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GC-MS, antibacterial and antioxidant potential of *Leucas lavandulifolia* Sm

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Abstract
Leucas lavandulifolia Sm. belonging to Lamiaceae is used extensively to treat various diseases in traditional medicine. Our aim of investigation to analysis the present of compounds and estimate the antioxidant and antibacterial activity of *L. lavandulifolia* floral extracts. Floral powder was successively extracted with various organic solvents in the order of increasing polarity, analysed for phytochemical constitution through GC-MS analysis. Screened for antioxidant activity DPPH and Ferric reducing ability and antibacterial activity was analysis by well diffusion methods. GC-MS analysis showed Tetracontane, Phenol, 2,4-bis(1,1-dimethylethyl)-, 5,7-dodecadiyn-1,12-diol and other 36 compounds. DPPH assay expressed IC_{50} values in the order of methanolic floral extract, ascorbic acid, ethyl acetate, chloroform and petroleum ether floral extracts such as 3.91, 0.24, 6.13, 5.38 and 69.75 µg/ml respectively. FRAP assay exhibited methanol floral extract and ascorbic acid respectively showed 0.2641 and 2.0088 (32 µg/ml). Methanol floral extract at 100 µg/ml concentration exhibited 17 mm (*Aeromonas hydrophila*), 18 mm (*Pseudomonas aeruginosa*), 19 mm (*Aeromonas caviae)*, 20 mm each (*Klebsiella pneumoniae* and *Streptococcus pyogenes*), 21 mm each (*Enterococcus faecalis* and *Enterobacter aerogenes*), 22 mm each (*Salmonella typhi* and *Corynebacterium diphtheriae*) and 23 mm against *Proteus mirabilis.* In this present study of plant species showed the present of biologically valuable secondary metabolites deducted by GC-MS analysis. It had strong antioxidant activity and exposed the significant antibacterial activity in methanolic extract. Further studies on the isolation and characterization of various bioactive principles from this herb as a potential source of natural antimicrobial agents will thereby be motivationally considered.

Keywords: gas chromatography, DPPH, FRAP, *Salmonella typhi*

Introduction
The World Health Organization (WHO) states that 80% (3,200 out of 4,000 million) inhabitants in the world depend mainly on traditional medicines to fulfill their primary health care requirements. Further, scientific screening of traditional medicines has led to identify 88 out of 119 plant-based drugs through isolation and structural elucidation of bioactive compounds [1]. It is estimated by the World Health Organization that currently there are 252 plant-based drugs wherein 11% of the drugs such as cardiotonic of digoxin (*Digitalis* spp.), antimalarial drugs of quinine and quinidine (*Cinchona* spp.), anticancer drugs of vincristine and vinblastine (*Catharanthus roseus*), anticholinergic drug of atropine (*Atropa belladonna*) and analgesic drugs of morphine and codeine (*Papaver somniferum*) are currently used worldwide [2]. Thirteen out of 69 small-molecule new drugs approved from 2005 to 2007 worldwide were of natural origin [3, 4]. About 75% or 131 small molecules approved as anticancer drugs from 1940 to 2014 are of either natural products or their derivatives [5]. Further, about 60% of anticancer and anti-infectious drugs currently available either in market or under clinical trials are derived from natural sources [6].
In traditional medicine, species of *Leucas* (Lamiaceae) distributed widely throughout Asia, including India and Africa, are treated to cure cough, cold, diarrhoea, and inflammatory skin disorder. *Leucas lavandulifolia* Sm. is used extensively in Mithila region of Bihar in India to treat various diseases such as cold, fever, headache, cough and loss of appetite, skin infections, and as antidotes for snakebite and scorpion sting in human and cattle [7]. Plant infusion has been used to treat psoriasis [8]. In pharmacology, it has been used variously such as entire plant in wound healing, antitussive, antipyretic and psychopharmacological and antidiarrheal [9, 10, 11, 12, 13]. Isolates of aerial part such as chrysoeriol-(OAC)-glucoside in analgesic and its derivative chrysoeriol-4′-O-α-L-rhamnopyranosyl (1>2) β-D-glucopyranoside in anti-inflammatory, antiulcer and floral parts in hypoglycaemia [9, 14, 15, 16, 17, 18].

Materials and Methods
Collection of plant materials
An herbarium of the plant material (P. Sakthidhasan and M.B. Viswanathan 3425) was collected from Sooriyur village in Tiruchirappalli, was prepared. Identity was authenticated by Prof. M.B. Viswanathan. Later, it was deposited in the Herbarium of the Centre for Research and Development of Siddha-Ayurveda Medicines (CRDSAM) of the Bharathidasan University in Tiruchirappalli, Tamil Nadu, India, for reference. Then, fresh plant materials required for the study were collected from the same location in enough quantities, washed thoroughly with distilled water, shade-dried, prepared coarse powder and extracted using Soxhlet apparatus with organic solvents from low to high polarity. The solvent extracts thus collected were concentrated using a Rotary Evaporator (Yamato VR300, Muromachi, Japan) and stored for further use.

GC-MS analysis
Various solvent floral extracts were used for GC-MS analysis. The column (HP-5) fused with silica 30 m x 0.25

mm I.D. was used. Conditions of analysis were 20 min at 100°C, 3 min at 230°C for column temperature, 260°C for injector temperature, helium was the carrier gas and split ratio was 10:1. One µl each of the solvent floral extracts was evaporated in a split less injector at 300°C. Run time was 32 min. Names, structures of the compounds and their molecular weight were ascertained by interpreting GC-MS mass spectrum database of the National Institute of Standards and Technology[19].

DPPH radical scavenging activity

A 0.1 mM of DPPH was dissolved with methanol. Each solvent floral extract was prepared in various concentrations such as 31.25, 62.5, 125, 250 and 500 µg/ml with DMSO. All the extracts were prepared similarly. In each well, 5 µl of the sample solution and 195 µl DPPH working solution was added to each well in a 96-well plate. After loading various concentrations of the floral extracts and a 20 min reaction time at room temperature, their absorbances were measured at 517 nm. The % of Inhibition was calculated by the formula of $(A_0 – A_1)/A_0 \times 100$ where A_0 (the absorbance of the control) and A_1 (the absorbance of the sample) [20].

Ferric reducing power assay

The FRAP reagent was prepared by mixing 300 mM sodium acetate buffer (pH 3.6), 10 mM TPTZ (2, 4, 6-tris (2-pyridyl)-s-triazine) solution and 20 mM ferric chloride. Various solvent floral extracts were prepared in different concentrations of 2, 4, 8, 16 and 32 µg/ml. Three ml each of FRAP reagent was added to them and permitted incubation at 37°C for 30 min. At 593 nm, their absorbances were measured and recorded [21].

Bacterial cultures

Bacterial strains of *Salmonella typhi* (733), *Klebsiella pneumoniae* (4030), *Aeromonas caviae* (6541), *Proteus mirabilis* (425), *Corynebacterium diphtheriae* (13812), *Enterococcus faecalis* (439), *Streptococcus pyogenes* (442), *Pseudomonas aeruginosa* (424), *Enterobacter aerogenes* (2822) and *Aeromonas hydrophila* (1739) were procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India, grown and maintained on Muller-Hinton medium at 37°C.

Antibacterial activity

Mueller-Hinton agar plates were prepared and inoculated with 17 h- old bacterial cultures using sterile cotton swabs. After 10 min, a cork borer was used to cut wells. Twenty µl each of the solvent floral extracts from 10, 20, 50 and 100 µg/ml concentration were loaded along with DMSO control and permitted 24 h incubation at 37°C. The diameters of the zones of inhibitions (ZOI) were measured for susceptibility and tabulated [22].

Results

GC-MS analysis

GC-MS analysis of the various solvent floral extracts in *L. lavandulifolia* showed 38 compounds. Number of compounds and exclusive to each extract were of 8 and 8 compounds to the petroleum ether floral extract, 17 and 17 compounds to the chloroform floral extract, 10 and 5 compounds to the ethyl acetate floral extract 4 and 3 compounds to the methanol floral extract respectively (Table 1).

Table 1: GC-MS analysis of various solvent floral extracts in *L. lavandulifolia*

S. No.	Compound name	Molecular weight	Extract	RT	Peak area
1	17-pentatriacontene	490	LCE	21.456	63,578,072.0
				24.442	17,803,748.0
2	1b,5,5,6a-tetramethyl-octahydro-1-oxa-cyclopropa[a]inden-6-one	208	LCE	20.155	21,461,698.0
3	1-docosene	308	LCE	16.549	11,150,498.0
4	1-hexyl-2-nitrocyclohexane	213	LME	21.176	17,824,420.00
5	2-dodecen-1-yl (0) succinic anhydride	266	LPE	22.666	111,540,232.0
			LPE	23.432	43,538,360.0
6	2-tert-butyl-4,6-bis(3,5-di-tert-butyl-4-hydroxybenzyl) phenol	586	LCE	30.384	12,358,852.0
7	3-piperidinol, 1,4-dimethyl-, cis-	129	LME	17.914	103,684,280.00
8	5,7-dodecadiyn-1,12-diol	194	LCE	21.811	170,621,296.0
9	5-eicosene, (e)-	280	LCE	18.189	21,007,370.00
10	6,10-dimethyl-4-undecanol	200	LEE	17.159	177,109,184.00
11	Acetoxyacetic acid, 4-pentadecyl ester	328	LEE	16.439	34,185,760.00
12	Alpha-d-galactopyranoside, methyl	194	LEE	16.774	75,249,088.00
				16.989	30,425,138.00
13	Behenyl chloride	344	LCE	21.811	29,474,950.00
14	Bicyclo[2.2.1]heptane-1-carboxamide, n-(2,5-difluorophenyl)-4,7,7-trime	321	LCE	23.847	34,494,400.00
15	Cyclohexene, 1-(2-nitro-2-propenyl)-	167	LCE	21.091	46,467,120.00
16	Cyclopropane, 1-cyclopropylethynyl-2-methoxy-3,3-dimethyl-	164	LCE	21.811	76,965,472.00
		322	LCE	23.001	65,605,836.00
17	Hentriacontane	436	LPE	26.383	202,566,992.00
18	Heptacosane	380	LEE	23.166	11,114,617.00
			LEE	26.458	9,344,070.00
			LPE	23.702	349,263,616.00
19	Heptacosanoic acid, methyl ester	424	LEE	17.839	106,633,936.00
20	Hexatriacontane	506	LEE	23.867	14,136,601.00
			LEE	25.197	14,223,143.00
			LPE	24.407	53,963,840.00
21	N-hexadecanoic acid	256	LEE	18.255	9,913,276.00
			LEE	18.585	8,495,868.00

			LME	19.77	4,682,071.50
			LCE	18.725	114,925,976.00
22	Octacosane	394	LEE	24.542	12,636,882.00
			LPE	22.241	56,002,884.00
			LPE	25.087	518,459,904.00
23	Octadecanal, 2-bromo-	346	LEE	20.565	31,789,348.00
24	Octanoic acid, 3-oxo-, methyl ester	172	LME	17.354	3,508,416.00
25	Pentadecanal-	226	LPE	24.862	21,938,202.00
26	Pentanoic acid, 2-(aminooxy)-	133	LEE	18.365	16,924,644.00
27	Pentatriacontane	492	LCE	23.747	9,365,859.00
28	Phenol, 2,4-bis(1,1-dimethylethyl)-	206	LCE	14.318	21,035,304.00
29	Preg-4-en-3-one, 17, alpha. - hydroxy-17. beta. – cyano -	313	LCE	19.695	96,740,104.00
30	Sulfurous acid, octadecyl 2-propyl ester	376	LCE	25.788	11,219,758.00
31	Tetracontane	562	LPE	25.747	40,567,944.00
32	Trans-chrysanthemal	152	LCE	20.685	285,833,728.00
33	Tritetracontane	604	LPE	27.008	24,440,820.00
				27.683	40,975,060.00

LPE: *L. lavandulifolia* petroleum ether floral extract**; LCE:** *L. lavandulifolia* chloroform floral extract**; LEE:** *L. lavandulifolia* ethyl acetate floral extract**; LME:** *L. lavandulifolia* methanol floral extract.

Antioxidant activity

DPPH radical scavenging assay

The DPPH assay of *L. lavandulifolia* showed IC_{50} values in the order of methanolic extract, ascorbic acid, ethyl acetate, chloroform and petroleum ether extracts such as 3.91, 0.24, 6.13, 5.38 and 69.75 µg/ml respectively (Table 2; Figure 1).

Table 2: DPPH assay of the various solvent floral extracts in *L. lavandulifolia*

DPPH assay	31.25	62.5	125	250	500	IC_{50} value
	Concentration in µg/ml					
LPE	0.01	2.09	1.76	1.55	3.46	69.75
LCE	6.99	14.21	22.19	32.79	49.88	5.38
LEE	7.99	16.10	20.32	31.31	42.42	6.13
LME	12.63	15.75	30.95	58.25	65.05	3.91
AA	68.42	79.02	80.86	81.77	83.11	0.24

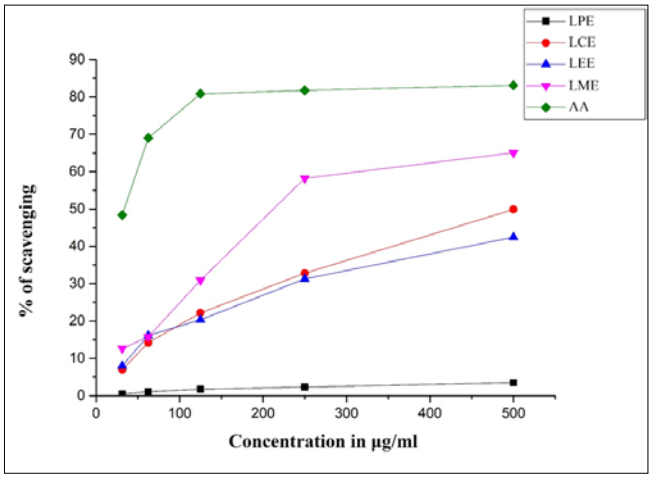

Fig 1: DPPH assay of the various solvent floral extracts in *L. lavandulifolia.*

Ferric reducing power assay

In FRAP assay of *L. lavandulifolia*, methanol floral extract and ascorbic acid respectively showed 0.2641 and 2.0088 to 32 µg/ml, 0.1308 and 1.7433 to 16 µg/ml, 0.0492 and 0.859 to 8 µg/ml, 0.0209 and 0.6805 to 4 µg/ml and 0.0111 and 0.4343 to 2 µg/ml (Table 3; Figure 2). Concentrations at 32 and 2 µg/ml showed 0.2445 – 0.0023 to ethyl acetate floral extract, 0.1862 – 0.0032 to chloroform floral extract and 0.0129 – 0.0009 to petroleum ether floral extract.

Table 3: FRAP assay of the various solvent floral extracts in *L. lavandulifolia*

FRAP assay	2	4	8	16	32
	Concentration in µg/ml				
LPE	0.0009	0.0029	0.0092	0.0114	0.0129
LCE	0.0032	0.0097	0.0330	0.0735	0.1862
LEE	0.0023	0.0073	0.0222	0.0998	0.2445
LME	0.0111	0.0209	0.0492	0.1308	0.2641
AA	0.4343	0.6805	0.8590	1.7433	2.0088

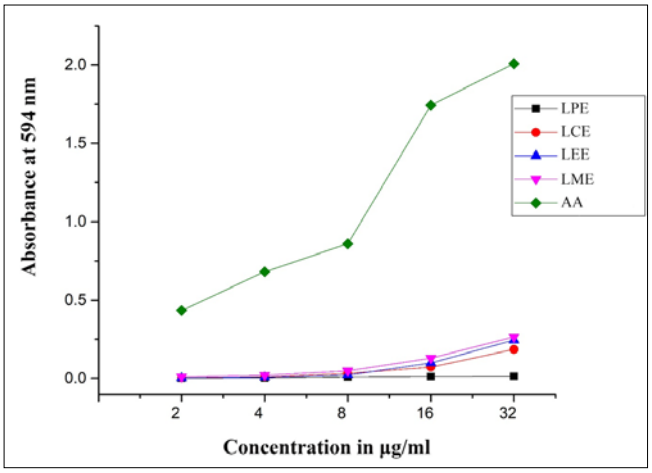

Fig 2: FRAP assay of the various solvent floral extracts in *L. lavandulifolia.*

Antibacterial activity

Antibacterial activity of the various solvent floral extracts of *L. lavandulifolia* exhibited good inhibitory activity against all the bacteria (Figure 3). Methanol extract at 100 µg/ml concentration recorded 23 mm (*P. mirabilis*), 22 mm each (*S. typhi*, *E. faecalis* and *C. diphtheriae*), 21 mm each (*E. aerogenes*), 20 mm each (*K. pneumoniae* and *S. pyogenes*), 19 mm (*A. caviae*), 18 mm (*P. aeruginosa*) and 17 mm (*A. hydrophila*).

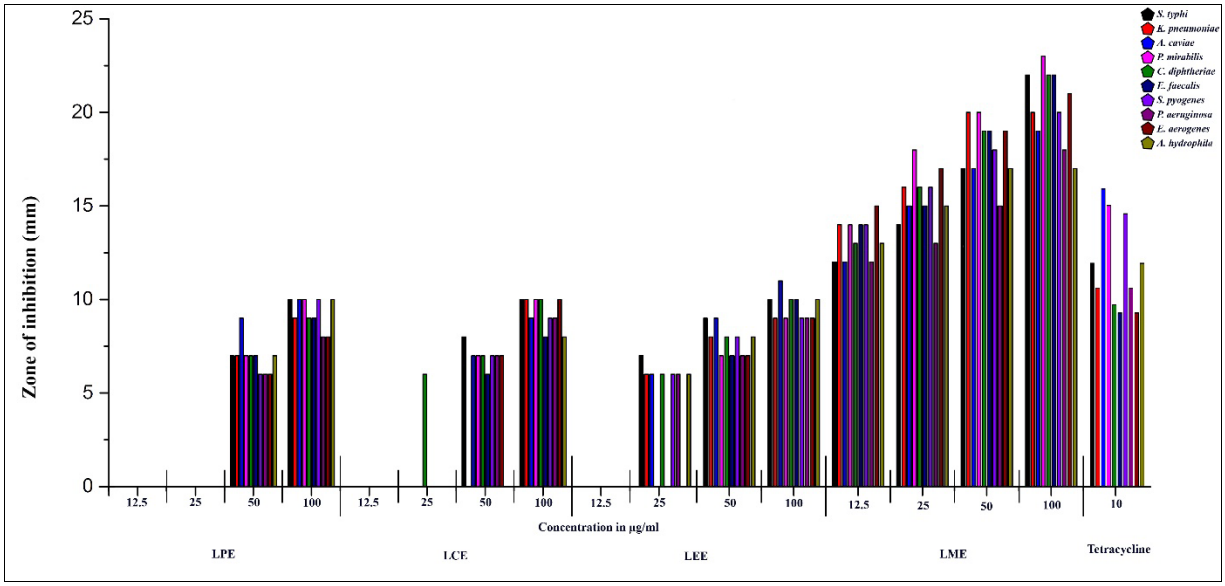

Fig 3: Antibacterial activity of the various solvent floral extracts in *L. lavandulifolia.*

Discussion

Aerial part of *L. virgata* was hydro-distillated and obtained essential oil. It had 43 compounds mainly constituted by oxygenated monoterpenes (50.8%) and camphor (20.5%) followed by minor quantities of β-eudesmol (6.1%), fenchon (5.4%), caryophyllene oxide (5.1%), exo-fenchol (3.4%) and borneol (3.1%) [23]. GC analysis of the flowering aerial part essential oil in *L. indica* contained 56 compounds wherein 71.8% were of sesquiterpene hydrocarbons mostly constituted by β-caryophyllene (51.1%) and α-caryophyllene (10.2%)[24]. GC–MS analysis of the methanolic extract in *L. aspera* showed 14 compounds wherein major compounds were of 6-octadecenoicacid (32.47%), n-hexadecanoicacid (25.97%) and 17-octadecen-14-yn-1-ol (14.22%) followed by minor composition of γ-sitosterol (2.45%) and stigmasterol (2%)[25]. The ethanolic stem extract of *L. aspera* recorded 13-docosenoic acid, 2-(9-octadecenyloxy), cyclopropaneoctanoic acid, pentadecanoic acid and 15,19-cyclo-5a-androst-6-ene-3[26]. Hydro-distilled essential oil of the aerial part of *L. aspera* showed 43 compounds wherein major constituents were of sesquiterpene hydrocarbons (47.7%), β-caryophyllene (34.2%) and 1-octen-3-ol (14.8%) and minor constituents were of α-humulene (6.3%), α-pinene (5.8%), epi-α-bisabolol (4.6%) and limonene (4.5%)[27]. GC–MS analysis of the methanol plant extract of *L. aspera* revealed the presence of 12 compounds such as 2, 6, 10, 14, 18, 22-tetracosahexane, oxiraneundecanoic acid, tetradecane, 1-hexadeconol, 3,7,11,15, tetramethyl-2-hexadec-1-ol, 9,12-octadecadienoic, 9,12,15- Octadecatrienoic acid, Catechin, Heptadecanoic acid, Aspidospermidne-17 ol, 9,12,15-Octadecatrienoic acid, Cholestan-3 ol, and eicosanoic acid[28]. Further, longifolene, 1,4,7-caryophyllene oxide, 1,6-hexadecanoic acid, 8-heptadecene, naphthalene, heptacosane, 1-chloro, phytol, 5-eicosene, IH-cyclopropa naphthalene, pentadecanal, and 19 other components were recorded from the essential oil of *L. aspera* by GC-MS analysis [29].

Antioxidant activity of *L. aspera* was reported such as 82.70 to 100 µl ethyl acetate flower extract and 80.69±3.68 to 500 µl methanolic extract [30, 31]. Its root methanolic extract exhibited IC_{50} of 6.552 mg/mL.[32] Essential oil of *L. virgata* aerial part demonstrated 31% reduction to 1000 µg/mL DPPH [32]. IC_{50} value of DPPH radical scavenging assay of leaf and root aqueous extracts of *L. aspera* showed 177.09 µg/mL and 228.35 µg/mL. Methanolic leaf and root extracts showed 136.17 µg/mL and 156.53 µg/mL. Hexane leaf and root extracts showed 169.17 µg/mL and 235.72 µg/mL [33]. Impressive antioxidant activity of *L. aspera* floral extract to nitric oxide scavenging assay was registered such as 50.27 at 10 µl than 50.81 to the corresponding standard of butylated hydroxy anisole (BHA). Further, the extract revealed concentration-dependent activity of 69.73 to 50 µl and 82.70 to 100 µl [34].

Antibacterial activity of *L. aspera* has been reported by various authors such as methanol floral extract exhibited 12.0, 11.5 and 10.0 mm to *B. subtilis*, *S. pyogenes* and *K. pneumoniae* respectively [35]. Ethyl acetate extract of *L. aspera* at 400 µg/disc recorded activity such as 24.0 mm (*S. epidermidis*), 21.0 mm (*K. pneumoniae*), 16.0 mm (*E. coli*), 13.5 mm (*B. subtilis*), 13.0 mm each (*P. aeruginosa* and *S. typhi*), 11.5 mm (*P. mirabilis*), 9.5 mm (*S. aureus*), 9.0 mm (*B. cereus*) and 8.5 mm (*P. vulgaris*) [36]. Root methanolic extract (100 µg/ml) showed 11±0.6 mm to *P. aeruginosa* and *S. typhimurium*, 11±0.5 mm to *S. choleraesuis*, and *S. flexneri*[32]. A 100 µl ethyl acetate floral extract recorded 23 mm to *V. cholerae*, 20 mm each to *B. polmyxa*, 16 mm *B. pumilus* and 13 mm to *E. coli* [34]. In the present study, methanol extract of *L. lavandulifolia* (Figure 3) at 100 µg/ml concentration exhibited 23 mm to *P. mirabilis*, 22 mm each to *S. typhi*, *C. diphtheriae* and *E. faecalis*, 21 mm each to *E. aerogenes*, 20 mm each to *K. pneumoniae* and *S. pyogenes*, 19 mm to *A. caviae*, 18 mm to *P. aeruginosa* and 17 mm to *A. hydrophila*. At similar concentration, ethyl acetate extract inhibited 11 mm to *A. caviae* whereas only 10 mm each to chloroform and petroleum ether extracts.

Conclusion

In this present study of plant species showed the present of biologically valuable secondary metabolites such as tetracontane, Phenol, 2,4-bis(1,1-dimethylethyl)-, 5,7-dodecadiyn-1,12-diol and other major constituents were deducted by GC-MS analysis. It had strong antioxidant activity and exposed the significant antibacterial activity in methanolic extract. Further studies on the isolation and characterization of various bioactive principles from this

herb as a potential source of natural antimicrobial agents will thereby be motivationally considered.

Declaration of Competing Interests

The authors have no conflict of interests to declare.

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References

1. World Health Organization. Guidelines for the study of dietary intakes of chemical contaminants / text prepared under the Joint FAO/WHO Food Contamination Monitoring Programme, in collaboration with the Joint FAO/WHO Food Standards Programme, WHO. World Health Organization; Geneva, 1985.
2. World Health Organization. Quality control methods for medicinal plant materials. World Health Organization; Geneva, 1998.
3. Newman DJ, Cragg GM, Snader KM. Natural products as sources of new drugs over the period 1981− 2002. Journal of Natural Products,2003:66:1022–1037.
4. Ngo LT, Okogun JI, Folk WR. 21st century natural product research and drug development and traditional medicines. Natural Product Reports,2013:30:584–592.
5. Newman DJ, Cragg GM. Natural products as sources of new drugs from 1981 to 2014. Journal of Natural Products,2016:79:629–661.
6. Shu YZ. Recent natural products-based drug development: a pharmaceutical industry perspective. Journal of Natural Products,1998:61:1053–1071.
7. Chopra RN, Chopra IC, Handa KL, Kapur LD. Indigenous drugs of India. UN Dhur and Sons; Calcutta, 1958.
8. Reddy MK, Viswanathan S, Thirugnanasambantham P, Kameswaran L. Analgesic activity of *Leucas aspera*. Fitoterapia,1993:64:151–154.
9. Saha K, Mukherjee PK, Das J, Pal M, Saha BP. Wound healing activity of *Leucas lavandulaefolia* Rees. Journal of Ethnopharmacology,1997:56:139–144.
10. Saha K, Mukherjee PK, Das J, Mandal SC, Pal M, Saha BP. Hypoglycaemic activity of *Leucas lavandulaefolia* Rees. in streptozotocin-induced diabetic rats. Phytotherapy Research,1997:11:463–466.
11. Mukherjee K, Saha BP, Mukherjee PK. Evaluation of antipyretic potential of *Leucas lavandulaefolia* (Labiatae) aerial part extract. Phytotherapy Research,2002:16:686–688.
12. Mukherjee K, Saha BP, Mukherjee PK. Psychopharmacological profiles of *Leucas lavandulaefolia* Rees. Phytotherapy Research,2002:16:696–699.
13. Tripathi KD. Essentials of medical pharmacology, (7th ed). Jaypee Brothers Medical Publishers (P) Ltd; New Delhi; 2013.
14. Chandrashekar KS, Satyanarayana D, Joshi AB. Analgesic activity of *Leucas lavandulaefolia* Rees. Indian Drugs,2004:42:78–80.
15. Chandrashekar K, Prasanna K, Anti-inflammatory potential of flavone glycoside from ethanol extract of the aerial parts of the plant *Leucas lavandulaefolia*. Der Pharma Chemica,2010:2:21-24.
16. Gupta JK, Upmanyu N, Patnaik AK, Mazumder PM. Evaluation of antiulcer activity of *Leucas lavandulifolia* on mucosal lesion in rat. Asian Journal of Pharmaceutical and Clinical Research,2010:3:110–120.
17. Saha K, Mukherjee PK, Pal M, Saha BP. Medicinal properties and chemical constituents of *Leucas lavandulaefolia*. Journal of Applied Research on Medicinal and Aromatic Plants,1997:19:1045-1050.
18. Chandrashekar KS, Prasanna KS. Hypoglycemic effect of *Leucas lavandulaefolia* Willd in alloxan-induced diabetic rats. Journal of Young Pharmacists,2009:1:326-329.
19. NIST Standard Reference Database 1A, NIST/EPA/NIH Mass Spectral Library (NIST 08) and NIST Mass Spectral Search Program (Version 2.0f), 2008.
20. Blois MS. Antioxidant Determinations by the Use of a Stable Free Radical. Nature,1958:181:1199–1200.
21. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: The FRAP assay. Analytical Biochemistry,1996:23:70–76.
22. Perez C. Antibiotic assay by agar-well diffusion method. Acta Biologiae et Medicinae Experimentalis,1990:15:113–115.
23. Mothana RA, Al-Said MS, Al-Yahya MA, Al-Rehaily AJ, Khaled JM. GC and GC/MS analysis of essential oil composition of the endemic Soqotraen *Leucas virgata* Balf. f. and its antimicrobial and antioxidant activities. International Journal of Molecular Sciences,2013:14:23129–23139.
24. Joshi RK. GC/MS analysis of the essential oil of *Leucas indica* from India. Natural Product Communications,2014:9:1607–1608.
25. Gopi K, Renu K, Jayaraman G. Inhibition of *Naja naja* venom enzymes by the methanolic extract of *Leucas aspera* and its chemical profile by GC–MS. Toxicology Reports,2014:1:667–673.
26. Keerthana PM, Shoba G, Padmini R. Screening of Novel MAPK Inhibitor from ethanolic extract of stem of *Leucas aspera* (Thumbai) using GCMS and *in silico* analysis. International Journal of Current Research and Academic Review,2016:3:195–206.
27. Joshi RK. *Leucas aspera* (Willd.) Link Essential Oil from India: β-Caryophyllene and 1-Octen-3-ol Chemotypes. Journal of Chromatographic Science,2016:54:295–298.
28. Elumalai D, Hemalatha P, Kaleena PK. Larvicidal activity and GC–MS analysis of *Leucas aspera* against *Aedes aegypti Anopheles stephensi* and *Culex quinquefasciatus*. Journal of the Saudi Society of Agricultural Sciences,2017:16:306-313.
29. Elumalai D, Kayalvizhi M, Kaleena PK, Vignesh A, Hemavathi M. Gas chromatography mass spectrometry analysis and larvicidal activity of leaf essential oil extract of *Leucas aspera* against dengue, malaria and filariasis vectors. The Journal of Basic and Applied Zoology | Home,2018:79:49.
30. Rahman MA, Islam MS. Antioxidant, antibacterial and cytotoxic effects of the phytochemicals of whole *Leucas aspera* extract. Asian Pacific Journal of Tropical Biomedicine,2013:3:273–279.

31. Tahareen S, Shwetha R, Myrene RD. Potential antioxidant, anti-inflammatory and antibacterial evaluation of extracts of *Leucas aspera* using *in vitro* models. International Journal of Pharmacy and Pharmaceutical Sciences,2016:8:292-297.
32. Chew AL, Jessica JJA, Sasidharan S. Antioxidant and antibacterial activity of different parts of *Leucas aspera*. Asian Pacific Journal of Tropical Biomedicine,2012:2:176–180.
33. Vasudha K, Archana D, Mutyalamma B, Kishori B. Phytochemical screening, antimicrobial, and antioxidant activities of root and leaf extracts of *Leucas aspera*. Asian Journal of Pharmaceutical and Clinical Research,2019:12:141–147.
34. Sabri G, Vimala Y. Antibacterial and antioxidant activity of *Leucas aspera* flowers from Bihar, India. Asian Journal of Pharmaceutical and Clinical Research,2018:11:223–226.
35. Mangathayaru K, Lakshmikant J, Sundar NS, Swapna RGXF, Grace XF, Vasantha J. Antimicrobial activity of *Leucas aspera* flowers. Fitoterapia,2005:76:752–754.
36. Ilango K. Ramya S. Gopinath G. Antibacterial activity of *Leucas aspera* Spreng. International Journal of Chemical Science,2008:6:526–530.