



# DISPARITIES AND SIMILARITIES IN THE PHYTOCHEMICAL PROFILING OF WHITE *CALOTROPIS GIGANTEA* LEAVES AMASSED FROM SIX COASTAL LOCALES ACROSS COLEROON DELTA

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## Abstract

*Calotropis gigantea* white is a medicinal shrub principally utilized by the rural population from the traditional period. The plant can grow in numerous environments such as fresh and saline water terrestrial plains notably most abundant in the sand dune associated coastal areas. However, the eco-geographical divergences and commonalities in the phytochemical composition of white *gigantea* leaves remain largely unexplained. In this context, the present study is aimed to identify the bioactive compounds present in the methanol leaf extract by using gas chromatography-mass spectrometry (GC-MS) analysis. The disease-free healthy leaves of the plant were collected from six different coastal locations across the Coleroon delta. The phytochemical analysis revealed the most abundant location-specific and common components among all locations. The predominance of alkanes, alkenes, heterocycles, alcohol, and silicon derivatives were also observed in the preliminary screening based on their functional groups. To the best of our knowledge, this is the first attempt to screen phytochemicals from the leaves of coastal white vegetation of *C. gigantea* among various locations across natural habitats. Further investigations are required for the comparative study of bioactive metabolites to establish the medicinal values of white *gigantea* leaves in ecological aspects.

**Key words :** *Calotropis gigantea*, dune flora, white leaves, Coleroon delta, GC-MS profiling, location-specific, commonality.

## Introduction

Medicinal plants, which form the backbone of traditional medicine and plant-derived drugs, serve as a prototype to develop more effective and less toxic medicines (Murugan *et al.*, 2008). According to the survey of the World Health Organization (WHO), 80% of the population depends on the traditional medicine for their primary health care needs. India has over one fourth (8000) of the world's familiar medicinal plant species (30,000) which are widely distributed in forests. As there is renewing interest in phytomedicine over the past few decades, many medicinal plant species are being screened for pharmacological activities. Perhaps, various secondary metabolites such as alkaloids, flavonoids, phenols, saponins, sterols, etc., the medicinal plants may play a pivotal role in the curative properties of the medicinal plants (Mallikharjuna *et al.*, 2007). There is growing attention in correlating the phytochemicals of a medicinal

plant with its pharmacological activity (Prachayasittikul *et al.*, 2008).

*Calotropis gigantea* which belongs to Asclepiadaceae family has been widely reported to possess number of medicinal properties from the whole plant (Kirtikar and Basu, 1935) identified the presence of A preliminary study on *C. gigantea* leaves has confirmed the presence of many biologically active primary molecules such as carbohydrates, proteins, amino acids, and secondary phytochemical components for instance, saponins, tannins, flavonoids, alkaloids, glycosides, polyphenols, triterpenoids and steroids in the leaves, flowers and stems in the methanol extract (Bhagavathy and Jancy Mary, 2015). Suchita *et al.*, (2014) have proven the synergistic action of leaf metabolites against bacteria. In particular, *Calotropis gigantea* leaves consist of some additional alkaloids such as catechol, calotropagenin and chrysin (Richa and Shikha, 2015) as well as cardenolides *viz.*, 19-nor- and

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18, 20-epoxy-cardenolides (Lhinhatrakool and Sutthivaiyakit, 2006), 15-beta-hydroxycardenolides and 16-alpha-hydroxycalactinic acid methyl ester (Seeka and Sutthivaiyakit, 2010). Additionally, *C. gigantea* leaves have expressed sapogenins, holarrhetine, cyanidin-3-rhamnoglucoside taraxasterol isovalerate, mudarine and three glycosides calotropin uscharin, calotoxin along with phenol (Misra *et al.*, 1993). The lignan, 9'-methoxypinoresinol, and glycosylated 5 hydroxymethyl furfurals, calofurfuralside A and calofurfuralside B have been isolated from the MeOH-soluble extract of *C. gigantea* leaves which are found to exhibit potent cytotoxic activity against PANC-1 human pancreatic cancer cell line (Nguyen *et al.*, 2017).

Generally, *Calotropis gigantea* 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 (Arulmoorthy and Srinivasan, 2017) in the conservation aspect of coastal biota. In the traditional medicine system, the roots and barks of *C. gigantea* are used to treat cardiovascular diseases (De and Datta, 1998) and cancer (Park *et al.*, 2002). It has anti-fertility (Upendra *et al.*, 1992) and anti scabetic (Kitagawa *et al.*, 1992) properties. Additionally, it is used as a remedial drug for epileptic convulsions in children and paralytic symptoms in adults (Nadakarni, 2002). The leaves of *C. gigantea* are used for the treatment of poisonous snake bites, periodic fever, intestinal worms and ulcers (Kumar *et al.*, 2011). The chloroform extracts of *C. gigantea* leaves have reported possessing antioxidant activity and hypoglycaemic activity respectively (Singh *et al.*, 2010; Rathod *et al.*, 2009). *Calotropis gigantea* leaves have also been screened for their antibacterial (Kumar *et al.*, 2010) and antifungal activities against *Candida albicans*, *C. parapsilosis*, *C. tropicalis* and *C. krusei*. (Senthilkumar *et al.*, 2012; Kumar *et al.*, 2010). Besides, the methanol leaf extract exhibits its insecticidal activity against *T. castaneum* (Asharaful Alam *et al.*, 2009), ovicidal activity (Prabhu *et al.*, 2012) and hepatoprotective activity (Usmani and Kushwaha, 2010).

Plants serve as a rich source of many novel biologically active compounds that are pervasive in plants and help them to maintain a labyrinthine balance with the environmental needs. The yield and composition of plant secondary metabolites are often variable due to the interplay among several ecological factors. 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 chemical compounds between individuals and groups (Moore *et al.*, 2013). Thus the knowledge on phytochemical profiles is not only useful in search of the ecological predominance of therapeutic curatives, but also reveals the novel and economic precursors to industrial applications.

Phytochemical analysis of medicinal plant parts is an important step in understanding the biological activities of a physiological system (Joseph and KumbhareKale, 2013). Since the beginning of two decades, studies on the different parts of *C. gigantea* purple variety have been explored much more than a white trait. Recently, the research on white-flowered trait has been started widening in various aspects like medicinal values associated with biological properties. 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). Also, Dhivya and Manimegalai (2013) have shown the abundance of hydrocarbons in the ethanolic flower extract that creates a baseline for the extensive research on *C. gigantea* white species. 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). However, there is limited information on phytochemical variation and commonalities in secondary metabolites of *Calotropis gigantea* white trait from different geographical locations as it has been less studied in the ecological aspects. Hence, the present investigation is aimed at the phytochemical characterization of leaf extracts collected from six different coastal populations of white *C. gigantea* across the Coleroon delta region.

## Materials and methods

### Collection of plant materials

Fully matured disease-free, aerial leaves of *Calotropis gigantea* white vegetation grown in natural habitat were randomly collected in from the east coastal villages in and around Chidambaram at Cuddalore region, which is closer to the shorelines of the Bay of Bengal with the geographical coordinates of 11.39° N 7970° E. This coastal zone has a tropical climate and the average annual temperature and rainfall have been reported as 28.4°C and 1248 mm respectively. Each population is located at least 2000 m apart from each other in the Coleroon (kollidam) river delta which is connected to the sea through an estuary outlet a unique assemblage of

marine and freshwater. Sampling was carried out at Saliyanthoppu (L1), Kadavacheri (L2), Velakudi (L3), Vallampadugai (L4), Kollidam (L5), and Usuppur (L6) situated at the distance range of 12790 - 15190 m far away from the coast and all sites are almost terrestrial proximity to Pichavaram mangrove forests. The topography of the locations is at an altitudinal range between 5.48 and 8.22 m from the sea level. The leaf specimens were authenticated in Centre for Floristic Research at Chennai and floral diversity manual of Botanical Survey of India, Southern Regional center at Coimbatore located in Tamil Nadu.

### Chemicals

The chemicals and solvents required for the study were purchased from Hi-Media Laboratories Pvt Ltd (Mumbai, India). Milli-Q ultrapure water was obtained from the central facility of the Department of Marine Biology, Annamalai University, Tamilnadu, India. All solvents used in the study were high-performance liquid chromatography grade.

### Processing and extraction

Fresh aerial leaves were collected and washed thoroughly with distilled water in order to remove the dirt and other foreign particles. The washed plant materials were dried under shade at lab temperature (25°C) so as to retain the fresh green color of the leaves, and also to prevent the decomposition of the potential active

compounds. The dried leaves 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 an analytical grade solvent of methanol for GCMS analysis. 25 g of crude powder was taken in a round bottom flask and 50 ml of methanol was added and refluxed for 5 h then cooled and centrifuged at 3000 rpm for 5 m. The residue obtained (1g) was dissolved in 5 ml of methanol and the supernatant was transferred into the GC vial and injected into the GC-MS port.

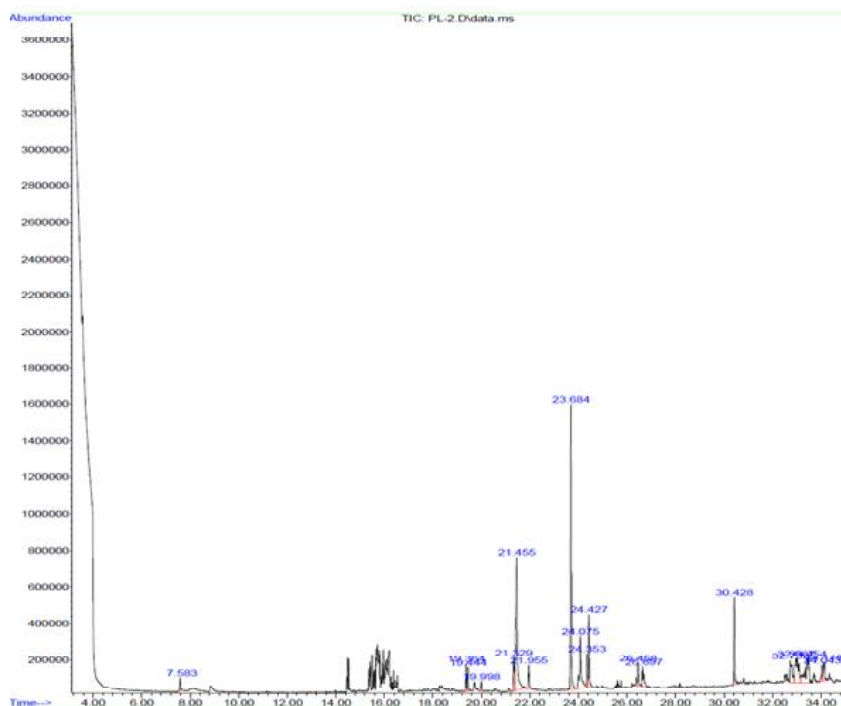
### GC-MS profiling of bioactive compounds

The GC-MS analysis was performed on an Agilent gas chromatograph model 7890A GC coupled to an Agilent 5975C N mass selective detector with a single quadrupole mass spectrophotometer and

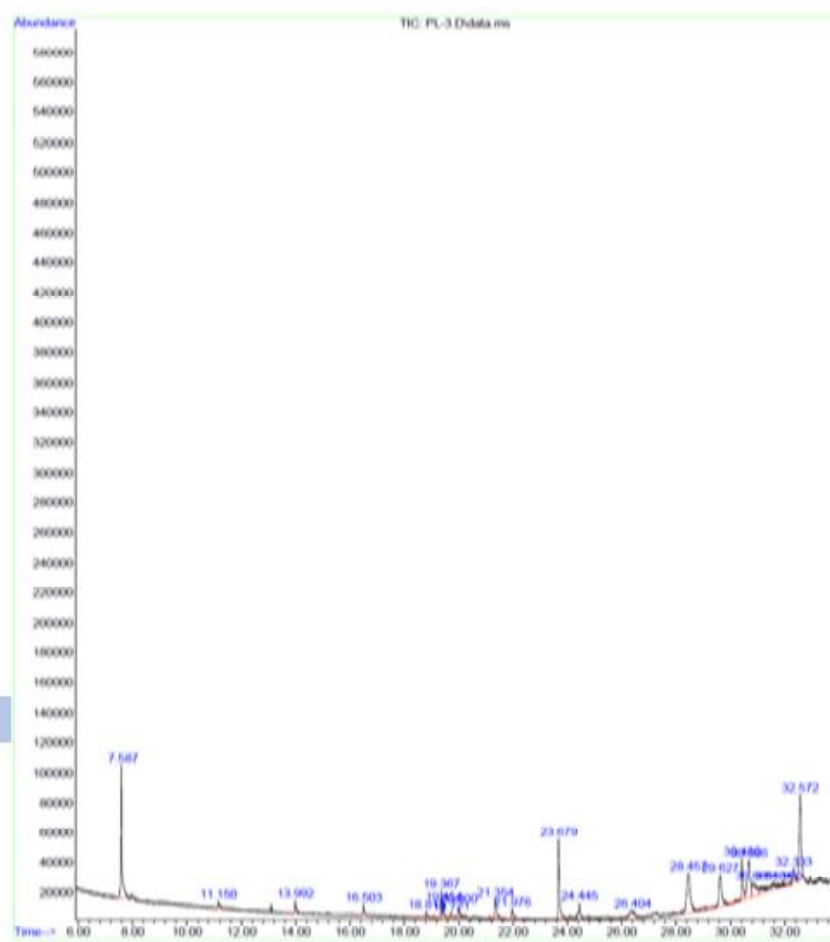
an electron ionization source. Analytes were separated on a wall coated open tubular ZB-5MS capillary column (30 m × 0.25mm × 0.25 μm composed of 5% phenyl polysiloxane) by applying for the temperature program: Quadruple and threshold temperature at 150°C; the oven temperature at 50°C for 3 min and then 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 at 280°C. Mass detector conditions were described below: electronic impact (EI) mode at 69.922 eV; injector temperature 250°C; mass scanning range m/z 35-700; scanning rate of 2.88 or 4s<sup>-1</sup>. The carrier gas was helium at 1.0 mL/min. An injection volume of 2 iL was employed (Split ratio of 5:1). The 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 NIST-MS Search II library (National Institute of Standards and Technology-Mass Spectrometry) supported by retention index data of available literature. Software adapted to handle mass spectra and chromatograms was Chem Station.

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

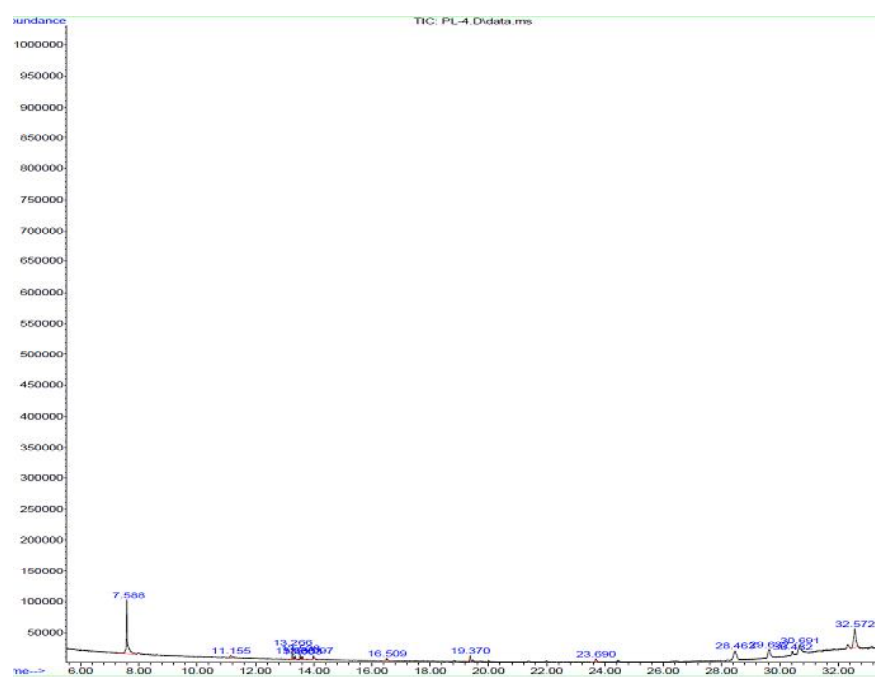
$$\% \text{ Relative Abundance} = \frac{\text{Peak area of the compound}}{\text{Total peak area of all compounds}} \times 100$$



**Fig.1a:** GC-MS chromatograms of methanol extract of white *Calotropis gigantea* leaves in six different locations from L1 to L6. **(a) L1.**



**Fig.1b:** GC-MS chromatograms of methanol extract of white *Calotropis gigantea* leaves in six different locations from L1 to L6. **(b) L2.**

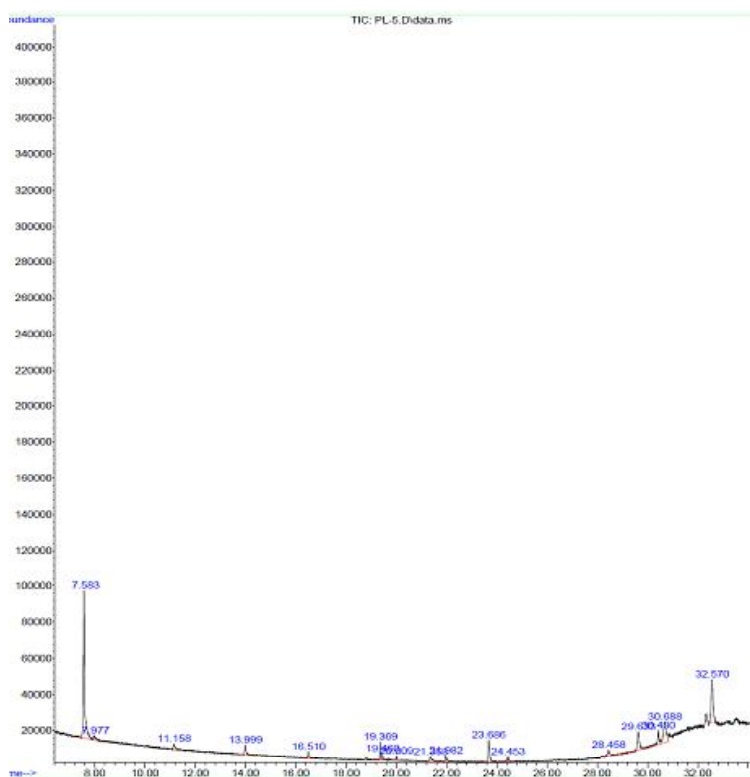


**Fig.1c:** GC-MS chromatograms of methanol extract of white *Calotropis gigantea* leaves in six different locations from L1 to L6. **(c) L3.**

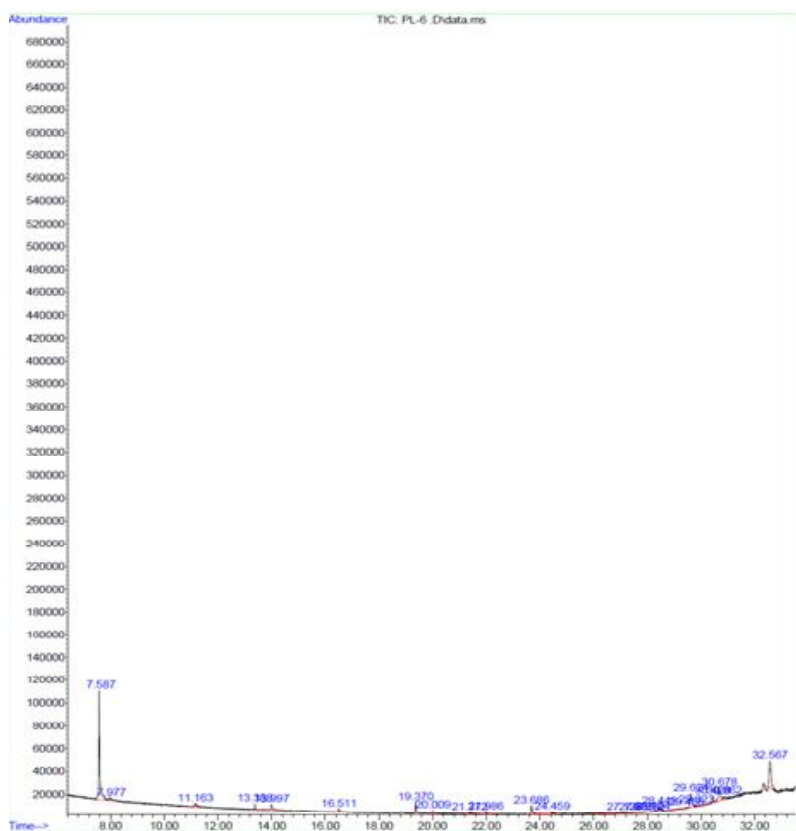
## Results and Discussion

*C. gigantea* white is a spreading shrub or medium-sized tree from pre-historic times to the modern era in many parts of the world. They have a profound influence on culture and have been worshipped in rural villages in India. The succulent species has the ability to grow near the high tidal range of coastal regions and are conserved as an environmental resource during natural catastrophes. The complete phytochemical composition of medicinal plants reveals a complex mixture of chemical groups with the greatest degree of variation in their abundance (Priyankar *et al.*, 2017). In our investigation, the phytochemical contents of the white *gigantea* leaves were identified by using GC-MS and categorized with reference to the ecological variations and commonalities.

The phytochemical analysis encompassed the presence of bioactive compounds in the methanolic extract of white *C. gigantea* leaves collected from six different sampling sites situated in the coastal belt of Bay of Bengal (L1 to L6). A total number of 19, 24, 15, 17, 24 and 17 compounds were eluted through GC-MS from the locations L1 to L6 respectively. The previous reports of Sharma *et al.*, (2016) have recorded 24 bioactive compounds in from the leaves of *C.gigantea*. Based on their concentration peaks, the major compounds in L1 were found to be phytol (20.29%) and n-hexadecanoic acid (20.16%) (Fig.1a). The GC-MS analysis of *C. gigantea* leaves confirmed the presence of phytol with peak area 1.05% in methanol (Sureshkumar, 2013) and 5.05% in ethanol extracts (Beena Thomas and Reshma Thampy, 2018). A'-neogammacer-22(29)-en-3-ol, acetate, (3.beta., 21. beta) (12.95%), tetraethyl silicate (10.34%) and alpha.-amyrin (10.21%) were found to have



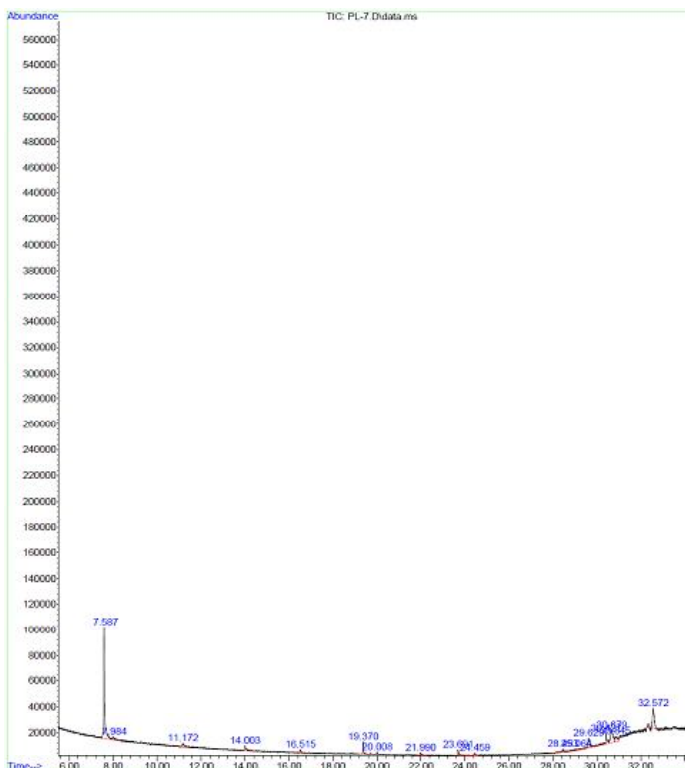
**Fig.1d:** GC-MS chromatograms of methanol extract of white *Calotropis gigantea* leaves in six different locations from L1 to L6. **(d) L4.**



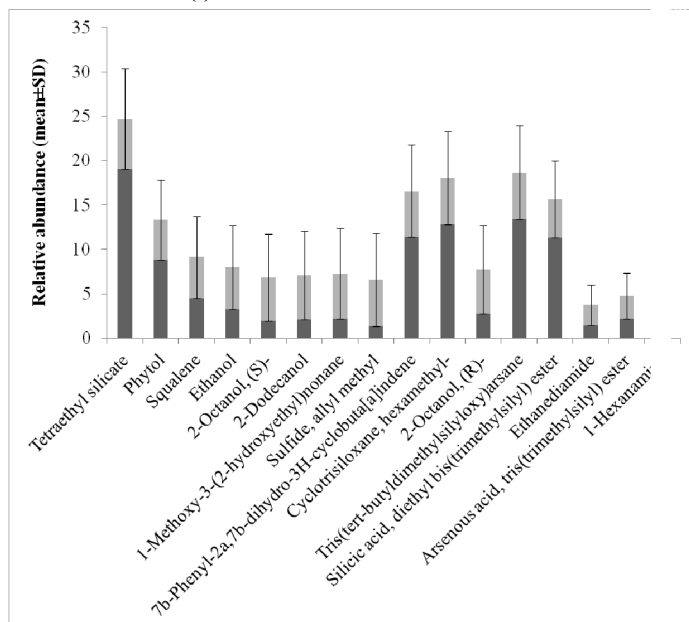
**Fig.1e:** GC-MS chromatograms of methanol extract of white *Calotropis gigantea* leaves in six different locations from L1 to L6. **(e) L5.**

high peak areas in L2 (Fig.1b). Location 3 revealed the prominent peaks for the silicone derivatives and hydrocarbons such as tris (tert-butyldimethylsilyloxy) arsane (22.64), 7b-phenyl-2a, 7b-dihydro-3H-cyclo buta [a] indene (21.55%) and tetraethyl silicate (11.34%) (Fig.1c). The predominant compounds in L4 location were tetraethyl silicate (31.02%) and 2-ethylacridine (16.33%) (Fig.1d). Likewise, location L5 detected tetraethyl silicate (32.23%) and 2-ethylacridine (13.66%) (Fig.1e) as the principal compounds with less variability. The most dominant compounds in L6 were as similar as L4 and L5 locations demonstrated tetraethyl silicate (34.21%), 2-ethylacridine (10.22%) along with cyclotrisiloxane, hexamethyl (10.73%) (Fig.1f). The secondary metabolite analysis demonstrated not only quite distinct but also significantly overlapping phytochemical profiles of the six white populations many of which are well explored for their potential therapeutic activities as well as other applications such as heavy metal indicators (Samantaray *et al.*, 1999). The screening results of the entire plant of *Gomphrena decumbens* Jacq attributed the elution of tetraethyl silicate (0.31%) (Yamuna *et al.*, 2017) and abundance of 2-ethylacridine (3.32%) could be explained in methanolic tuber extract of *Momordica cymbalaria* (Manikandan *et al.*, 2019).

Among six populations, 16 compounds were identified as common compounds that were present in all locations with variations in their cumulative relative abundances (Fig. 2). Tetraethyl silicate ( $19.00 \pm 5.64$ ), tris (tert-butyldimethylsilyloxy) arsane ( $13.37 \pm 5.27$ ), cyclotrisiloxane, hexamethyl- ( $12.79 \pm 5.26$ ), 7b-phenyl-2a, 7b-dihydro-3H-cyclobuta [a] indene ( $11.39 \pm 5.19$ ) and silicic acid, diethyl bis (trimethylsilyl) ester ( $11.34 \pm 4.29$ ) were the common compounds characterized as silicon derivatives present with optimum abundance and high variability in *C.gigantea* leaf extracts among six locations. The least abundance was observed in ethane diamide ( $1.43 \pm 2.30$ ), arsenous acid, tris (trimethylsilyl) ester ( $2.20 \pm 2.58$ ) and 1-hexanamine ( $1.44 \pm 3.39$ ). The occurrence of



**Fig. 1f:** GC-MS chromatograms of methanol extract of white *Calotropis gigantea* leaves in six different locations from L1 to L6. **(f) L6.**



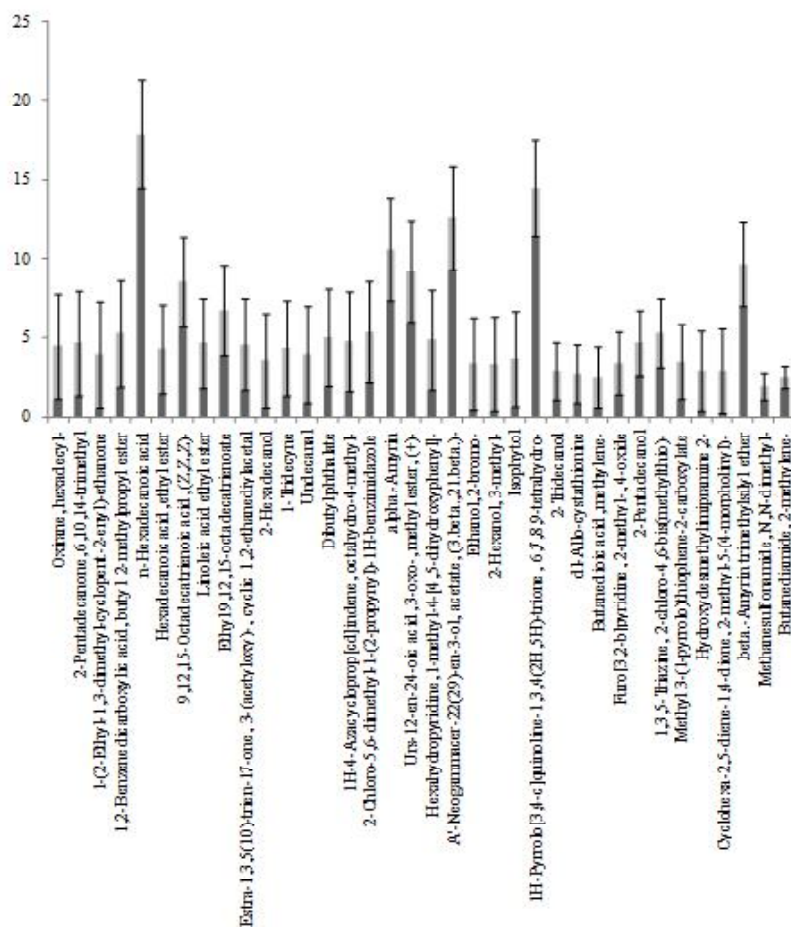
**Fig. 2:** The variation in relative abundance of different phytochemicals commonly found in six locations (L1-L6) based on their presence in 2 or more white *Calotropis gigantea* species. Data are represented as mean + SD of relative abundance.

tris (tert-butyl)dimethylsilyloxy) arsane has been reported earlier in *Terminalis arjuna* (Salim, 2018) and the aerial parts of *Alternanthera philoxeroides* (Mart.) (7.27%) (Arul Pamila and Karpagam, 2018). The petroleum ether extracts

of *Pulicaria crispa* has revealed the presence of 7b-phenyl-2a, 7b-dihydro-3H-cyclobuta[a] indene with the peak area of 5.514% (Elshiekh and Mona, 2015). Phytoconstituents of chloroform extract from *Shorea robusta* resin contain cyclotrisiloxane, hexamethyl-(6.37%) higher than methanol extract (0.25%) (Sushma *et al.*, 2017) and has been reported to have antioxidant activity (Alok Prakash and Suneetha, 2014). The GCMS analysis of methanol extract of *Cadaba fruticosa* consists of silicic acid, diethyl bis (trimethylsilyl) ester (Sharmila Juliet *et al.*, 2018) proven to have antibacterial activity (Hema *et al.*, 2011).

On the other hand, 36 compounds were found as location-specific phytochemical mixture in the leaf extracts of white variety from each location. The variation of cumulative relative abundance was shown in Fig. 3. Among 36 compounds, n-hexadecanoic acid ( $14.45 \pm 3.43$ ), 1H-pyrrolo[3,4-c]quinoline-1,3,4(2H,5H)trione,6,7,8,9,tetrahydro ( $11.38 \pm 3.08$ ), A'-neogammacer-22(29)-en-3-ol, acetate, (3.β.,21.β.)- ( $9.28 \pm 3.27$ ), α-amyrin ( $7.32 \pm 3.27$ ) and β-amyrin trimethyl silyl ether ( $6.99 \pm 2.66$ ) were the most abundant and highly variable phytochemicals, whereas the least abundant compounds were found to be methane sulfonamide, N, N-dimethyl-( $1.03 \pm 0.87$ ), butane diamide, 2-methylene-( $1.78 \pm 0.70$ ), butanedioic acid, methylene-( $0.53 \pm 1.92$ ) and dl-allo-cystathionine ( $0.81 \pm 1.87$ ) The methanol leaf extract has revealed the presence of n-hexadecanoic acid, A'-neogammacer-22(29)-en-3-ol, acetate, (3.β., 21. β.) and α-amyrin, the *C. gigantea* derived terpene which shows a great potentiality against AeSCP-2. (Sureshkumar, 2013). Among the 40 compounds identified in the ethanolic extract, the major phytoconstituents are hexadecanoic acid, ethyl ester (19.70%), oxirane (butoxymethyl)- (1.62%), and 9, 12, 15-octadecatrienoic acid, (Z, Z, Z)-(2.81%) which shows similarity with our results respective of eluted compounds (Beena Thomas and Reshma Thamby, 2018). The investigation of Kravchenko *et al.*, (2005) revealed the role of 1H-Pyrrolo[3, 4-c] quinoline-1, 3-diones as potent caspase-3 inhibitors in apoptotic cell process that supports our GC-MS outcomes.

The previous GC-MS analysis has reported the occurrence of potential phytosterols in *C. gigantea* leaves which could be a supplement in the conventional drug development. (Sureshkumar *et al.*, 2012). The predominance of chemical classes in each location and the cumulative analysis in all six white



**Fig. 3:** The variation in relative abundance of different location-specific phytochemicals found in six locations (L1-L6) based on their presence in 2 or more *Calotropis* species. Data are represented as mean + SD of relative abundance.

*C. gigantea* species were presented in Fig. 4 (a-g). Location L1 expressed the dominance of heterocyclic compounds while L6 showed silicon derivatives as the dominant group. Alcohol was the major chemical group represented by the locations L2, L3, L4, and L5 respectively. The overall abundance of phytochemicals in leaves among six populations of *C. gigantea* white clearly indicated the alcohols ( $26.91 \pm 15.66$ ) silicone derivatives ( $16.21 \pm 10.77$ ) and heterocycles ( $11.70 \pm 4.73$ ) were the major classes contributing the greatest share with the highest variability, whereas alkanes and alkenes were found to be the minor classes. Moreover, for the first time, this study reports silicone derivative in the leaves of *Calotropis* white populations with the greatest variability. The mass spectrum pertaining to GC-MS analysis displays the identification of 22 compounds disclosing hydrocarbon, methyl, hydroxyl, nitrogen, and carbonyl functional groups from the ethanol extract of *C. gigantea* leaves (Dhivya and Manimegalai, 2016). Despite their less prevalence in each location, the classes such as ketones, fatty acids, aldehydes, sulfides, metal hydrides, cyclic ether, arsenous acid, amines, and amides

contributed considerable share ( $15.15 \pm 9.90$ ) cumulatively, which were closer to the alcohols and silicones. The chemical profiles of methanol leaf extract in *C. gigantea* have exhibited the maximum peaks for amines, ketones, alcohols, and aldehydes with strong antimicrobial activity (Sachin *et al.*, 2018; Singh and Javed, 2015) correlating the trait variations of the species. Although the essentiality of plants is still controversial, silicon derivatives and heterocyclic compounds play an eminent role against plant pathogens (Khalaphallah, 2015) and attenuate the biotic and abiotic stress responses on plants (Frew *et al.*, 2018) respectively. In addition to, the GC-MS chromatogram of methanol leaf extract showed cyclohexane as a major compound with peak area of 43.28% (Sharma *et al.*, 2016) bolstering the presence of alkanes in our phytochemical screening.

## Conclusion

The present methanobotanical investigation on white *Calotropis gigantea* leaves proved the existence of bioactive compounds with their possible functional groups. In fact, significant differences and similarities were

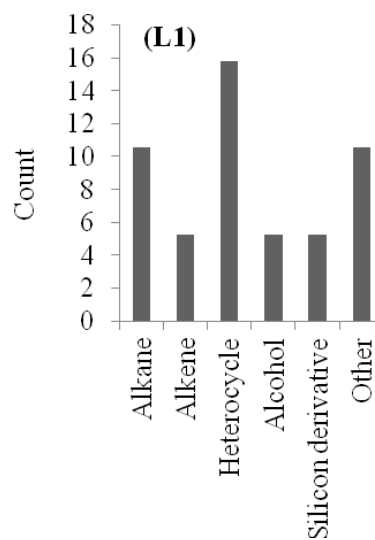


Fig. 4(a)

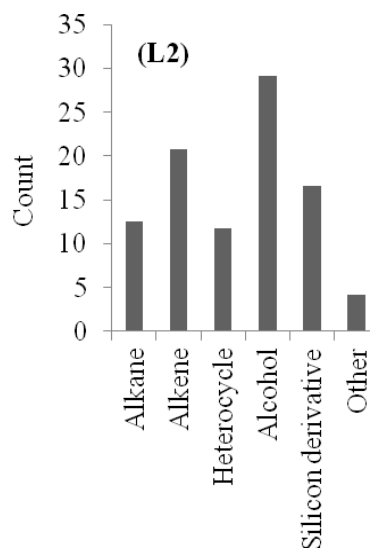


Fig. 4(b)

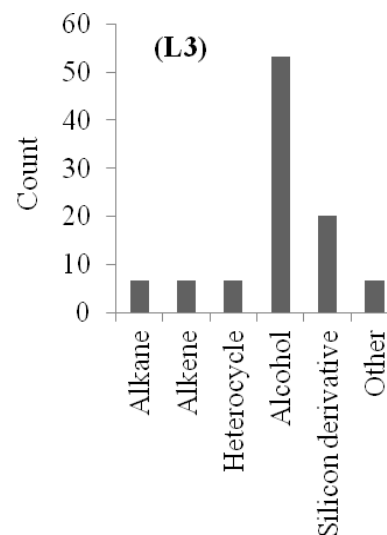


Fig. 4(c)

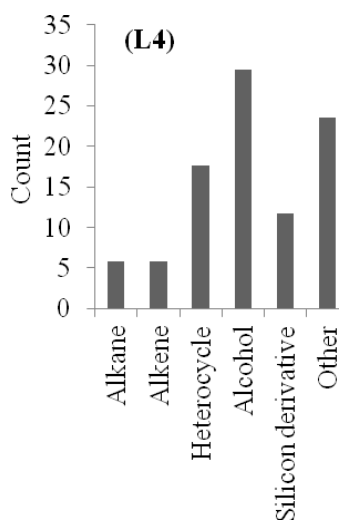


Fig. 4(d)

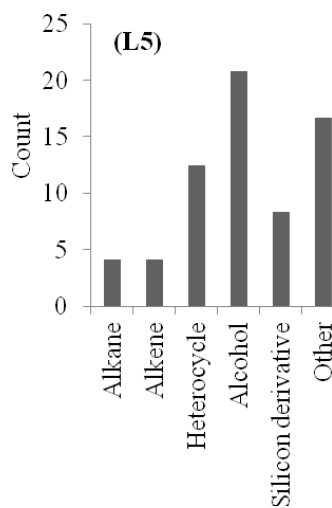


Fig. 4(e)

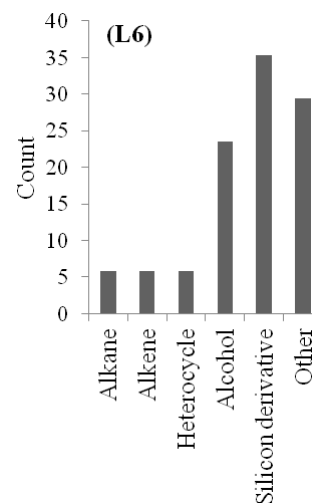


Fig. 4(f)

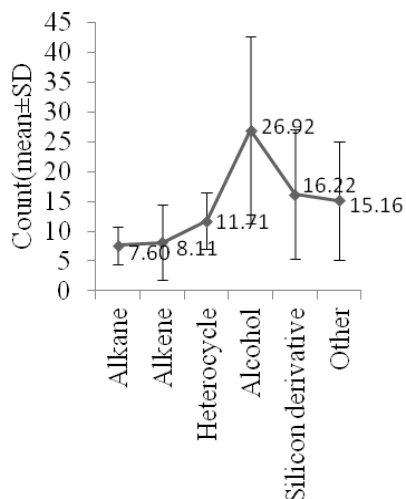


Fig. 4(g)

**Fig. 4:** The count of different phytochemicals based on their functional groups. (a) L1 (b) L2 (c) L3 (d) L4 (e) L5 (f) L6 and (g) cumulative count of phytochemicals in all six white *Calotropis gigantea* species.



observed in their relative abundance resulting in a synergy of phytochemical profiles between the same species from various coastal locations. Besides, the leaf extracts of *C. gigantea* white from six populations across the Coleroon delta were characterized by a high percentage of alcohol and silicone derivatives as the dominant classes of compounds. However, it was found that some of the major constituents identified were notably different from documented literature which could be linked to plethora of medicinal activities ensuring an effective conservation and utilization of white *gigantea* species. Hence, the present study reflects how the overall chemical composition in leaves of white species through the divergence and coexistence may play a key role in the selective preference of a beneficial compound among all ecological sites. Further investigations would be emphasized to explore the therapeutical importance of leaf metabolites and the other anatomical parts of the sand dune flora with respect to their phytochemistry to select sustainable novel drugs.

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### Conflict of interest

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

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