PHYTOCHEMICAL SCREENING AND BIOCHEMICAL ANALYSIS OF A FEW TRADITIONALLY IMPORTANT MEDICINAL PLANTS AGAINST DERMATOPHYTES

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ABSTRACT

Human skin diseases, caused by dermatophytes, are common in North East India due to the high humid condition. The medicinal plants play the major role of treating the skin diseases in the region, especially among the ethnic people. The present study was undertaken to analyse different phytochemicals and biochemical properties of ten different medicinal plants used by traditional healers against dermatophytes. Based on the phytochemical constituents, four plants viz Dendrocnide sinuata (Blume) Chew, Meyna laxiflora Robyns, Sterculia villosa Roxb, and Eupotorium odoratum L were carried forward for further analyses. The result showed that the methanol, chloroform and aqueous leaf extracts of D. sinuata, M. laxiflora, S. villosa and E. odoratum hold promises as a source of pharmaceutically important phytochemicals like alkaloid, tannin, flavonoid and phenol. Methanol, chloroform and aqueous leaf extracts of M. laxiflora recorded higher content of phenol and flavonoid. Higher content of alkaloid was recorded in all the four selected plants. Total tannin content was found higher in E. odoratum followed by S. villosa, M. laxiflora and D. sinuata. Major biochemical compounds identified through GC-MS analysis are neophytadiene, linalool, indoles, terpenes, acetogenins, phenols, Z-28-Heptatriaconten-2-one, oxirane, hexadecyl, phytol, squalene and 2,4-DI-tert-butylphenol. These findings would be useful for further exploitation of the selected experimental plants for bioprospection.

Keywords: - Phytochemical screening, biochemical analysis, traditional use, medicinal plant, dermatophytes

INTRODUCTION

The incidence of dermatophytosis has increased considerably day by day (Jessup *et al*., 2000). It occurs in various forms as non-contagious and contagious diseases. The primary cause of skin diseases is fungal but sometimes bacterial, viral and parasitic infection also occurred. The sub-tropical humid condition in North East India facilitates the incidence of fungal diseases accounting 50% of total skin diseases (Das, 2003). Moreover, the majority of the people of Assam is engaged in agriculture and related activities and is thereby frequently exposed to different dermatological infections (Saikia *et al*., 2006). Plants, animals and minerals are natural products that have been the basis in the treatment of many diseases from ancient time (Süntar, 2006). In India and other

developing countries, low-income group people, and ethnic/native communities use folk medicine for the treatment of various diseases (Fabricant and Farnsworth, 2001). The people of the region also have developed a rich ethnomedical practice. Investigations of the indigenous remedies and their possible effects have attracted attention to many researchers for years.

Traditional knowledge is still relevant, firstly, because of highly profitable and today's best selling drugs or classes of drugs are being obtained from such learning; secondly, because the pharmaceutical industry is dependent on the renewing of past discoveries, some of which will definitely have traditional knowledge origins; thirdly, research on natural product, whether plant or animal origin, continues despite the promise of new chemical, biotechnological and screening technologies, to be absolutely essential for the industry (Keswani *et al*., 2017). There is enough evidence to demonstrate that loss of traditional knowledge means loss of a potentially huge and priceless stock of complex biological substances. There is a need for efforts to support the ethnoecological systems that conserve this knowledge through everyday use and generate further knowledge (Dutfield, 2010). Since the dawn of civilization, humans have learned to use plants and plant-derived products as remedies for various ailments, leading to the discovery of various home-made remedies. Such practices are seen in traditional cultures, often followed by village shamans or tribal medicine men.

Although the synthetic medicines are effective, their regular use led to development of the resistance of microbes (Hamdache *et al*., 2010; Katan, 1982). There is a constant need for novel and effective therapy (Bhavnani and Ballow, 2000). Researchers, therefore, searched for alternate source of medicine (Shih *et al*., 2010; Singleton *et al*., 1999). Medicinal plant plays a key role in world health and use of these plants has attained a commanding role in world health system all over the world (Oladeji, 2016). Almost 25% of prescribed medicines are directly or indirectly derived from plants (Parekh and Chanda, 2007). The presence of different phytochemicals and bioactive compounds of the medicinal plants play the major role of treating the skin diseases caused by dermatophytes. The plants consider as a rich resource of ingredients which can be used in drug development (Rakotoarivelo *et al*., 2015; Yuan *et al*., 2016). Some of the medicinal plants are important source of nutrition and have therapeutic values (Abdul and Hassan, 2012). The major categories of plant-derived compounds having medicinal properties are the flavonoids, phenolics, terpenoids, the glycosides and the alkaloids (Sahoo and Marar, 2018). The secondary metabolites are accountable for the biological properties of plants and used for various purposes, including treatment of diseases (Singh, 2015).

Keeping view of the facts, the present study therefore, was conducted to determine the phytochemical constituents and detailed biochemical analysis of a few traditionally used medicinal plants from Assam, India against dermatophytes.

MATERIALS AND METHODS

Collection and identification of plant samples

Based on the secondary information and direct interactions with the traditional healers, 10 plants which are extensively used against dermatophytes were selected and collected from different areas of Kamrup district of Assam, India (Fig. 1; Table 1). The plant samples were identified following the standard literature i.e., Flora of Assam and authenticated by Department of Botany, Gauhati

University. The collected plants include Meyna laxiflora Robyns, Dendrocnide sinuata (Blume) Chew, Vitex negundo L, Centella asiatica (L.) Urban, Sterculia villosa Roxb. Ex Sm., Nymphaea nouchali Burn. f., Dioscorea pentaphylla (L.), Houttuynia cordata Thunb., Ocimum gratissimum (L.) and Eupotorium odoratum L. (Fig. 2; Table 1). The local names, site of collection, distribution, latitude, and longitude of the plant species have been shown in Table 1 (Kanjilal *et al*., 1940; Jain and Rao, 1977).

Preparation of plant samples

After identification of the specimen healthy leaves were collected and washed with tap water, prior to distilled water and dried in the shade for 40-45 days till it turns into moisture free. After drying, the leaves were ground to a coarse powder using mixture grinder and kept in an airtight container. The powdered material was then extracted with methanol, chloroform and water separately. For preparing the organic extract, 20 gm of powdered plant material was soaked in 200 ml solvent for 24 hrs in an orbital shaker at 150rpm in 300 C and macerate were filtered through the Whatman No. 1 filter paper to obtain filtrate. The filtrate was allowed to evaporate to dryness using a rotary evaporator (Buchi R-124). For the aqueous extract, 100 gm of powdered samples were heated in 1000 ml water for one hour in a water bath at 400C, filtered and finally lyophilized to dryness. The extracted samples were stored in closed glass vials in the refrigerator at 4^0 C till further investigation (Khan and Javaid, 2020; Harborne, 1973).

Table 1. Different species of medicinal plants collected from various locations

Sl. No.	Plant species	Family	Local Name	Site of Collection	Distribution (Regional)	GPS coordinates
1	*Centella asiatica* (L.) Urban	Apiaceae	Bor manimuni	Rani	Very common herb	26.07316° N 91.59463° E
2	*Dendrocnide sinuate* (Blume) Chew	Urticaceae	Sorat	Gamarimura, Boko	Common plant found throughout Assam	25.86844° N 91.12830° E
3	*Houttuynia cordata* Thunb.	Saururaceae	Masundari	Muduki	Common in kitchen garden	25.87744° N 91.46994° E
4	*Vitex negundo* (L.)	Lamiaceae	Posotia	Rani	Found near water bodies, grasslands, and mixed open forests.	26.07316° N 91.59463° E
5	*Sterculia villosa* Roxb. Ex Sm.	Malvaceae	Odal	Gunpati	Commonly found in deciduous forest	25.99499° N 91.55124° E
6	*Dioscorea pentaphylla* (L.)	Dioscoreaceae	Pachpotia alu	Bherbheri	Lakhimpur, Degraded deciduous forests and wet places	26.01561° N 91.39182° E
7	*Eupotorium odoratum* (L.)	Asteraceae	Jarmoni bon	Chandubi	Common plant found throughout Assam in low watery area	25.87134° N 91.42303° E
8	*Meyna laxiflora* Robyns	Rubiaceae	Kutkura	Chandubi	Open areas of Barak Valley, Darrang, Goalpara, Kamrup	25.87134° N 91.42303° E
9	*Ocimum gratissimum* (L.)	Lamiaceae	Ram tulsi	Garopara	Plains to Low Altitude, Moist localities throughout Assam	25.96175° N 91.48278° E
10	*Nymphaea nouchali* Burm.f	Nympheaceae	Bhet	Ukium	Common aquatic plant	25.84445° N 91.34402° E

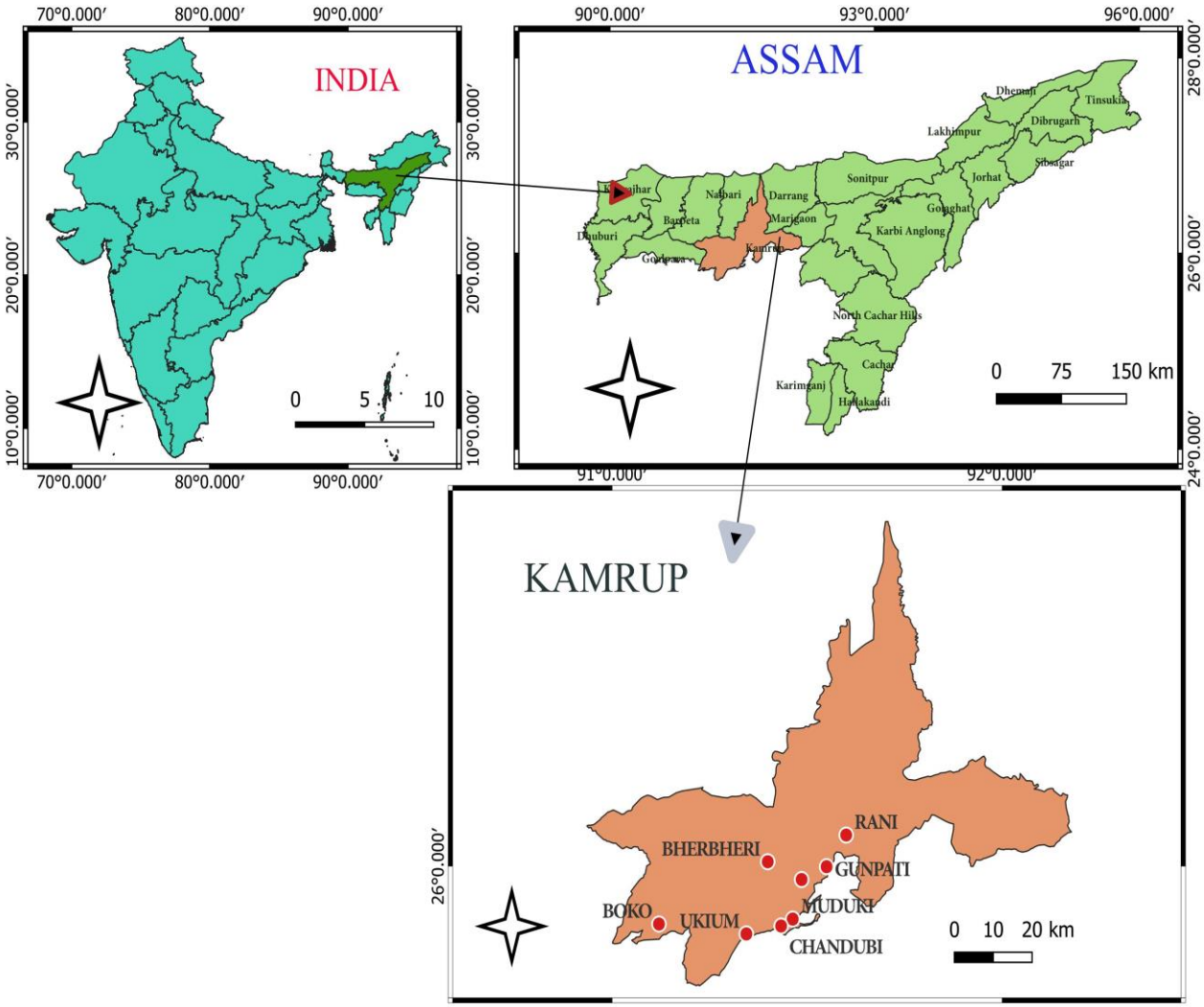


Fig. 1. Map showing collection sites of the medicinal plant species

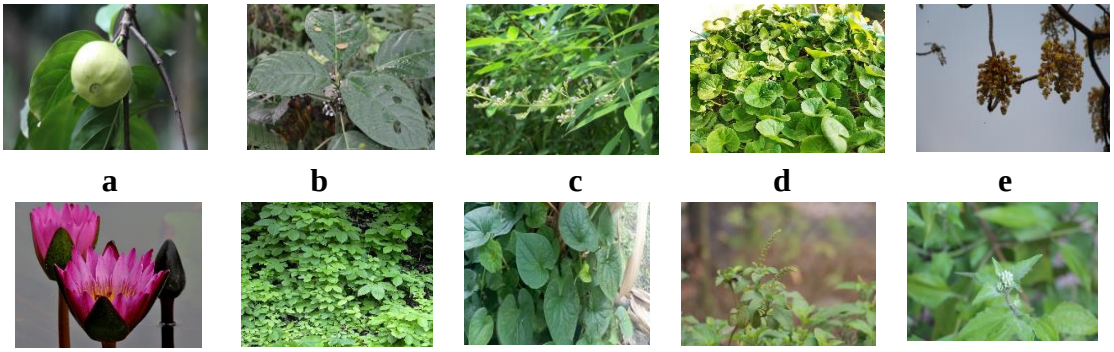


Fig. 2. Medicinal plant species used in this study; a) *M. laxiflora* Robyns, b) *D. sinuata* (Blume) Chew, c) *V. negundo* L, d) *C. asiatica* (L.) Urban, e) *S. villosa* Roxb. Ex Sm., f) *N. nouchali* Burm. f., g) *D. pentaphylla* (L.) h) *H. cordata* Thunb., i) *O. gratissimum* (L.) and j) *E. odoratum* L.

Qualitative phytochemical screening

The qualitative phytochemical screening was determined by following standard methods. Alkaloid, tannin, flavonoid, carbohydrate, protein, glycoside, saponin, steroid, phenol and terpenoid were tested.

Quantitative phytochemical screening

For quantitative phytochemical evaluation total phenolic content, total flavonoid content, alkaloids and tannins were estimated. The total phenolics in the extracts were estimated by spectrophotometric assay (Barreira *et al*., 2008) with minor modification. Gallic acid was used for constructing the standard curve and the results were expressed as μg of gallic acid equivalents/mg of extract (GAEs).

Flavonoid contents in the extracts were determined by method of Ebrahimzadeh *et al*., 2008 (Ebrahimzadeh *et al*., 2008) with slight modification. The total flavonoid contents in the extract in quercetin equivalents were calculated by the following formula:

T= CV/M Where, T= total flavonoid content in mg/mL of plant extract, C= the concentration of quercetin established from the calibration curve in mg/mL, V= the volume of extract in mL, M= the weight of methanolic plant extract in mg.

Alkaloids were determined using Harborne, 1978 method and calculated using the following formula: % Alkaloid = (W3 − W2 W1) ∗ 100; Where: W1 =initial weight of sample, W2 =weight of the extract, W3 = final weight of the residue.

Tannins were evaluated by using the method of Peri and Pompei, 1971. A standard graph (gallic acid1mg/ml) was plotted for determining the tannin content of the extracts. The total tannin content was expressed in mg/g of extract.

GC- MS analysis

For the GC-MS analysis, methanol and chloroform extracts of selected plants were analysed for possible compound identification and characterisation of samples.

A 30 × 0.25 mm × 0.25 μmdf a 5% diphenyl 95% dimethyl polysiloxane column; was used in Clarus 500 Perkin–Elmer gas chromatograph with a Turbo mass gold-Perkin–Elmer detector. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1 mL/min. and an injection volume of 3 mL was employed (split ratio of 10:1) (Roy *et al*., 2019). Injector temperature was 250 °C and ion-source temperature was 180 °C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450 Da. Total GC running time was 36 min. GC-MS mass spectrum analysis was performed using the National Standard and Technology Institute (NIST, 2014) database and a library search (Casuga *et al*., 2016). The name, retention time (RT), molecular formula (MF), molecular weight (MW), a peak area in percentage and structure of the compound have been established (VasudhaUdupa *et al*., 2012).

Statistical analysis

The experiments were performed in a complete randomized way with three replications. The findings were calculated mean ± SE using Microsoft excel. Significant differences among the plant extracts were estimated by ANOVA with Duncan's multiple range test and Tukey hsd was performed ($p<0.05$) using R-programme.

RESULTS

Plant contains a variety of phytochemicals which have biological activity. These substances represent the main source of active components which are very important in pharmaceutical industry. The present investigation was carried out to determine the phytochemical and biochemical analysis of selected medicinal plants which are used against dermatophytes by traditional healers.

Qualitative phytochemical screening of crude extracts of plants

The results of the phytochemical studies depicted the presence or absence of different types of phytochemicals such as alkaloid, tannin, flavonoids, carbohydrate, steroid, terpenoid etc. were present in almost all the plants which are summarized in the (Fig 3).

The result showed that the methanol, chloroform and aqueous leaf extracts of *D. sinuata, M. laxiflora, S. villosa* and *E. odoratum* hold promises as a source of pharmaceutically important phytochemicals like alkaloid, tannin, flavonoid and phenol. Hence, quantitative determination of these four plants for important phytochemicals become crucial.

Biochemical analysis

In the biochemical analysis the total content of phenol, flavonoid, alkaloid and tannin present in various leaf extract of screened plants were determined. The results demonstrate that *D. sinuata, M. laxiflora, S. villosa* and *E. odoratum* hold promises as a source of pharmaceutically important phytochemicals like alkaloid, tannin, flavonoid and phenol (Table 2 and Fig 4).

The total phenol content was found in a range from 2.58 (µg/ml GAE) for methanol leaf extract of *S. villosa* (Roxb.) to 14.64 (µg/ml GAE) for methanol leaf extract of *M. laxiflora* (Robyns). A notable difference was also observed in case of total flavonoid content.

The total flavonoid content ranges from 0.76 (mg/g quercetin) for aqueous leaf extract of *S. villosa* (Roxb.) to 8.00 (mg/g quercetin) for chloroform leaf extract of *M. laxiflora* (Robyns).

Total alkaloid content in the investigated plants ranges from 0.65 mg/g to 1.37 mg/g. The lowest amount was found in aqueous leaf extract of *E. odoratum* (Linn.) and highest in methanol leaf extract of *M. laxiflora* (Robyns).

Significant difference was found in case of total tannin content which ranges from 0.05 mg/g in aqueous leaf extract of *E. odoratum* (Linn.) to 4.02 mg/g in methanol leaf extract of *E. odoratum* (Linn.).

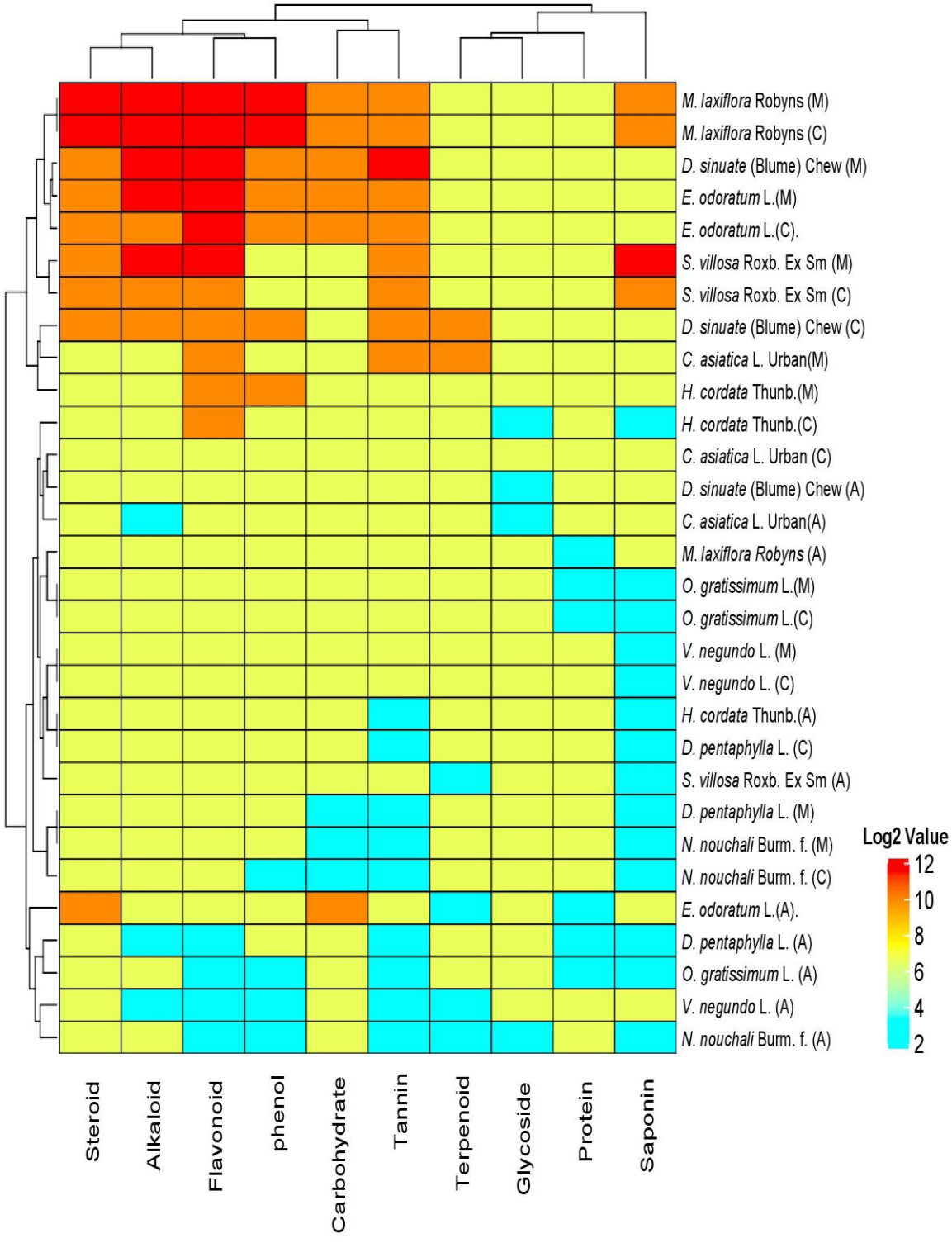
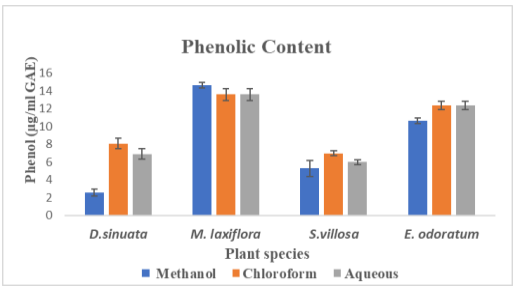


Fig 3. Heatmap showing qualitative phytochemical screening of three different crude extracts of selected plants (M= Methanol, C= Chloroform A= Aqueous

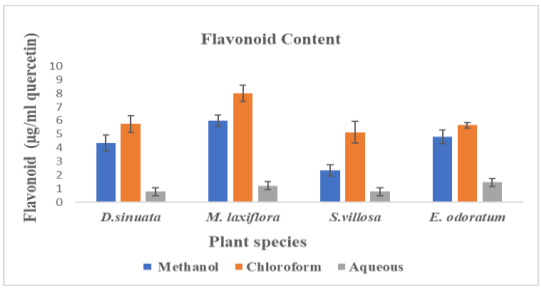
Table 2. Phenolic, flavonoid, alkaloid and tannin content of *D. sinuata*, *M. laxiflora*, *S. villosa* & *E. odoratum* in methanol, chloroform & aqueous extract (GAE & CE)

Extract	Plant (Leaf)	Total Phenol content (mg/g)	Total Flavonoid content (mg/g)	Total Alkaloid content (mg/g)	Total Tannins content (mg/g)
Methanol extract	*D. sinuata* (Blume)	2.58±0.4	4.34±0.6	0.99±0.3	2.59±0.1
	M. laxiflora Robyns	14.64±0.3	5.99±0.4	1.37±0.1	2.93±0.1
	S. villosa (Roxb.)	5.31±0.9	2.34±0.4	0.88±0.27	3.53±0.1
	E. odoratum (Linn.)	10.63±0.3	4.81±0.5	1.12±0.12	4.02±0.5
Chloroform extract	*D. sinuata* (Blume)	8.09±0.6	5.75±0.6	1.37±0.13	0.81±0.09
	M. laxiflora Robyns	13.59±0.7	8.00±0.6	1.32±0.1	1.21±0.6
	S. villosa (Roxb.)	6.99±0.3	5.14±0.8	0.92±0.12	0.87±0.4
	E. odoratum (Linn.)	12.37±0.5	5.67±0.2	0.94±0.14	0.48±0.2
Aqueous extract	*D. sinuata* (Blume)	6.92±0.6	0.77±0.03	0.68±0.1	0.39±0.08
	M. laxiflora Robyns	13.18±0.7	1.21±0.03	0.74±0.1	0.36±0.5
	S. villosa (Roxb.)	5.99±03	0.76±0.03	0.68±0.08	0.27±0.6
	E. odoratum (Linn.)	12.37±0.5	1.46±0.03	0.65±0.07	0.05±0.7

Samples were analyzed in three replicates and data are as an average of three tests i.e., n=3, mean ± standard error.



(A)



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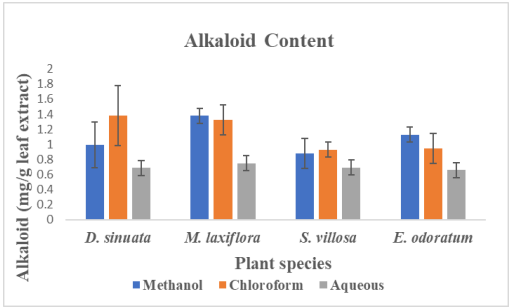

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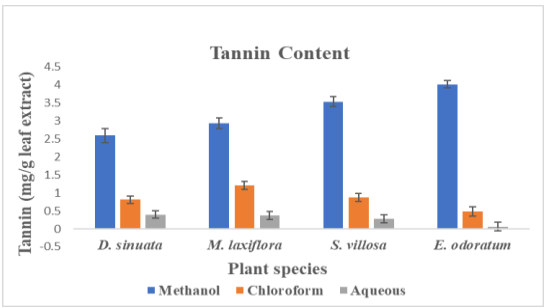

(D)

Fig. 4. Biochemical properties of the plant species; A. Total phenolic content (µg/ml GAE); B. Total flavonoid content (µg/ml quercetin); C. Alkaloid content (%); D. Tannin content (mg/g)

GC-MS analysis

Gas chromatography-mass spectrometry is the best techniques to detect the components of the volatile substance, long chain, branched chain hydrocarbons, alcohols, acids, esters etc. In the present study, GC-MS chromatogram analysis of the methanol and chloroform extracts of *D. sinuata*, *M. laxiflora, S. villosa* and *E. odoratum* showed several peaks which indicate the presence of numerous phytochemical compounds (Fig 5 & Fig 6). The name, retention time (RT), molecular formula (MF), molecular weight (MW), a peak area in percentage and bioactivity of the compounds have been presented in (Table 3).

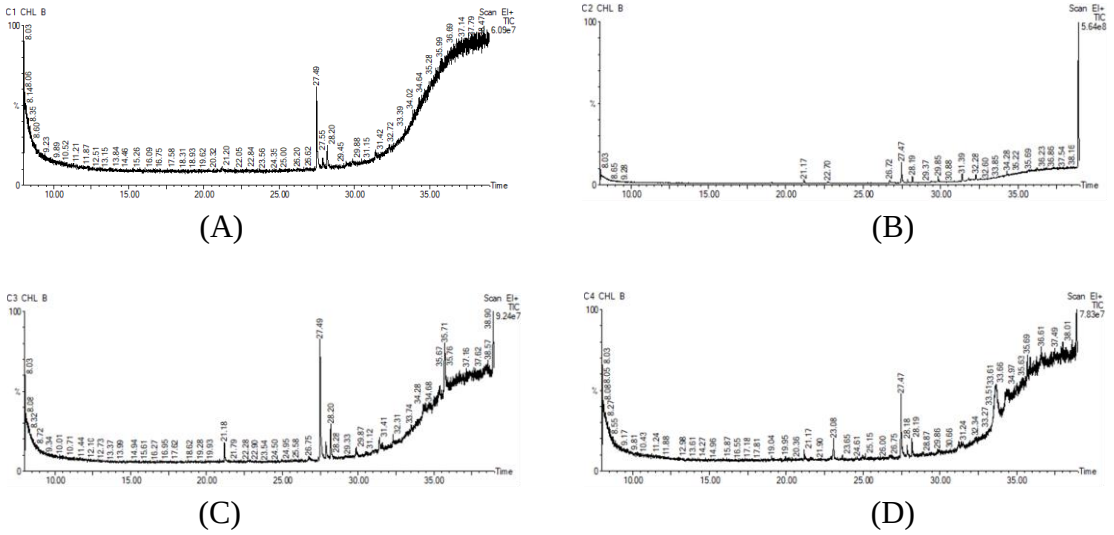

Fig. 5. GC-MS chromatogram of chloroform leaf extract of (A) *D. sinuata*, (B) *M. laxiflora*, (C) *S. villosa*, (D) *E. odoratum*

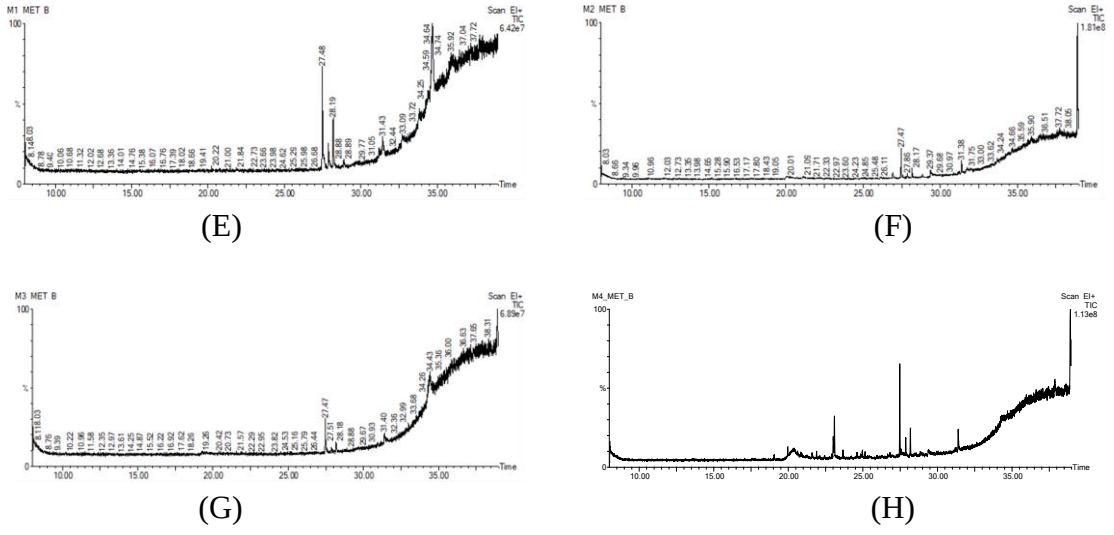

Fig. 6. GC-MS chromatogram of methanolic leaf extract of (E) *D. sinuata*, (F) *M. laxiflora*, (G) *S. villosa*, (H) *E. odoratum*

Table 3. Important phytocomponents identified in chloroform and methanol leaf extract of *D. sinuata*, *M. laxiflora*, *S. villosa* and *E. odoratum* by GC-MS analysis (RT= retention time, MW= molecular weight, MF= molecular formula)

Compound Name	MF	MW	RT	Peak Area (%)	Bioactivity
Chloroform extract					
D. sinuata					
Neophytadiene	$C_{20}H_{38}$	278	27.491	3.206	It has a role as an anti-inflammatory agent, an antimicrobial agent, a plant metabolite and an algal metabolite. It is an alkene and a diterpene (Ratheesh et al., 2022).
3,7-Dimethyl-1,6-octadiene	$C_{10}H_{18}$	138	27.891	0.351	It has a role as an anti-inflammatory agent, an antimicrobial agent, a plant metabolite and an algal metabolite. It is an alkene and a diterpene (Ratheesh et al., 2022).
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	28.196	0.860	The compounds with antifungal activity were identified as indoles, terpenes, acetogenins, phenols, and volatile halogenated hydrocarbons (Nithya *et al*., 2018).
Z-28-Heptatriaconten-2-one	$C_{37}H_{72}O$	533	31.418	0.201	This study suggested that FH could have high concentration of bioactive compounds like rutin and ellagic acid or its analogues compared to MFE which may be responsible for its strong antioxidant and antibacterial activity (Olasunkanmi *et al*., 2022).
Oxirane, hexadecyl	$C_{18}H_{3}$	268	31.418	0.201	Antidiabetic and antioxidant agents (Musa *et al*., 2015).
M. laxiflora					
Neophytadiene	$C_{20}H_{38}$	278	27.471	3.531	It has a role as an anti-inflammatory agent, an antimicrobial agent, a plant metabolite and an algal metabolite. It is an alkene and a diterpene (Ratheesh *et al*., 2022).
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	28.196	0.860	The compounds with antifungal activity were identified as indoles, terpenes, acetogenins, phenols, and volatile halogenated hydrocarbons (Nithya *et al*., 2018).
Phytol	$C_{20}H_{40}O$	296	31.393	1.613	Phytol is an approximately good

					antimicrobial agent. The antimicrobial efficacy of phytol is comparable with other traditional disinfectants (Ghaneian *et al*., 2015).
Squalene	$C_{20}H_{50}$	410	38.906	1.330	It is a natural 30-carbon isoprenoid compound and intermediate metabolite in the synthesis of cholesterol. It is not susceptible to lipid peroxidation and provides skin protection. Squalene is investigated as an adjunctive cancer therapy (Lozano-Grande *et al.*, 2018).
S. villosa					
2,4-DI-tert-butylphenol	$C_{20}H_{40}O$	206	21.179	0.771	2,4-di-tert-butylphenol is a member of the class of phenols. It has a role as a bacterial metabolite, an antioxidant and a marine metabolite (VasudhaUdupa *et al*., 2012).
Neophytadiene	$C_{20}H_{38}$	278	27.491	5.014	It has a role as an anti-inflammatory agent, an antimicrobial agent, a plant metabolite and an algal metabolite. It is an alkene and a diterpene (Ratheesh *et al*., 2022).
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	27.491	5.014	The compounds with antifungal activity were identified as indoles, terpenes, acetogenins, phenols, and volatile halogenated hydrocarbons (Nithya *et al*., 2018).
E. odorata					
Neophytadiene	$C_{20}H_{38}$	278	27.471	2.009	It has a role as an anti-inflammatory agent, an antimicrobial agent, a plant metabolite and an algal metabolite. It is an alkene and a diterpene (Ratheesh *et al*., 2022).
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	27.491	5.014	The compounds with antifungal activity were identified as indoles, terpenes, acetogenins, phenols, and volatile halogenated hydrocarbons (Nithya *et al*., 2018).
9-Eicosyne	$C_{20}H_{38}$	278	27.881	0.422	Anti-microbial and cytotoxic properties (Paul *et al*., 2022).
Methanol extract					
D. sinuata					
Neophytadiene	$C_{20}H_{38}$	278	27.471	2.009	It has a role as an anti-inflammatory agent, an antimicrobial agent, a plant

					metabolite and an algal metabolite. It is an alkene and a diterpene (Ratheesh *et al*., 2022).
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	27.491	5.014	The compounds with antifungal activity were identified as indoles, terpenes, acetogenins, phenols, and volatile halogenated hydrocarbons (Nithya *et al*., 2018).
Phytol	$C_{20}H_{40}O$	296	31.428	1.295	Phytol is an approximately good antimicrobial agent. Moreover, it had no remarkable toxicity and had high stability. Compared with other studies, the antimicrobial efficacy of phytol is comparable with other traditional disinfectants (Musa *et al*., 2015).
M. laxiflora					
Neophytadiene	$C_{20}H_{38}$	278	27.471	2.009	It has a role as an anti-inflammatory agent, an antimicrobial agent, a plant metabolite and an algal metabolite. It is an alkene and a diterpene (Ratheesh *et al*., 2022).
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	27.491	5.014	The compounds with antifungal activity were identified as indoles, terpenes, acetogenins, phenols, and volatile halogenated hydrocarbons (Nithya *et al*., 2018).
Squalene	$C_{30}H_{50}$	410	38.896		It is a natural 30-carbon isoprenoid compound and intermediate metabolite in the synthesis of cholesterol. It is not susceptible to lipid peroxidation and provides skin protection. Squalene is investigated as an adjunctive cancer therapy (Lozano-Grande *et al.*, 2018).
S. villosa					
Neophytadiene	$C_{20}H_{38}$	278	27.471	2.009	It has a role as an anti-inflammatory agent, an antimicrobial agent, a plant metabolite and an algal metabolite. It is an alkene and a diterpene (Ratheesh *et al*., 2022).
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	27.491	5.014	The compounds with antifungal activity were identified as indoles, terpenes, acetogenins, phenols, and volatile halogenated hydrocarbons (Nithya *et al*., 2018).

E. odorata					
Neophytadiene	$C_{20}H_{38}$	278	27.471	2.009	It has a role as an anti-inflammatory agent, an antimicrobial agent, a plant metabolite and an algal metabolite. It is an alkene and a diterpene (Ratheesh *et al*., 2022).
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	27.491	5.014	The compounds with antifungal activity were identified as indoles, terpenes, acetogenins, phenols, and volatile halogenated hydrocarbons (Nithya *et al*., 2018).
Phytol	$C_{20}H_{40}O$	296	31.428	1.295	Phytol is an approximately good antimicrobial agent. Moreover, it had no remarkable toxicity and had high stability. Compared with other studies, the antimicrobial efficacy of phytol is comparable with other traditional disinfectants (Musa *et al*., 2015).

DISCUSSION

The plant kingdom has proved to be most important source of components required for therapeutic uses, like treatment of various diseases and thus are important sources for most of the pharmaceuticals. Medicinal plants contain different compounds like alkaloid, tannin, flavonoid, carbohydrate, protein, glycoside, saponin, steroid, phenol, diterpenoid etc. which provide specific physiological action on human body (Roy *et al*., 2019; Kabir *et al*., 2020). Keeping this important perspective in mind, the investigations were carried out on phytochemical analysis of methanol, chloroform and aqueous leaf extract of *D. sinuata, M. laxiflora, S. villosa* and *E. odoratum*. In the study, the phytochemical analysis of methanol, chloroform and aqueous leaf extracts of *D. sinuata, M. laxiflora, S. villosa* and *E. odoratum* showed the presence of various groups of secondary metabolites like alkaloid, tannin, flavonoid, carbohydrate, protein, glycoside, saponin, steroid, phenol, terpenoid etc. which are potential source of diverse range of medicines or important bioactive compound for human benefits (Kabir *et al*., 2020; Tanti *et al*., 2010).). By GC-MS analysis, several compounds were detected but some of the identified major biochemical compounds through GC-MS analysis are neophytadiene, linalool, indoles, terpenes, acetogenins, phenols, Z-28-Heptatriaconten-2-one, oxirane, hexadecyl, phytol, squalene and 2,4-DI-tert-butylphenol (Ratheesh *et al*., 2022; Guo *et al*., 2021; Nithya *et al*., 2018; Olasunkanmi *et al*., 2022; Musa *et al*., 2015).

Methanol, chloroform and aqueous leaf extract of *C. asiatica* showed the presence of tannin, flavonoid carbohydrate, protein, glycoside, saponin, steroid and terpenoid (Jacinda *et al*., 2009). While, methanol leaf extract exhibited higher content of tannin, flavonoid and terpenoid than both chloroform and aqueous leaf extract. Methanol and chloroform leaf extracts of *D. sinuata* recorded the presence of alkaloid, tannin, flavonoid, carbohydrate, protein, steroid, phenol and diterpenoid (Tanti *et al*., 2010). On the other hand, methanol and chloroform leaf extract showed the higher content of alkaloid, tannin, flavonoid, steroid, phenol and terpenoid than aqueous extract. Methanol, chloroform and aqueous leaf extract of *H. cordata* showed the presence of alkaloid, tannin,

flavonoid, steroid and terpenoid. While, methanol and chloroform recorded extract the higher content of flavonoid and phenol. Methanol, chloroform and aqueous leaf extracts of V. negundo recorded the presence of alkaloid, tannin, flavonoid, steroid, phenol and terpenoid. Methanol, chloroform and aqueous leaf extracts of *S. villosa* indicated the presence of alkaloid, tannin, flavonoid, carbohydrate, protein, saponin, steroid, phenol and terpenoid but methanol and chloroform extract exhibited the higher content of alkaloid, tannin, flavonoid, saponin and steroid (Das *et al*., 2017). Methanol and chloroform leaf extract of *D. pentaphylla* showed the presence of alkaloid, flavonoid, protein, glycoside, steroid, phenol and terpenoid, while aqueous extract of the same exhibits the presence of carbohydrate, glycoside, steroid, phenol and terpenoid (Prakash and Hosetti, 2012). Methanol, chloroform and aqueous leaf extracts of *E. odoratum* recorded the presence of alkaloid, tannin, flavonoid, carbohydrate, protein, steroid, phenol and terpenoid but methanol and chloroform leaf extract recorded the higher content of alkaloid, tannin and flavonoid (Mishra *et al*., 2010). Methanol, chloroform and aqueous leaf extracts of *M. laxiflora* indicated the presence of alkaloid, tannin, flavonoid, carbohydrate, protein, glycoside, saponin, steroid, phenol and terpenoid, while methanol and chloroform extract showed the higher content of alkaloid, tannin, flavonoid, steroid, phenol and terpenoid (Dhodade *et al*., 2019). All the three leaf extracts of *O. gratissimum* and *N. nouchali* indicated the mere presence of alkaloid, tannin, flavonoid, steroid, phenol and terpenoid, the presence of alkaloid, flavonoid, saponin, tannin, carbohydrate, phenol and glycoside (Prabhu *et al*., 2019; Islam and Ahmed, 2017) were recorded in methanol and aqueous extract of *Cerbera odollam*, a medicinal plant having antimicrobial activity as reported by Sahoo and Marar, 2018.

The total phenol content for dry weight of *M. laxiflora* was estimated to be 14.64 mg/g for methanol extract and 10.63 mg/g for *E. odoratum*. Phenols are reactive species towards oxidation and pose biological activity. The process of oxidation and free radicals' generation leads to cancer and other diseases. The activity of phenols against this cancer-causing process can have therapeutic application in anti-cancer therapies. Plants having more phenol content exhibits good antioxidant activity.

The total flavonoid content was found to be 8.00 mg/g for chloroform extract and 5.99 mg/g for methanol extract mg/g of *M. laxiflora*, while, 5.75 mg/g for chloroform extract of *D. sinuata* and 5.67 mg/g for chloroform extract mg/g of *E. odoratum*. The antioxidative properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper, and inhibition of enzymes responsible for free radical generation.

The total alkaloid of *M. laxiflora* for methanol extract and *D. sinuata* for chloroform extract was found to be 1.37 mg/g, whereas, that of *M. laxiflora* for chloroform was 1.32 mg/g and for methanol extract of *E. odoratum* was 1.12 mg/g (Tanti *et al*., 2010). Most of the alkaloids have local anaesthetic and stimulant properties. These show cytotoxic activity even in low concentration and other biological activity, showing a wide use in the medical application. The total tannin content of methanol extract of *E. odoratum* was 4.02 mg/g and of *S. villosa* was 3.53 mg/g. On the other hand, total tannin content of methanol extract of *M. laxiflora* was 2.93 mg/g and of *D. sinuata* was 2.59 mg/g. Most of the tannins have antibacterial, antifungal and anti-cancer properties. Tannin is an astringent, bitter plant polyphenolic compound that binds to and precipitates proteins and various

other organic compounds including amino acids and alkaloids. This tannin-protein complex can provide persistent antioxidant activity.

More than 70 compounds were obtained from methanol and chloroform extracts. The compounds having antioxidant, antimicrobial significance has been listed in the Table 4. Two compounds Neophytadiene and 3,7,11,15-Tetramethyl-2-hexadecen-1-ol were found in both methanol and chloroform extracts of *D. sinuata, M. laxiflora, S. villosa* and *E. odoratum*. These two compounds with antimicrobial activity were identified as indoles, terpenes, phenols etc. 3,7-Dimethyl-1,6-octadiene also known as Linalool is a volatile oil component, an antimicrobial agent was present in chloroform extract of *D. sinuata*. Phytol is an approximately good antimicrobial agent was present in chloroform extract of *M. laxiflora* and methanol extract of *D. sinuate* and *E. odoratum*. Squalene present in chloroform and methanol extract of *M. laxiflora* is investigated as an adjunctive cancer therapy (Bacanl *et al*., 2018). 9-Eicosyne, an anti-microbial agent, a plant metabolite was obtained from chloroform extract of *E. odoratum*.

CONCLUSION

It can be concluded that plants under study have a potential source of phytochemical constituents that would be useful for future pharmaceutical research. The GC-MS analysis justifies the presence of important biological and pharmacological properties such as antimicrobial and antioxidant activities. In the study, the phytochemical analysis of methanol, chloroform and aqueous leaf extracts of *D. sinuata, M. laxiflora, S. villosa* and *E. odoratum* established the presence of alkaloid, tannin, flavonoid, carbohydrate, protein, glycoside, saponin, steroid, phenol, terpenoid etc. which are potential source of diverse range of medicines or important bioactive compound for human benefits. By GC-MS analysis, several compounds were detected but some of the identified major biochemical compounds through GC-MS analysis are neophytadiene, linalool, indoles, terpenes, acetogenins, phenols, Z-28-Heptatriaconten-2-one, oxirane, hexadecyl, phytol, squalene and 2,4-DI-tert-butylphenol. Further studies are suggested to confirm more active compounds for pharmacological uses.

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