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POTENTIAL IMPACTS OF VARIOUS COASTAL LOCALES ON THE PHYTOCHEMICAL LANDSCAPE IN SAND DUNE FLORA *CALOTROPIS GIGANTEA* WHITE ACROSS THE COLEROON VALLEY

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Abstract

Calotropis gigantea has been well recognized as sand dune flora in the conservation aspect of coastal biota. However, little is known about the eco-geographical diversities in the phytochemical composition of white floral variety. The present study deals with gas chromatography mass spectrometry (GC-MS) phytochemical profiling in methanol extracts of white flower collected from seven different coastal locations across Coleroon valley. The intraspecific diversity was observed in 37 phytoconstituents whereas 23 common profiles were found in *C. gigantea* white. The most dominant classes were found as alcohols, terpenoids, fatty acid esters, aliphatic hydrocarbons, and amines. The major common peaks included n-hexadecanoic acid and glycerin (13.2%), A'-neogammacer-22(29)-en-3-ol, acetate, (3.beta., 21.beta.) (10.42%), 12-oleanen-3-yl acetate, (3.alpha.) - (10.28 %), Hop-22(29)-en-3-ol, acetate, (3.beta.) - (9.83%), .beta.-Amyrin (7.53 %), 3-ethoxy -1, 2, propane- diol (15.82%) and ethyl Oleate (7.07%). The location-specific compounds such as erythritol, tetrahydrocannabinol carbonic acid-D3-HFBA-PFPOH-derivative (THC), 1-nitro-propanol, oleic acid, Urs-20-en-3-ol, (3.beta. 18.alpha.19.alpha), thiodiglycol, mercaptamine were obtained with major peaks from the respective locations (L1-L7). To the best of our knowledge, this is the first screening study in coastal white vegetation respective of various locations and the first report of a psychoactive compound THC with 98.28% peak area in *gigantea* species. From this preliminary approach, we found richness in diversified and common phytochemicals that may uncover the biological underpinnings of medicinal property at realistic ecological and geographical scales.

Key words: Coastal locales, *Calotropis gigantea,* white flower, GC-MS screening, THC-derivative

Introduction

Calotropis gigantea white flower shrub which belongs to Asclepiadaceae family, commonly known as giant milkweed, has been chiefly seen in rural home yards and temples in northern and southern parts of India (Singh *et al.,* 1996). Generally, it has become naturalized in dry tropical regions as well as sandy coastal habitats along the sheltered shores of the lagoons. In the recent past, it has been well recognized as sand dune flora in the conservation aspect of coastal biota. A study conducted at Coleroon delta throws some light on the importance of coastal mangrove vegetations as concrete sea wall structures, suggesting *Calotropis gigantea* and *Calotropis procera* as more suitable species to mitigate the effect of the tsunami (Kathiresan and Rajendran, 2005). Another dune floral survey was done in the coastal Cuddalore region mentioning *gigantea* species are characteristic in supporting the dunes with their roots, anchoring them temporarily in place, and tend to expand the dune formation while their leaf trap sand. (Arulmoorthy and Srinivasan, 2017).

The sand dune flora produces clusters of waxy flower (white) helps to relieve asthma due to its analgesic activity and also has been reported for its anti-microbial action and cytotoxicity (Habib and Karim, 2009). The biochemical investigations explicitly elucidate the antidiabetic activities proving *C. gigantea* white flower extract as a therapeutic target in diabetes research (Manivannan and Shopna, 2017). Thus the traditional healers suggested that, when using *Calotropis gigantea* for medication, white flowering should be preferred over purple flowering.

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Nonetheless, it is known for its various medicinal properties in the traditional folkloric health care system (Varier, 2003) and used to cure a variety of diseases; their floral phytochemical compositions with respect of eco-geographical variations are poorly understood especially in terms of medicinal values. Thus it forms a gap in understanding the diversity, ecological, functional and economical values for conservation of this coastal dune vegetation worldwide, especially in Indian coasts. Phytochemical analysis of medicinal plant parts is an important step in understanding the many biological activities in physiological systems (Joseph *et al*., 2013). The previous phytochemical studies on *Calotropis* white have bestowed with intraspecific diversity in different parts of the plant such as roots, bark and leaves consisting of hydrocarbons, fatty acids, terpenes and sterols (Shilpkar *et al*., 2007; Singh and Rastogi, 1972; Lhinhatrakool and Sutthivaiyakit, 2006 and Rasilingam *et al*., 2009). The results showed the presence of fourteen major compounds in the ethanolic extract of *Calotropis gigantea* Linn. that creates a platform to screen many bioactive constituents from traditional plants (Dhivya and Manimegalai, 2013). However, there is limited information on phytochemical variation in *Calotropis gigantea* white trait from different whereabouts leaving the question open, whether intraspecific diversity in plant secondary metabolites (PSMs) among different geographical locations can be inherently beneficial for future research.

Plant secondary metabolites (PSMs) are pervasive in plants and help them to maintain a labyrinthine balance with the environmental needs. Phytochemical diversity is a key component of functional diversity also highly variable in composition as well as quantity within and among individuals. This chemo diversity is resulted by the natural selection process which allows structural modifications on the basis of mechanistic stress. The underlying great variation permits the biochemical system to evolve rapidly in the number, evenness and quality of PSMs between individuals and groups (Moore *et al*., 2013). The yield and composition of plant secondary metabolites are often variable due to the interplay among several environmental factors such as edaphic, climatic, geographical altitude and latitude of growth and other topographical conditions (Purohit and Vyas, 2004; Rahimmalek *et al.,* 2009 and Pirbalouti *et al.,* 2011).

Plants at various altitudes tend to have adapted in order to overcome the stress conditions, thus conferring evolution in resistant genotypes. A significant chemodiversity within a narrow range of altitude in relation to the sea level was observed by Moses (2012) and Majuakim *et al.,* (2014) where the synthesis of phytochemical contents was strongly influenced by environmental factors such as soil pH and nutrient, rainfall, moisture, light intensity, and atmospheric temperature. Although researchers have acknowledged the influence of the surrounding environment on genetic and phenotypic diversity among species, the only recent approach has evidenced the ecological importance of variation within species. Besides, it demonstrated the pivotal role of two facets in diversity through intraspecific and species effects for an understanding of community and ecosystem dynamics (Roches *et al.,* 2018). The findings of Gololo *et al.,* (2018) demonstrated the effect of geographical location on accumulation of phytochemicals in the leaves of *S. Italica*. Richards *et al.,* (2015) showed that high phytochemical diversity not only enhances the variation in plant-associated insects, but also contributes the ecological predominance of specialized insect herbivores.

Thus the knowledge on the divergence of phytochemical profiles is not only useful in search of therapeutic curatives, but also reveals the novel and economic precursors to industrial applications. Hence taking the locational influence and significance of phytochemical diversity into consideration, the present study is entailed to determine the variations in the metabolite profiles of *Calotropis* white flower extracts from different peripheral coastal populations across the Coleroon valley.

Materials and Methods

Sampling site of plant materials

Fully matured white flowering vegetation of *Calotropis gigantea* growing in natural habitat was randomly collected in October month from the east coastal villages in and around Chidambaram at Cuddalore Taluk, the locale of latitude 11.39º N 7970º E longitude which is closer to the shorelines of the Bay of Bengal. It has a tropical climate and the average annual temperature and rainfall have been reported as 28.4°C and 1248 mm respectively in this coastal zone. Each population is located at least 2000 m apart from each other in the Coleroon (kollidam) river valley which is connected to the sea through an outlet supports a unique assemblage of marine and fresh water known as estuary; besides all sampling sites fall within 10000 m of driving scopes. Sampling was carried out at C. Thandeswaranallur (L1), Saliyanthoppu (L2), Kadavacheri (L3), Velakudi (L4), Vallampadugai (L5), Kollidam (L6) and Usuppur (L7) situated away from the sea at the distance range of 12790-15190 m (Table 1). The topography of the locations (Fig. 1) is almost plain nearby Pichavaram wetlands with second largest mangrove forests around and the elevation ranges

between 5.48 and 8.22 m a.s.1. from the sea level (Table 1). The flower specimens were authenticated at Centre for Floristic Research, Madras Christian College, Chennai, Tamilnadu.

Table 1: Geographic coordinates of *C.gigantea* white flower sampling sites (L1-L7) in Coleroon river valley (Bay of Bengal coast).

Sampling Locations	Latitude	Longitude	Altitude (m a.s.1)
L1	11°23'14.23" N	79°41'33.52 ''E	6.09
L2	11°21'51.78" N	79°41'16.14 ''E	5.49
L3	11°22'09.74" N	79°42'21.92 ''E	5.48
L4	11°21'23.68" N	79°42'05.94 ''E	6.11
L5	11°20'46.83" N	79°43'02.31 ''E	6.12
L6	11°19'51.83" N	79°43'12.72 ''E	8.22
L7	11°22'41.98" N	79°42'26.62 ''E	5.79

Preparation and extraction

Fresh aerial flowers were collected and washed thoroughly with distilled water in order to remove the dirt and other contaminations. The washed plant materials were dried under shade, at room temperature so as to retain the freshness of the flowers, and also to prevent the decomposition of the potential active compounds. The dried flowers were powdered using an electric pulverizer and stored in an airtight, dark, glass container to prevent photochemical reactions. The crude powder was subjected to extraction with analytical grade solvent of methanol (1g/5ml) under reflux for 5 h then cooled and centrifuged at 3000 rpm for 5 m. The supernatant thus obtained was transferred into the GC vial and injected into the GC-MS port.

GC-MS Identification of bioactive constituents

The compound profiles present in the extracts of *C.gigantea* flower were determined using gas chromatography-mass spectrometry (GC-MS) following the protocol of Karau *et al.,* (2014) reported earlier. GC-MS analysis was performed by using an Agilent 7890A GC gas chromatograph coupled to an Agilent 5975C N mass selective detector (Agilent Technologies, Germany) with a single quadruple mass spectrophotometer and an electron ionization source. The gas chromatograph is equipped with a wall coated open tubular ZB-5MS capillary column (30 m × 0.25mm × 0.25 µm composed of 5% phenyl polysiloxane); Quadruple and threshold temperature 150°C. The oven temperature was

Table 2: The relative abundance (%) of common bioactive constituents in methanol extract of *C.gigantea* white flower among seven populations.

S. No.	Area (%)	Relative abundance(%)	Common bioactive constituents	Molecular formula	Molecular weight (g/mol)
1	2.36	1.96	Tetraethyl silicate	$C_8H_{20}O_4Si$	208.32
2	0.57	0.47	2-Dodecanol	$C_{12}H_{26}O$	186.33
3	2.9	2.41	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.47
4	1.68	1.4	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.44
5	1.9	1.58	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308.51
6	4.17	3.47	9,12-Octadecadienoic acid, ethyl ester	$C_{20}H_{36}O_2$	308.49
7	7.07	5.88	Ethyl Oleate	$C_{20}H_{38}O_2$	310.51
8	1.56	1.2	Hentriacontane	$C_{31}H_{64}$	436.83
9	0.61	0.51	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.55
10	7.53	6.26	.beta.-Amyrin	$C_{30}H_{50}O$	426.71
11	10.28	8.55	12-Oleanen-3-yl acetate, (3.alpha.)-	$C_{32}H_{52}O_2$	468.75
12	1.01	0.84	Benzo[h]quinoline, 2,4-dimethyl-	$C_{15}H_{13}N$	207.27
13	1.21	1.01	2-Ethylacridine	$C_{15}H_{13}N$	207.27
14	1.42	1.81	Cyclotrisiloxane, hexamethyl-	$C_6H_{18}O_3Si_3$	222.46
15	1.52	1.27	Eicosane	$C_{20}H_{42}$	282.54
16	9.83	8.81	Hop-22(29)-en-3.beta.-ol	$C_{30}H_{50}O$	426.71
17	0.4	0.33	1-methoxy-3-(2) hydroxy ethyl nonane	$C_{12}H_{26}O_2$	202.33
18	6.13	5.1	Urs-12-en-24-oic acid,3-oxo-, methyl ester	$C_{31}H_{48}O_3$	468.71
19	15.82	13.15	3-ethoxy-1,2,propane- diol	$C_5H_{12}O_3$	120.15
20	13.22	10.99	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	255.42
21	3.81	3.17	6-Octadecenoic acid	$C_{18}H_{34}O_2$	282.46
22	13.2	10.97	Glycerine	$C_3H_8O_3$	92.09
23	10.42	8.67	A'-Neogammacer-22(29)-en-3-ol, acetate,	$C_{32}H_{52}O_2$	468.77
			(3.beta.,21.beta.)-		

programmed from 50°C for 3 min and then 10°C/min increased to 100°C for 3 min, then held at the temperature of 300°C for 4 min with the retention time of 35 min; Equilibrium time 0.5 min; Transfer line temperature 280°C. The ion source has set at 230°C and electron ionization at 69.922 eV. Helium was used as the carrier gas at a flow rate of 1 ml/min and an injection volume of 2 µL was employed (Split ratio of 5:1); injector temperature 250°C. The scanning range was m/z 35-700 with a minimum scan rate of 2.88 or 4 s^{-1}; EMV mode gain factor 1 and voltage was 506 v. The tentative identification of unknown volatile components was achieved by comparing the mass spectra with the database of known components stored in (National Institute of Standards and Technology-Mass Spectrometry (NIST-MS Search II) library. Software adapted to handle mass spectra and chromatograms was ChemStation.

The percentage (%) relative abundance of each compound in the plant extract was calculated by the formula (Abuto *et al.,* 2018) as shown below:

$$\%\,Relative\,Abundance = \frac{Peak\,area\,of\,the\,compound}{Total\,peak\,area\,of\,all\,compounds} \times 100$$

Results

Our present study reported 167 chemical profiles of compounds in total, comprising with qualitative and quantitative differences as well as similarities in wild *Calotropis gigantea* white flower collected from narrow altitude and latitudinal gradients in close proximity to the seashore. The chromatography peaks of identifying compounds from methanol flower extract, matched with a NIST spectral database are shown (Fig.2 a-g). Each location in this study, revealed the presence of a complex mixture of compounds varying from 14 to 35 (Fig. 3) and it can be arranged as L7(35)>L6(30)> L1(27)>L5(24)> L3(22)>L4(15)>L2(14) as per the total number of compounds eluted.

The 23 characteristic peaks shared by all seven white flower populations of *C.gigantea* from different loci found to be common were classified into different classes as aliphatic hydrocarbons, fatty acids and esters, terpenoids, heterocyclic compounds, alcohols and silicone derivatives based on their functional groups (Fig. 4). The percentage of chemical classes varied greatly for instance, the terpenoids and fatty acid esters (21.73%) were the most dominant, whereas heterocyclic compounds and silicon derivatives (8.69%) were the least dominant classes of common bioactive substances based on their relative abundance. The alcohols, fatty acids, and hydrocarbons were considerably abundant (13%) in the white flower extract. Also based on the area peaks, terpenoids (44.19%), alcohols (29.57%), fatty acids (18.71%) and fatty acid esters (16.65%) were found in high concentrations. The silicone derivatives and hydrocarbons were shown with peak area of 3.78% and 3.48%, while heterocyclic compounds were found in low concentrations (2.22%) respectively. The predominant

Table 3: Common phytochemicals from *C.gigantea* white flower populations with maximum peak area (%) and reported medicinal properties.

Maximum peak area (%)	Common phytochemicals	Nature of the compound	Bioactivities
7.07	Ethyl oleate	Fatty acid ester	Antibacterial (Ankita *et al*., 2015)
7.53	.beta.-Amyrin	Pentacyclic	Antibacterial,antioxidant,sedative, hypoglycemic,
		Triterpenoid	hypolipidemic effects, antiplatelet components
			hepatoprotective activities (Duke, 2015)
9.83	Hop-22(29)-en-3-ol,	Terpenoid	Antibacterial, anticancer (Kanika *et al*., 2016)
	acetate,(3.beta.)		
10.28	12-Oleanen-3-yl		Antioxidant, antibacterial, antiinflammatory
	acetate, (3.alpha.)-	Triterpene	antitumor activities, anti-diabetic, and anti-amylase
			inhibitor activities (Fabiyi *et al.,* 2012)
10.42	A'-Neogammacer		
	22(29)-en-3-ol, acetate,	Triterpenoid	Anticancer efficacy (Bishayee, 2011)
	(3.beta.,21.beta.)-		
13.22	n-Hexadecanoic acid	Fatty acid	Antiinflammatory drug for rheumatic
			symptoms (Aparna *et al.,* 2012)
13.22	Glycerine	Sugar alcohol	Acts as an osmotic diuretic in cerebral
			edema (Chaudhary, 2017)
15.82	3-ethoxy-1,2,		Renewable solvent for aquatic
	propane-diol	Fatty acid ester	bioindicators (Eduardo *et al.,* 2017)

plant metabolites are represented with their concentration (% Area), molecular formula (MF) and molecular weight (MW) and their relative abundance (% RA) (Table 2). Based on the concentration peaks, the major compounds were n-hexadecanoic acid and glycerin (13.2%), A'-Neogammacer-22(29)-en-3-ol, acetate, (3.beta.,21.beta.) (10.42%), 12-Oleanen-3-yl acetate, (3.alpha.)- (10.28 %), Hop-22(29)-en-3-ol, acetate,(3.beta.)- (9.83%), .beta.-

Table 2: The relative abundance (%) of common bioactive constituents in methanol extract of *C.gigantea* white flower among seven populations.

S. No.	Area (%)	Relative abundance(%)	Location-specific bioactive constituents	Molecular formula	Molecular weight (g/mol)
1	1.06	0.84	Methyl nonadecane	$C_{20}H_{42}$	282.57
2	0.58	0.46	Phytol	$C_{20}H_{4O}O$	296.53
3	2.22	1.76	4,4,6a,6b,8a,11,12,14b-Octamethyl- 1,4,4a,5,6,6a, 6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one	$C_{30}H_{48}O$	424.71
4	0.59	1.18	Ethyl 8-p-[[[diethylsulfamyl]phene thyl] amino] -3-methylpyrido[2,3-b]pyrazine-6-carbamate	$C_{23}H_{30}N_6O_4S$	486.59
5	8.01	6.33	Erythritol	$C_4H_{10}O_4$	122.12
6	1.96	1.55	Thieno[2,3-b]pyridine-2-carbonitrile, 3-amino-4-methylamino-	$C_{10}H_{10}N_4S$	218.28
7	0.62	0.49	Atomoxetine	$C_{17}H_{21}NO$	255.36
8	0.85	0.67	4-Fluoro benzoic acid undec-10-enyl ester	$C_{18}H_{25}FO_2$	292.39
9	5.49	4.34	.alpha.-Amyrin	$C_{30}H_{50}O$	426.72
10	2.56	2.02	Cyclopropaneoctanol,2-octyl	$C_{11}H_{22}$	154.17
11	98.28	77.63	Tetrahydrocannabinolcarbonic acid-D3-HFBA-PFPOH-Derivative	$C_{28}H_{28}F_{12}O_5$	675.52
12	2.63	2.08	Pentacosane	$C_{25}H_{52}$	352.69
13	1.38	1.09	1,3-Dioxan-5-ol	$C_4H_8O_3$	104.11
14	1.84	1.45	(E)-2-bromobutyloxychalcone	$C_{19}H_{19}BrO_2$	359.27
15	0.71	0.56	Trichloroacetic acid, hexadecyl ester	$C_{18}H_{33}Cl_3O_2$	387.81
16	4.11	3.25	1-Nitro-Propanol	$C_3H_7NO_3$	105.09
17	0.34	0.27	1-tridecyne	$C_{13}H_{24}$	180.33
18	1.09	0.86	Tris(tert-butyldimethylsilyloxy)arsane	$C_{18}H_{45}AsO_3Si_3$	468.73
19	1.34	1.06	Silicic acid, diethyl bis(trimethylsilyl) ester	$C_{10}H_{28}O_4SiO_3$	296.58
20	14.83	11.71	Oleic acid	$C_{18}H_{34}O_2$	282.47
21	1.49	1.18	Ethyl 13-methyl-tetradecanoate	$C_{17}H_{34}O_2$	270.45
22	0.36	0.28	Carbonic acid, octadecyl 2,2,2-tri chloroethyl ester	$C_{21}H_{39}Cl_3O_3$	445.89
23	1.78	1.41	1,2-Benzenedicarboxylic acid, 4-(1,1-dimethylethyl)-	$C_{12}H_{14}O_4$	222.24
24	0.72	0.57	N-Carbethoxy-N-methoxymethylamine	C_2H_7NO	61.08
25	1.62	1.28	Benz[b]-1,4-oxazepine-4(5H)-thione, 2,3 -dihydro-2,8-dimethyl-	$C_{11}H_{13}NOS$	207.29
26	2.96	2.34	Thiodiglycol	$C_4H_{10}O_2S$	122.21
27	0.5	0.4	Nonadecyl trifluoroacetate	$C_{21}H_{39}F_3O_2$	380.54
28	0.64	0.51	2-Undecanol	$C_{11}H_{24}O$	173.31
29	0.82	0.65	11-Octadecenoic acid,methyl ester	$C_{19}H_{36}O_2$	296.51
30	4.06	3.21	Mercaptamine	C_2H_7NS	77.15
31	7.1	5.61	Urs-20-en-3-ol, (3.beta.,18.alpha.,19.alpha.)-	$C_{30}H_{50}O$	426.48
32	0.61	0.49	Supraene	$C_{30}H_{50}$	410.73
33	0.67	0.53	Adenosine,2-methyl-	$C_{11}H_{15}N_5O_4$	281.27
34	0.38	0.3	1,2-Propanediol,3-methoxy-	$C_4H_{10}O_3$	106.12
35	1.84	1.45	Nickel,bis(dipentylcarbamodithioate-S,S')-,(SP-4-1)-	$C_{22}H_{44}N_2NiS_4$	523.56
36	0.21	0.17	Trans-(2-Chlorovinyl)trichlorosilane	$C_2H_2Cl_4Si$	195.93
37	1.89	1.49	1-deoxy-d-arabitol	$C_5H_{12}O_4$	136.15

Table 5: Location-specific phytochemicals of *C.gigantea* white flower with maximum peak area (%) and reported medicinal properties.

Location	Maximum peak area (%)	Location-specific phytochemicals	Nature of the compound	Bioactivities
L1	8.01	Erythritol	Sugar alcohol	Acts as a hydroxyl radical scavenger to protect endothelial cells in hyperglycemic conditions (Boesten *et al*., 2013)
L2	98.28	Tetrahydrocannabinol carbonic acid-D3-HFBA-PFPOH- derivative (THC)	Cannabinoid	Psychoactive drug to treat the people with multiple sclerosis (Koppel *et al*., 2014; Whiting *et al*., 2015)
L3	4.11	1-nitro-propanol	Alcohol	Bactericidal activity (Cobo *et al*., 2009)
L4	14.83	Oleic acid	Fatty acid	Antioxidant property (Wei *et al*., 2016)
L5	2.96	Thiodiglycol	Alcohol	Antioxidant and antibacterial activity (Nanyonga *et al*., 2013)
L6	4.06	Mercaptamine	Amino thiol	Acts as a dietary additive;cystine depleting agent in the treatment of cystinosis (Barnett and Hegarty, 2016)
L7	7.1	Urs-20-en-3-ol, (3.beta. 18.alpha.19.alpha)	Pentacyclic triterpenoid	Anticancer activity and antiinflammatory activity (Sharma and Zafar, 2015)

Table 6: Location-specific unknown compounds of *C.gigantea* white flower species.

Location	Area (%)	RT (s)	Unknown Compound	Number of compounds/ location	Relative Abundance(%)
L1	Matches Not found			NA	NA
L2	0.05	6.177	Unknown	14	57.14
	0.12	6.342	Unknown		
	0.11	6.409	Unknown		
	0.02	21.54	Unknown		
	0.07	23.83	Unknown		
	0.07	23.89	Unknown		
	0.05	31.38	Unknown		
	0.23	31.57	Unknown		
L3	Matches Not found			NA	NA
L4	Matches Not found			NA	NA
L5	Matches Not found			NA	NA
L6	Matches Not found			NA	NA
L7	0.52	27.366	Unknown	35	11.42
	2.11	29.027	Unknown		
	8.41	29.147	Unknown		
	7.12	29.394	Unknown		

Note: NA-not applicabl

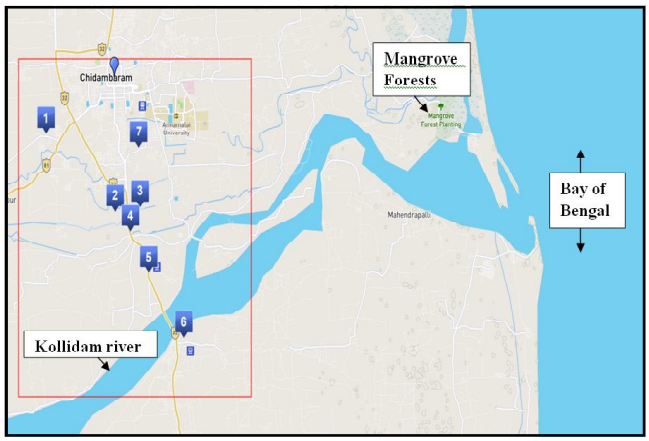

Fig. 1. Geographical origin of the seven *C.gigantea* white flower populations analyzed in this study. **L1**– C. Thandeswaranallur; **L2** – Saliyanthoppu; **L3** – Kadavacheri; **L4** – Velakudi; **L5** – Vallampadugai; **L6** – Kollidam; **L7** – Usuppur

Amyrin (7.53 %), ethyl Oleate (7.07%) and 3-ethoxy -1,2, propane- diol (15.82%) whereas cyclotrisiloxane, hexamethyl-(1%) as well as 2-dodecanol (1.42 %) were the minor compounds shown with biological activities reported earlier (Table 3).

Interestingly, there were substantial variations in the chemical profiles of 37 compounds through GC-MS analysis, which were found only in specific locations situated across all seven white blossoming populations. All the location-specific bioactive constituents were classified as aliphatic hydrocarbons, fatty acids and esters, terpenoids, ketones, alcohols, silicon derivatives, carbamic acid esters and amines on the basis of functional groups present. Additionally, heterocyclic compound, cannabinoid, aminothiol, metal hydride and inorganic acid ester were present (Fig.5). On the whole, the most abundant classes were categorized by the high percentage of alcohols (21.62 %), terpenoids and fatty acid esters (13.51 %), aliphatic hydrocarbons and amines (8.108%) whereas the lowest percentage were found with carbamic acid esters, silicon derivatives, fatty acids, and ketones (5.4%). The least abundant classes were amino thiols, inorganic

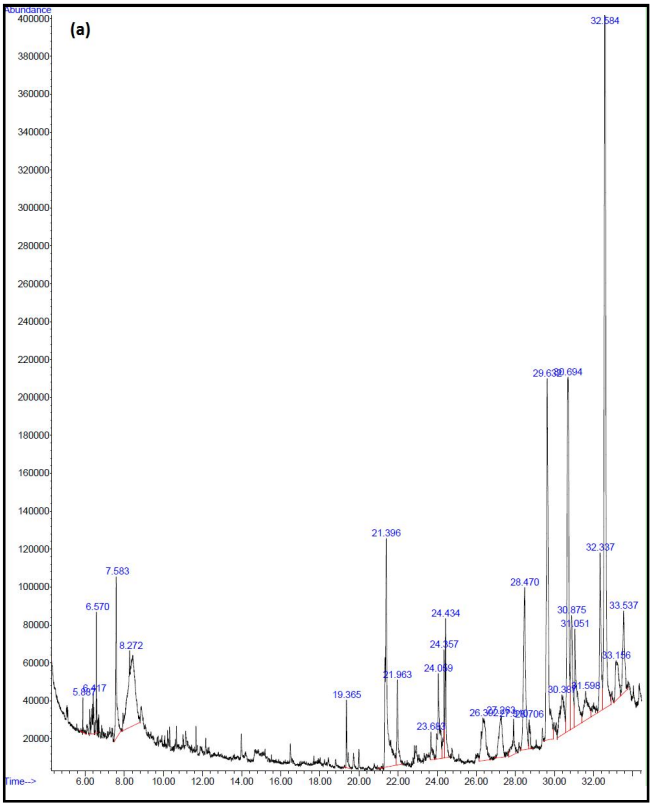

Fig.2a. GC-MS chromatograms of methanol extract of *Calotropis gigantea* white flower in 7 different locations from L1 to L7. **(a) L1**

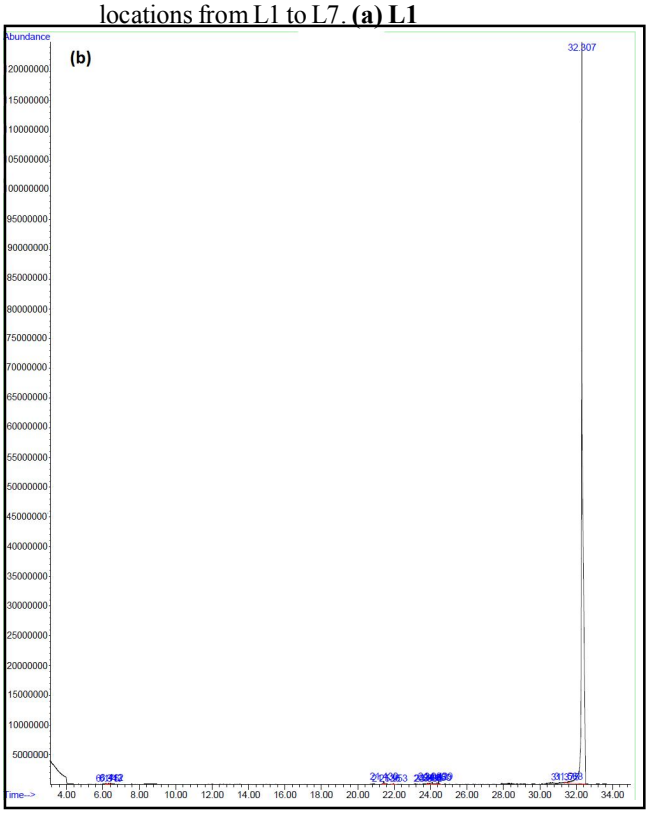

Fig.2b. GC-MS chromatograms of methanol extract of *Calotropis gigantea* white flower in 7 different locations from L1 to L7. **(b) L2**

esters, metal hydrides, cannabinoid, and heterocyclic compounds (2.7%). The concentration peak was predominantly high in cannabinoid (98.28%) while inorganic acid esters (0.36%) were identified with very low concentrations in the chromatogram.

The location wise specific bioactive metabolites with their peak area, molecular formula, molecular weight, and relative abundance are expressed in (Table 4). The major compound present in high concentration in location L1 was erythritol (8.01 %) while the minor compounds were phytol and ethyl 8-p-[[[diethylsulfamyl] phenethyl] amino]-3-methylpyrido [2, 3-b] pyrazine-6-carbamate (0.58 %). This is the first study reported a cannabinoid, tetrahydrocannabinol carbonic acid-D3-HFBA-PFPOH-derivative (THC) in *Calotropis* species from L2 with the highest peak of 98.28%. Consequently, L3 was reported with 1-nitro-propanol (4.11%) as the major compound and 1-tridecyne (0.34%) as the minor one. Location 4 was largely composed of oleic acid (14.83%) and tris (tert-butyldimethylsilyloxy) arsane was relatively small as 1.09 %. In addition, thiodiglycol (2.96 %), mercaptamine (4.06 %), Urs-20-en-3-ol, (3.beta. 18.alpha.19.alpha.)- (7.1%) showed high peaks, whereas carbonic acid, octadecyl 2,2,2-tri chloroethyl ester (0.36%), nonadecyl trifluoroacetate (0.5%), and trans-(2-Chlorovinyl) trichlorosilane (0.21%) showed low peaks in L5, L6, and L7 respectively. All the compounds found are reported by previous studies for their profound medicinal properties (Table 5).

It is worth noting that, the methanol extract consists of phytochemicals (Table 6) comprising as 8 unidentified peaks in L2 and 4 peaks in L7 locations, which have not been matched with the known components stored in the NIST database. The percentage relative abundance of unknown compounds was 57.14% in L2 and 11.42% in L7 locations.

Discussion

The high accumulation of bioactive compounds was recorded in methanol extracts of *C. gigante*a white from all locations underlining the efficacy of high polar solvent extraction followed by gas chromatography. Over a decade, the recognition of phytoconstituents from medicinal plants has been practicing and GC-MS analysis is found as an ideal technique for volatile and semi-volatile compounds (Sharma *et al.,* 2016). Also, the previous reports suggested methanol, as a better solvent system for various bioactive compound extraction from *C.gigantea* leaves and flowers (Sachin *et al.,* 2018; Singh and Javed, 2015). In this context, the present study was aimed for the recovery of a maximum number of

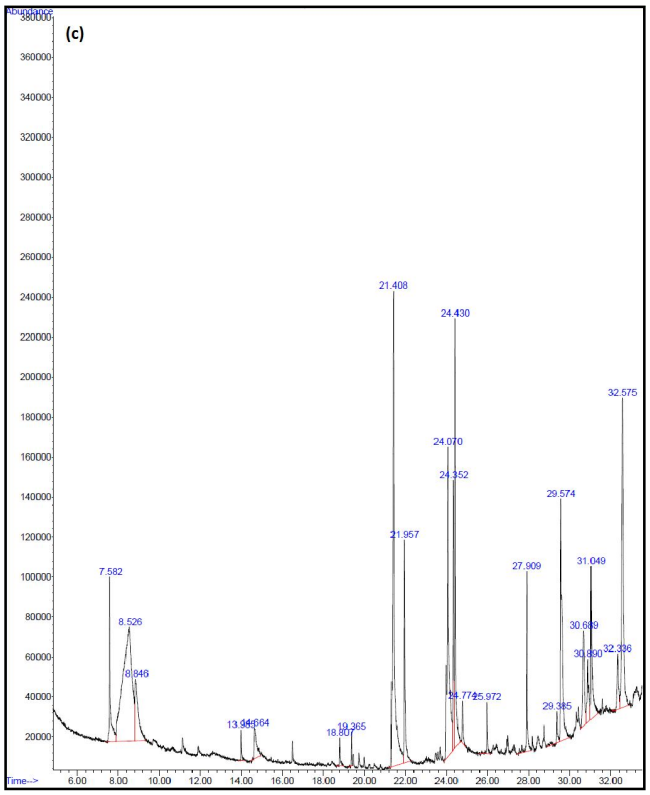

Fig.2c. GC-MS chromatograms of methanol extract of *Calotropis gigantea* white flower in 7 different locations from L1 to L7. **(c) L3**

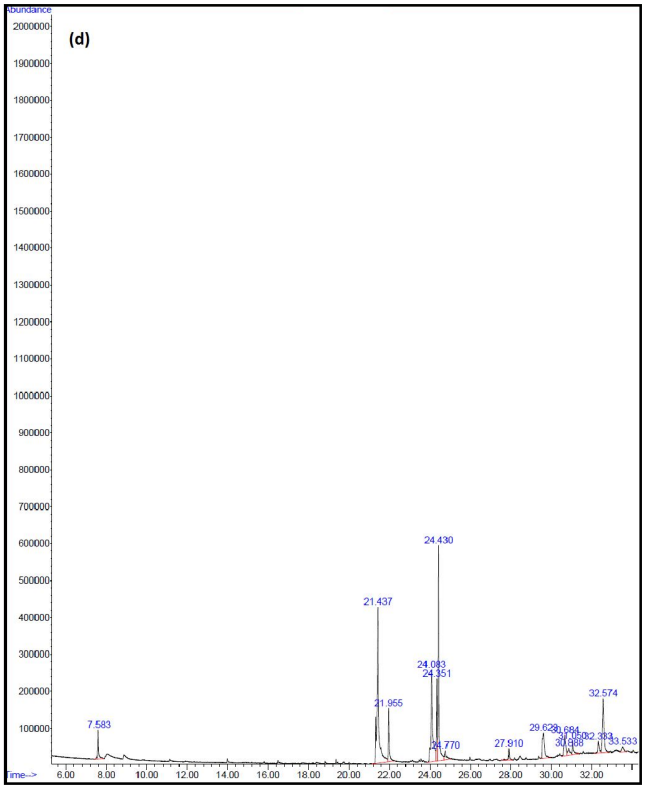

Fig.2d. GC-MS chromatograms of methanol extract of *Calotropis gigantea* white flower in 7 different locations from L1 to L7. **(d) L4**

the compound profiles through GC-MS analysis with high polar solvent.

There were variations in the number of compounds present in the white flower extracts of *C, gigantea* from seven peripheral populations located (L1-L7) between 2 and 10 km across the Coleroon river valley. For instance, the number of compounds in L7 and L6 was higher compared to other populations, whereas L2 showed less count of phytocompounds. These results are in agreement with the findings of Priyanka *et al.,* (2013) who noted the discrepancies in the total number of phytoconstituents which could be associated with specific ecological conditions of the regions including both biotic and abiotic factors. The phytochemical screening of *Pinus halepensis* barks collected from four ecological sites showed significant differences depending on the origin of the biomass (Refifa *et al.,* 2015).

Some compounds observed in current research were common even though their proportions varied in concentrations from all locations. Such changes in concentration of common plant secondary metabolites (PSMs) in plants may be as a result of specific up- or down-regulation of their biosynthesis (Moore *et al.,* 2013). The high peaks found in chromatograms for all these compounds were reinforced by the earlier reports confirming the presence of A'-neogammacer-22(29)-en-3-ol, acetate, (3.beta. 21. beta.), 12-oleanen-3-yl acetate (3.alpha.)-, .beta.-amyrin and n- hexadecanoic acid in ethanol extract of *Calotropis gigantea* purple flowers with the peak areas of 14.3, 10.28, 7.53 and 15.03 % respectively (Bhagavathy and Jancy Mary, 2015). Likewise, the methyl alcohol extract of *Calotropis gigantea* (purple) leaves has been reported to contain Hop-22(29)-en-3-ol, acetate, (3.beta.)- (30.86%) (Suresh Kumar, 2013). Hexadecanoic acid shows the discriminatory peaks about 1.55% (Suresh Kumar, 2013) and 11.74% (Sachin *et al.,* 2018) in GC-MS spectrum. Dhivya and Manimegalai (2016) found ethyl oleate, a fatty acid ester in ethanol extract *C.gigantea* leaves with 7.07% peak area which evidences our reports. Plant glycerin mostly found in vegetable oils has been identified with a peak around 0.9% in GC-MS chromatogram of methanol extract of *Mimosa pudica* Linn. (Sridharan *et al.,* 2011). Hussein *et al.,* (2018) determined a major peak of 3-ethoxy -1, 2, propane diol at the retention time of 3.31s slightly differs from our RT value of 8.74 s. The occurrence of compounds common either among the plant parts within *C.gigantea* species or in specific plant part among different populations could be a result of sharing

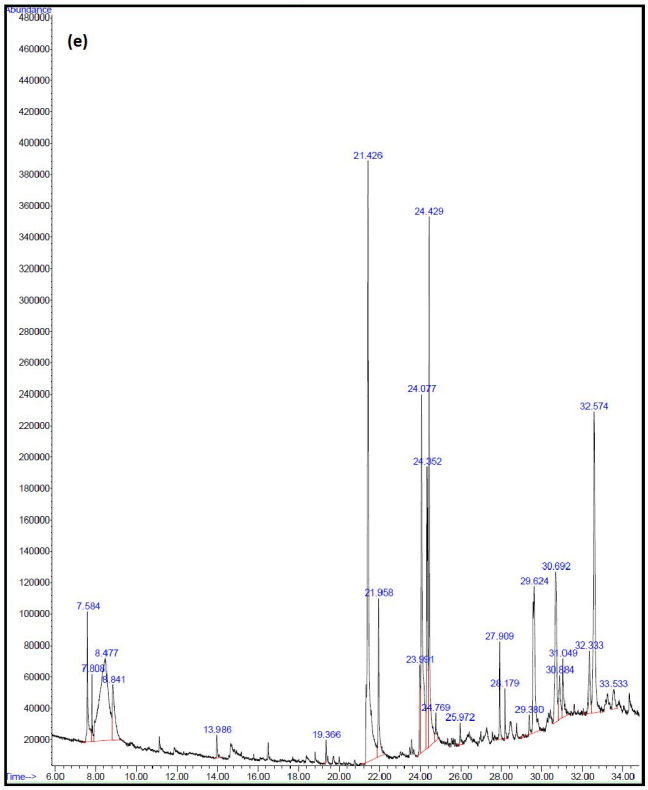

Fig.2e. GC-MS chromatograms of methanol extract of *Calotropis gigantea* white flower in 7 different locations from L1 to L7. **(e) L5**

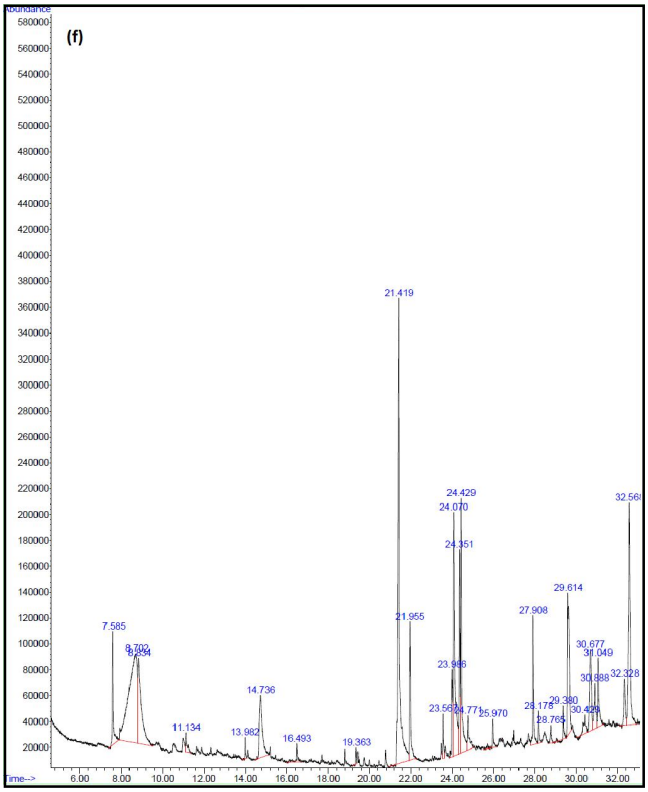

Fig.2f. GC-MS chromatograms of methanol extract of *Calotropis gigantea* white flower in 7 different locations from L1 to L7. **(f) L6**

of similar constitutive genes that influence phenotypic characteristics of the plant (Koricheva, 1999). Another conceivable reason for this commonality may be the proximity between the sampling sites located in the identical altitude coastal range (Majuakim *et al.,* 2014).

The GC-MS analyses of methanol extracts from white flower populations also revealed diversity in the phytochemical profiles with varying concentrations. This intraspecific diversity reported the peak area's abundance from each location across seven white flower populations. The past studies in the methanol extract of *Acacia nilotica* L. leaves (Gupta and Bhat, 2016) revealed the presence of erythritol in GC-MS analysis. A similar trend was observed in our findings with 4.08% of erythritol from L1. For the first time, a signature compound tetrahydro-cannabinol carbonic acid-D3-HFBA-PFPOH-derivative (THC) with a sky-high peak (98.28%) and relative abund-ance (77.62%) is found in location L2 which is a psychoactive tetrahydrocannabinol generally found in cannabis plant also known as marijuana. GCMS analysis has shown THC with a peak area range from 6.69 to 14.41% in the ethanol extract of *Cannabis sativa* L. from four different areas in Pakistan (Tayyab 2014). *Cannabis* spp. contains a highly complex mixtue of phytocannabinoids, and up to 568 unique molecules are identified in the cannabis to date (Hanus *et al.,* 2016). Among these compounds, Δ9-THC, cannabinol (CBN), and cannabinodiol (CBND) are known to be psychoactive. (Pertwee, 2014). The THC seems to bind non-specifically with cannabinoid receptors in the human and animal brain nerves and muscles to relieve the pain (Galal *et al.,* 2009). Since the drug has increasingly been seen as a novel bioactive metabolite with medical evidences, its possession has been legalized or decriminalized in Portugal and Canada (Castaneda, 2018; Congreso aprobó, 2016). These testimonies are stepping stones for a comprehensive chemical analysis in *C. gigantea*, however, bring attention to look upon the maximum compatibility of THC with the variety of physicochemical parameters along with an environmental framework of specific L2 location so as to gobble up the drug for medicinal reasons.

GC-MS analysis of *Lepidium sativum* leaves (Hussein *et al.,* 2017**)** revealed the existence of 1-nitro-propanol disclosing the efficacy of methanol solvent in the extraction of novel materials present with 4.11% concentration peak in L3. The occurrence of oleic acid, and Urs-2-en-3-ol, (3.beta. 18.alpha. 19.alpha) in respective locations L4 and L7 has been confirmed in

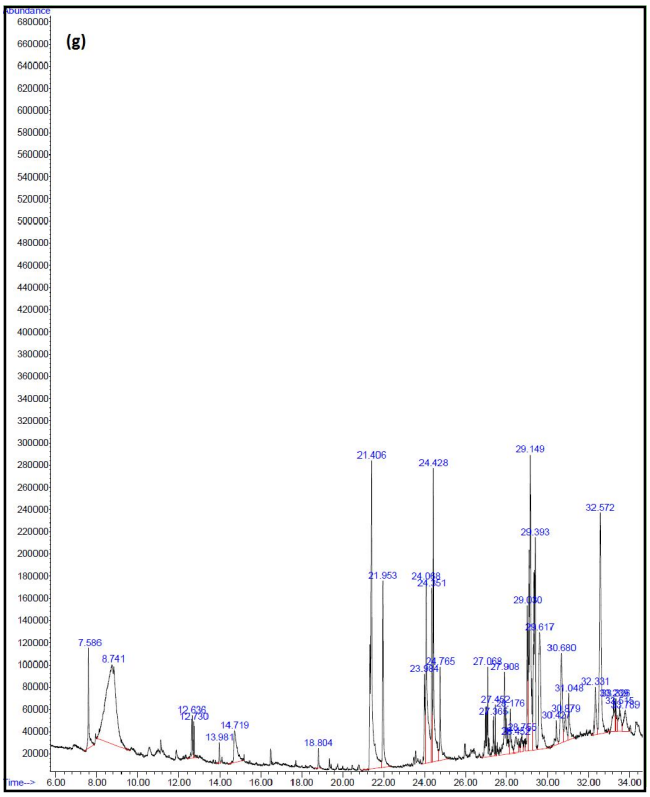

Fig.2g. GC-MS chromatograms of methanol extract of *Calotropis gigantea* white flower in 7 different locations from L1 to L7. **(g) L7**

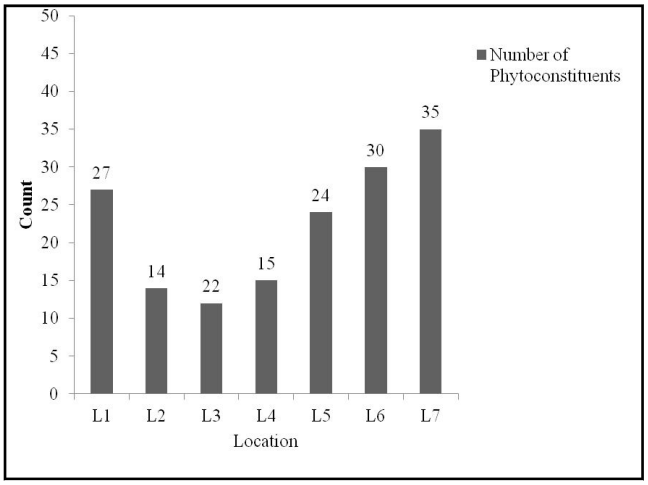

Fig.3. Distributional variations in phytoconstituent count of *C.gigantea* white flower populations from different locations.

earlier findings of *C. gigantea* flowers with a percentage difference in contents (Bhagavathy and Jancy Mary, 2015). Valli and Gokulshankar (2013) reported thiodiglycol with 0.21% peak area in the ethanol extract of *Terminalia chebula* fruit constitute rigorous support for the screening of sulfur mustard precursor in L5. Mercaptamine in L6 has been previously shown in the methanol extract of wild *Cissus quadrangularis* var. *rotundus* with the peak area of 2.75% at 10.41s retention time (Rosy and Rosakutty, 2012). However, our results showed striking differences in the percentage content of secondary metabolites thus because of the type of solvent used, its polarity index, the solubility of the compounds in the extraction solvent and the origin of the plant specimens (Iloki-Assanga *et al*., 2015). The quantitative divergence could be attributed to different expression levels of genes that confer the biosynthesis of various plant secondary metabolites (PSMs) in *C.gigantea* white (Kombrink and Somssich, 1995).

Additionally, locations L2 and L7 were comprised of unknown compounds with a percentage abundance of 57.14 and 11.42 respectively among all locations. The unidentified spectra with utmost peaks of 8.41% and 7.12% in L7 location have to be ana-lyzed to ascertain their name, molecular weight, and structure for future reference. Regardless of their percentage composition being less than 2% in L2 location, the remaining 8 unknown components might be anticipated as the novel compounds, further emphasizing the need for detailed studies in order to establish their nature in various aspects.

On the whole, white *C.gigantea* flower has been recognized as a rich source of several classes of compounds in methanol extracts. The common and location-specific metabolites are characterized by a high percentage of terpenoids and fatty acid derivatives marked as the dominant classes of compounds in *Warburgia ugandensis* Sprague (Abuto *et al.,* 2018). The different expression levels of key structural enzymes especially involved in the biosynthesis of fatty acids and terpenoids could be the possible reason for the occurrence of diverse classes in *gigantea* white (Wang *et al.,* 2015). The chemical profiles of the essential oil from *C. gigantea* flower reveals the dominance of oxygenated diterpenes, aromatic alcohol and linear chain alcohol (Singh and Javed, 2015) that bolster our results. Furthermore, our observations support the findings of previous studies which report the abundance of hydrocarbons (Dhivya and Manimegalai, 2013) in *C. gigantea* flower. Other classes of compounds identified were heterocyclic compounds and silicon derivatives, which were expressed in low concentrations. Although the essentiality of these compounds to plants is still debated, they act as versatile players against plant pathogens (Khalaphallah, 2015) and alleviate biotic and abiotic stress responses on plants (Frew *et al.,* 2018) respectively.

We herein provide the first phytochemical screening report with respect of similarities and intraspecific differences in secondary metabolites of *C.gigantea* white

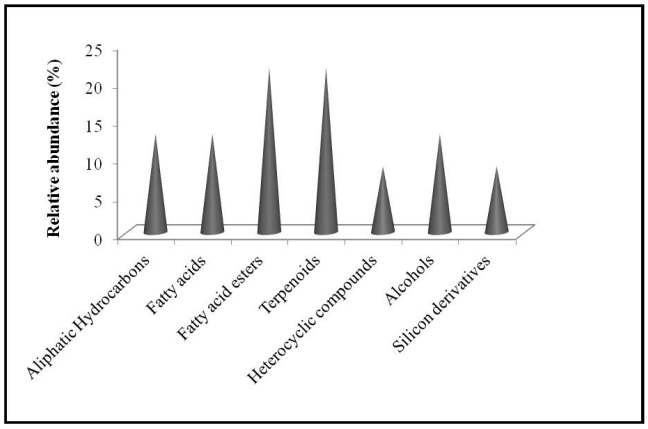

Fig.4. The relative abundance (%) of different chemical classes in common phytoconstituents extracted from various *C.gigantea* white flower species.

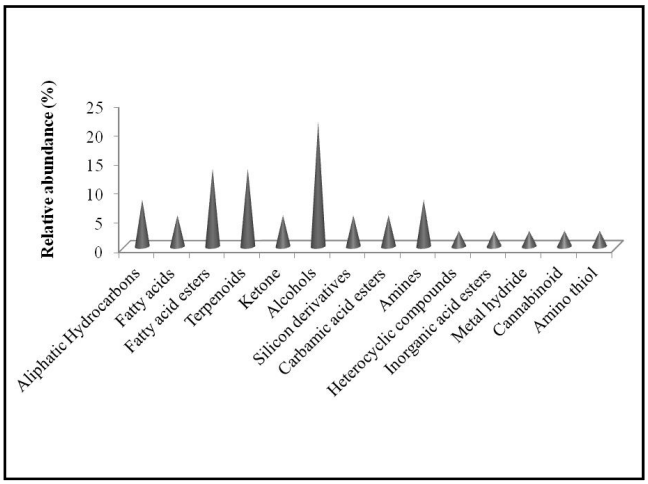

Fig.5. The relative abundance (%) of different chemical classes in location-specific phytoconstituents extracted from *C.gigantea* white flower species.

flower populations. The general chemical profile of flower extracts from the seven populations was found to be quite different from what was previously reported. Compounds that were earlier reported to occur in chloroform and ethyl acetate extracts of wild type *C.gigantea* white flower randomly collected from Kumbakonam in Tamilnadu on the basis of biological characterization by GC-MS analysis (Rajamohan *et al*., 2014). This observation revealed the presence of 4 major peaks; three compounds in the chloroform extract namely 2-methoxy-4-vinylphenol, phenol-4-methoxy-3-(methoxy methyl), 8-octadecenoic acid, methyl ester (E) and benzhydrazide, 4-methoxy-N2-(5-bromo-2-methoxy benzylideno) in ethyl acetate which were absent in the present study. The major compounds identified in our study were notably different from 14 major compounds observed in the ethanol extract of C.*gigantea* flower variety collected from a natural habitat at Coimbatore in Tamilnadu. (Dhivya and Manimegalai, 2013).With respect of retention time (RT), they reported 1-tetradecene(RT=14.74s), 3-ethyl-1-tetradecene (18.81), 3-octadecene, (E) (22.93), ethyl linoleate (30.72), tricosane (37.38), 4' methyl-2 phenylindole (44.35), and 5,12-naphthacenedione, 8-ethyl-7,8,9,10-tetrahydro-1,6,8,11-tetrahydroxy (45.81) as the major compounds while in this study, 1-deoxy-d-arabitol (RT=14.721) 1-tridecyne (19.37), 11-octadecenoic acid, methyl ester (23.56), 12-oleanen-3-yl acetate, (3.alpha.)- (30.68), Urs-20-en-3-ol, (3.beta, 18.alpha, 19.alpha.)- (32.57), and cyclotrisiloxane, hexamethyl- (33.79) were the fractioned compounds in *C.gigantea* white flower extracts from all 7 locations contradicts the documented literature. These variations in literature data may be linked to the dissimilarity in altitudes analyzed, climatic conditions, seasons, soil types, solvents and other environmental-factors. However, the quantification of phytochemicals within a species from different locations even with less altitude and latitudinal variations will help in establishing the potential medicinal plants as they may stimulate adaptability to the external factors.

The differences in the geographical location parameters such as altitudes (Gololo *et al*., 2017), soil types (Jayanthy *et al*., 2013) and the magnitude of exposure to sunlight (Tabin *et al.,* 2016) of the sample collection sites, maybe the major contributing factors to the observed disparities in the phytochemical composition of the flower samples of *C. gigantea* from seven sites across Coleroon delta. The reports of Demasi *et al*., (2018) possibly explained the influence of sea distance on morphological and phytochemical traits while elevation from sea level seemed to affect mainly the phytochemical composition of *L. angustifolia* plant grown under uniform cultivation conditions. The latitudinal and altitudinal gradients may have induced different adaptation mechanisms, genotypic sorting and selection across physical and biotic environments in white flower traits leading to different phytochemical contents.

The intra specific phytochemical diversity of abundant metabolites may be either solely or partly linked to the medicinal properties of the plant species (Wang *et al*., 2015). The seven peripheral populations of white flower in the present study located closer to Bay of Bengal were expected for the higher yield of valuable secondary metabolites therefore could be conserved to develop new breeds with antimicrobial property when treating the

human pathogens (Peela and Porana, 2017). The presence of apparently low and variable amounts of bioactive metabolites from different geographical origins is the major limiting factor in the determination of their therapeutic quality assessment that eventually causes challenges for quality assurance of herbal medicinal products (Dhami and Mishra, 2015). The location-dependent manner has been shown in comparative analysis of phytochemical quantity from different locations in South Africa (Gololo *et al*., 2018) hence highlights the importance of harvesting phytochemical rich plants from specific locations for the usage in traditional medicine. It also provides knowledge of the suitable habitation area that affords optimum concentrations or quantities of specific active ingredients (Inbathamizh and Padmini, 2013). Undoubtedly, the present investigation of the phytochemical content of *C.gigantea* white flower at varying altitudes can help select elite genotype on the basis of the occurrence, abundance and medicinal values.

Conclusion

Notwithstanding our stance on site-specific diversity is still in its infancy in *Calotropis* white trait, this exploration warrants the abundance of flavonoids, alkaloids, fatty acids, terpenoids along with a cannabinoid consequently associating it as a standardized drug with the cutting-edge treatment regimens. The outcome of the present findings concluded that the Coleroon delta in the Bay of Bengal coastal belt is a rich source of driving economically and therapeutically important bioactive compounds. Also, these perspectives could emphasize the future research with more cultivation protocols of coastal white flora in marine habitats respective of protection against natural disasters. It is anticipated that the screening with bio-geographical variation will expand the formulation strategies with more specific novel mutants to conserve the ecology and evolution of plant secondary metabolites.

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Conflict of Interest

The authors declare that there is no conflict of interest to reveal.

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