-hexane on the 30th cycle of circulation (39.45%) (Fig. 3). From this, it is implied that oil yield is increased initially with increasing number of circulations and steady with increase in number of circulations.

Fig. 2. Effect of solvents for oil extraction; maximum oil yield was obtained from n-hexane

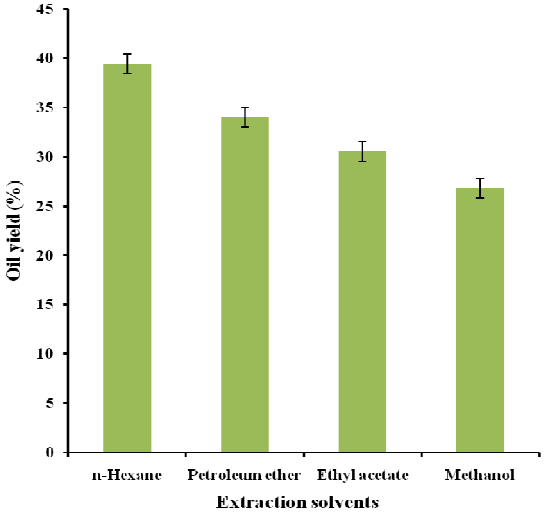
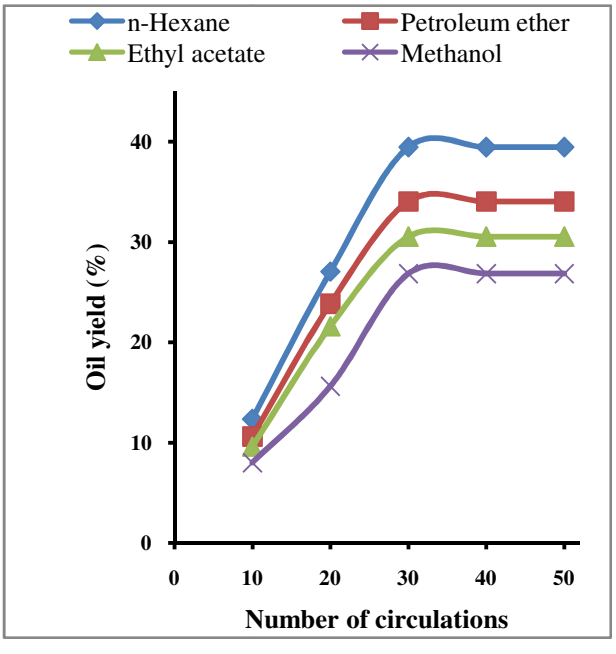


Fig. 3. Effect of solvent circulation; optimum yield was obtained in the 30th cycle



Characterization of RSO

Acid value and FFA

After cleaning, the rubber seeds were kept in cold room and the oil was extracted by soxhlet extraction method. The acid value was determined following standard protocol of EN 14104 titration method. The acid value of RSO was found as 45.32mg KOH/g oil. Free fatty acid content was calculated as 22.66 (half of acid value). At the initial stage it was less but increased with the time of keeping the rubber seeds before extracting oil. The freshness of the oil is related to acid value of the feedstock, while the feedstock/seeds may generate free fatty acids during longer storage due to enzyme activities. Hence the acid value becomes one of the important quality targets to determine the purity of oil. Acid value measures the degree of unsaturation of oil. It corresponds to the amount of potassium hydroxide needed to neutralize the free fatty acids. (Pianthong and Thaiyasuit, 2009). Comparison of the acid value of RSO with that of other commercial oils like rice bran oil, palm oil, sun flower oil, olive oil and ground nut oil (Table 2) showed that RSO had high acid value. This indicates the presence of higher content of unsaturated fatty acids. The acid value (45.32) obtained in this study is similar to that obtained at RRIN (43.62) (Abdullah and Salimon, 2009). The lower the acid value of an oil, the lower is the presence of free fatty acids which makes it less exposed to the phenomenon of rancidification (Asuquo *et al*., 2010) (Tabe 2).

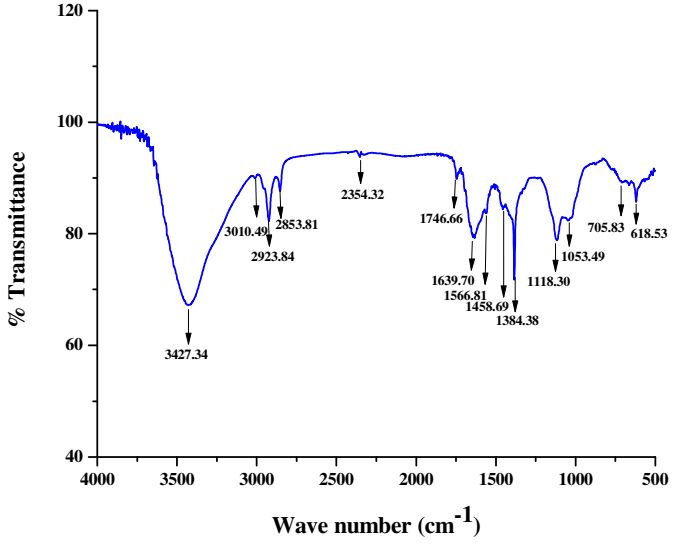
Table 2. Comparison of acid value of RSO and commercial oils

Sample oil	Acid value (mg KOH/g)	FFA
Rice bran oil	1.73	0.865
Palm oil	0.966	0.483
Sunflower oil	7.645	3.822
Olive oil	0.716	0.358
Ground nut oil	0.477	0.238
Rubber seed oil	45.32	22.66

FTIR analysis

The structural characterization of the extracted RSO reveals its chemical nature as well as the compositional identity and it was carried out by FTIR spectroscopy. FTIR clearly indicated the presence of peaks characteristic to the unsaturated fatty acids. For instance, the broad and significant peak at 3010-3427 cm^{-1} corresponds to –OH stretching of the glycolipid; whereas multiple peaks at 2923-2853 cm^{-1} indicate the aliphatic CH_3, CH_2 vibrations. The major peak at 1746 cm^{-1} is contributed by the C=O group due to the functional ester group, and the vibration at 1639 cm^{-1} indicates the presence of COO– in the sample. Similarly, the peaks at 1458-1384 cm^{-1} correspond to the bending vibrations of –OH on carboxylic group; whereas peaks at 1050-1118 cm^{-1} stand for the C–O–C vibrations that can be used as an analytical tool to detect RSO adulteration (Choe and Min, 2007; Ogbu *et al*., 2016). Spectrum shows that triglyceride (TG) was the main component of RSO. The FTIR spectrum represents a fingerprint region of 1458–618 cm^{-1}.

Fig. 4. FTIR spectrum of rubber seed oil



Fatty acid profile of RSO

Vegetable oils are mainly triglycerides of three fatty acid chains with a glycerol backbone. The fatty acid composition of the RSO is shown in Table 3. It can be seen that the refined RSO comprises of linoleic acid (33.43%), oleic acid (29.73%), linolenic acid (15.90%), palmitic acid (12.59%), and stearic acid (8.35%) which agrees with the result of previous studies (Ramadhas *et al*., 2005). The total unsaturated fatty acid content of the RSO is found to be 79.06%.

Table 3. Fatty acid composition of rubber seed oil

Fatty acid	Formula	Area (%)
Palmitic acid ($C_{16:0}$)	$C_{16}H_{32}O_2$	12.59
Stearic acid ($C_{18:0}$)	$C_{18}H_{36}O_2$	8.35
Oleic acid ($C_{18:1}$)	$C_{18}H_{34}O_2$	29.73
Linoleic acid ($C_{18:2}$)	$C_{18}H_{32}O_2$	33.43
Linolenic acid ($C_{18:3}$)	$C_{18}H_{30}O_2$	15.90

CONCLUSION

Rubber (*Hevea brasiliensis*) seed oil is golden yellowish in colour and has a pleasant nut like odour. It contains moisture of 3.45% and extraction using non-polar solvents gives higher oil yield. Besides, the best extraction time is 3 hours/30 circulations and the yield is 39.45%. The physicochemical properties obtained in the present study agreed with reported values in literature. The high content of unsaturated fatty acids (79.06%) and moderately low content of saturated fatty acids (20.94 %) adversely affect the shelf life of the oil compared to other oils. Closer examination of results of GC-MS shows a satisfactory agreement that rubber seeds have an insignificant proportion of polyunsaturated fatty acids (linoleic, linolenic, etc.). If RSO like vegetable oils could be used for industrial applications including

biodiesel production, it would provide additional source of income to farmers as well as it would be a boost to the rubber industry.

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SUSTAINABLE UTILIZATION OF *CINNAMOMUM* SPECIES FROM THE WESTERN GHATS OF INDIA

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Abstract: The genus *Cinnamomum* is an important group of aromatic plants in the Western Ghats, with 25 species reported from the region, of which 16 are endemic. The present study evaluates the potential utility of *Cinnamomum* species from the Western Ghats as source of valuable aroma compounds. Volatile chemical analysis of 21 *Cinnamomum* species (*C. agasthyamalayanum, C. alexei*, *C. chemungianum, C. dubium, C. filipedicellatum, C. heyneanum, C. keralaense, C. litseifolium, C. macrocarpum, C. malabathrum, C. mathewianum, C. mohanensis, C. nicolsoniamum, C. nilagiricum, C. palghatensis, C. riparium, C. sulphuratum, C. travancoricum, C. verum, C. walaiwarense* and *C. wightii*) from the Western Ghats was done by GC-MS and GC-FID with regard to the distribution and potential utility of the aroma compounds. Monoterpenoids and sesquiterpenoids respectively were the predominant volatile chemicals in the *Cinnamomum* species studied, followed by phenyl propanoids. The study led to the discovery of novel natural sources of aroma compounds such as camphor (*C. nilagiricum*), linalool (*C. malabathrum*), safrole (*C. alexei*), and benzyl benzoate (*C. sulphuratum*). The present discovery of the wild native plants as source of economically important chemicals highlights the importance of the floristic diversity of the Western Ghats and its inventorisation and sustainable utilization.

Keywords: Western Ghats, *Cinnamomum* species, Essential oils, Aroma compounds

INTRODUCTION

The genus *Cinnamomum* (Lauraceae) is represented by 250 species in the world and 45 species in India. The Western Ghats hosts 25 *Cinnamomum* species of which 16 species are endemic to the region (Kostermans 1983; Nayar *et al*., 2014; Remyakrishnan *et al*., 2014). *Cinnamomum* species are well known for their aromatic nature and essential oil potential. *Cinnamomum* species of economic importance in India are *C. verum* (Syn. *C. zeylanicum*), *C. tamala, C. bejolghota, C. impressinervium* and *C. malabathrum*. Dried inner bark of *C. verum* (cinnamon) is a widely used spice all over the world. Volatile chemical studies on *C. verum, C. camphora, C. tamala, C. pauciflorum* and *C. glanduliferum* have been carried out from North East India (Ravindran *et al*., 2004; Baruah and Nath 2008). Among the 25 *Cinnamomum* species in the Western Ghats, only a few species like *C. verum, C. malabathrum, C. sulphuratum, C. chemungianum, C. filipedicellatum, C. agasthyamalayanum* and *C. heyneanum* have been studied in detail for their volatile chemicals (Rameshkumar *et al*., 2007; Sriramavaratharajan *et al*., 2015). Previous studies show that essential oils of *Cinnamomum* species are sources of bioactive aromatic compounds such as eugenol, linalool, safrole, benzyl benzoate, methyl cinnamate and cinnamaldehyde (Jayaprakasha and Rao, 2011). *Cinnamomum* species show biogeographic variation in their secondary metabolite content which results in the occurrence of various chemotypes for same species. Three

chemotypes (eugenol type, cinnamaldehyde type, linalool type) were reported for *C. tamala* and four chemotypes (linalool type, cinnamaldehyde type, methyl cinnamate type, citral cinnamaldehyde type) were reported for *C. sulphuratum* from North East India (Baruah and Nath, 2008; *Kumar et al*., 2012), while the benzyl benzoate type of *C. sulphuratum* was reported from southern Western Ghats (Rameshkumar *et al*., 2006). The essential oils and aroma compounds from *Cinnamomum* species exhibit potential biological activities such as antimalarial, antibacterial, antifungal, immunomodulatory and anti-inflammatory (Jayaprakasha and Rao, 2011).

The Western Ghats host 25 *Cinnamomum* species and so far essential oil studies on only ten species have been reported. In this context, the present paper describes the diversity of volatile chemicals of 21 *Cinnamomum* species occurring in the Western Ghats, their inventorisation and sustainable utilization.

MATERIALS AND METHODS

Plant material

Fresh leaves of *Cinnamomum* species were collected from different parts of southern Western Ghats of India. The species were identified by Dr. E. S. Santhosh Kumar, JNTBGRI, Thiruvananthapuram. Voucher specimens of the collected accessions were deposited in the herbarium of Jawaharlal Nehru Tropical Botanic Garden and Research Institute (TBGT).

Isolation of essential oils

Fresh leaves (100 g each) of the *Cinnamomum* species were subjected to hydrodistillation using a Clevenger-type apparatus for 4 h. The essential oils collected were dried over anhydrous Na_2SO_4 and kept at 4^0C in sealed vials until analysed. Oil yields of each species are specified in Table 1.

Essential oil analysis

GC-FID and GC-MS analysis of 1 µL of the leaf oils (1:50 dilution in diethyl ether) were carried out on Shimadzu TQ triple quadrupole gas chromatograph fitted with a Cross bond 1,4-bis (dimethyl siloxy) phenylene dimethyl polysiloxane Rxi-5 Sil MS capillary coupled with a 8030 series mass selective detector (Shimadzu, Japan). The analyses were repeated thrice under similar experimental conditions. Relative percentages of individual components were obtained from the peak area percent report of volatiles from GC-FID data.

The essential oil components were identified by comparison of their retention indices (*RIs*) in a Rxi-5 Sil MS capillary column calculated using standard series of C_8-C_{30} hydrocarbons (Aldrich Chemical Company, USA), by Wiley 275.L and NIST 05.L database matching and by literature comparison (Adams 2007).

RESULTS AND DISCUSSION

Essential oil yield and composition

Leaf essential oil yield of *Cinnamomum* species varied from 0.1 %v/w to 2.2 %v/w. Essential oil analysis through capillary GC-FID and GC-MS led to the identification of economically important aroma compounds (Fig.1, Table 1). Monoterpenoids,

sesquiterpenoids and phenylpropanoids derived from DOX, MVA, and shikimate pathways respectively were the major class of compounds in the essential oils.

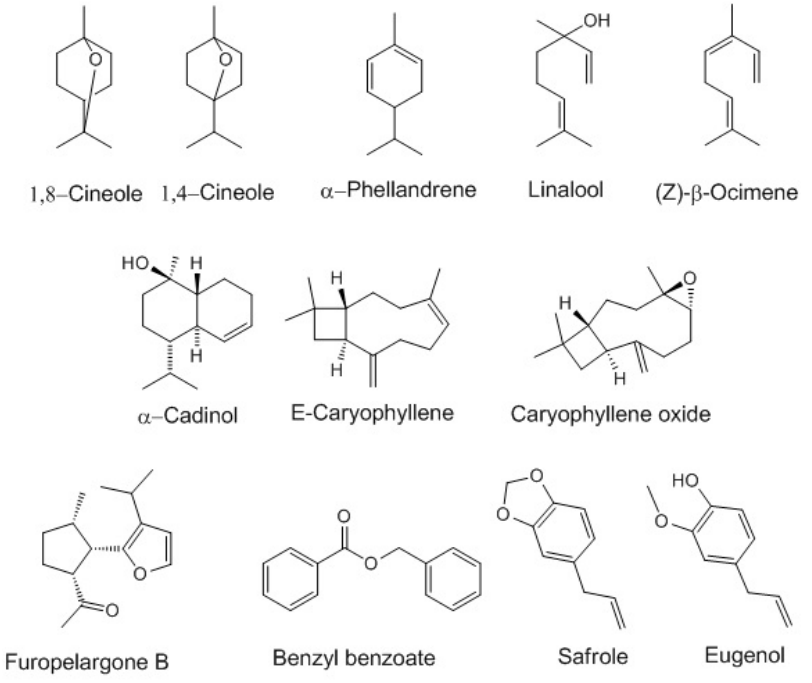
Table 1. Major compounds identified from essential oils of *Cinnamomum* species

Sl No	*Cinnamomum* species	Major compounds
1	*C. agasthyamalayanum*	β-Phellandrene
		Eugenol
2	*C. alexei*	Safrole
3	*C. heyneanum*	Safrole
4	*C. mathewianum*	Safrole
5	*C. walaiwerense*	Safrole
6	*C. chemungianum*	β –selinene
7	*C. dubium*	caryophyllene oxide
8	*C. filipedicellatum*	Cryptone
		Cuminaldehyde
9	*C. keralaense*	α-Cadinol
		epi- α-Cadinol
10	*C. litseifolium*	α- Phellandrene
		α-Cadinol
11	*C. malabathrum*	Linalool
12	*C. mohanense*	1,8-Cineole
		α-Terpineol
13	*C. palghatensis*	Bicyclogermacrene
		E-Caryophyllene
14	*C. riparium*	Shyobunol
		α-Cadinol
15	*C. sulphuratum*	Benzyl benzoate
16	*C. travancoricum*	α- Terpinene
		Z-β-Ocimene
17	*C. verum*	Eugenol
18	*C. wightii*	1,4- Cineole
		1,8-Cineole

Major compounds identified from *C. agasthyamalayanum* and *C. travancoricum* were the monoterpene hydrocarbons, *β*-phellandrene (33.2%) and (Z)-*β*-ocimene (31.1%) respectively, while in *C. mohanense* and *C. wightii,* oxygenated monoterpenes 1, 8-cineole (50.1%) and 1,4-cineole (59.4%) respectively were the major constituents. *C. litseifolium* was marked by high content of the monoterpene hydrocarbon, *α*-phellandrene (32.1%). Volatile oils containing 1,8-cineole and its derivatives with characteristic spicy taste and aroma are used in confectioneries, beverages, insect repellents, and also in aromatherapy (Ravindran *et al*., 2004; Sacchetti *et al*., 2005). Oxygenated sesquiterpenes contributed to the major part of essential oils of *C. dubium*, *C. keralaense* and *C. riparium*. *C. dubium* was rich in caryophyllene oxide, humulene epoxide II and germacra-4(15),5,10(14)-trien-1-*α*-ol (64.4, 6.7 and 5.9% resp.), while in *C. keralaense, epi-α*-cadinol, and *α*-cadinol (10.3, and 24.4% resp.)

were in higher proportions. In *C. riparium* α-cadinol (12.5%) and shyobunol (22.0%) were the major volatile compounds. Sesquiterpene hydrocarbon derivatives, bicyclogermacrene (25.0%) and (E)-caryophyllene (12.9%) were characteristic to *C. palghatensis*.

Fig. 1. Structures of major compounds identified from *Cinnamomum* species



Unique furan derivatives furopelargone A (5.1%) and furopelargone B (27.1%) present in *C. macrocarpum* were reported for the first time from *Cinnamomum* species. The phenyl propanoid safrole was the major compound in *C. alexei* (95.4%) and also in *C. mathewianum* (93.2%). Essential oil analysis of *C. heyneanum* from the Western Ghats also revealed high content of safrole (93.3%); however, the oil yield was very less (0.2 %v/w) compared to *C. alexei*. In *C. verum* and *C. sulphuratum,* eugenol (94.6%) and benzyl benzoate (88.5%) were the prominent constituents.

The findings were in agreement with that of earlier reports of these species (Ravindran *et al*., 2004; Rameshkumar *et al*., 2006). Benzyl benzoate and eugenol have been reported as antimicrobial, antifungal, acaricidal and insecticidal. The present study revealed that *Cinnamomum* species can be considered as alternative sources of the bioactive and aromatic compounds such as camphor, linalool, 1,8-cineole, eugenol, safrole and benzyl benzoate which finds use as antioxidants, antimicrobials, antifungals, insect repellents, condiments and are of high demand in international trade (Hiltunen and Holm, 1999; Maciel *et al*., 2010).

CONCLUSION

The present investigation on essential oil profiling of *Cinnamomum* species in the Western Ghats indicates that the genus is a rich repository of high value aroma compounds

like linalool, 1,8-cineole, eugenol, safrole and benzyl benzoate. The information can be used to support their possible application in developing new perfume concoctions, food additives and medications. The study also highlights the potential of the floristic wealth of the Western Ghats in India, which hosts diverse *Cinnamomum* species, and the need for its conservation and sustainable and effective utilization.

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