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IN VITRO ANTIMICROBIAL EFFICACY AND GC- MS ANALYSIS OF BIOACTIVE COMPONENTS FROM *LEPIDAGATHIS KERALENSIS* (ACANTHACEAE)

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ABSTRACT

Lepidagathis keralensis, a medicinal plant belonging to the family Acanthaceae is endemic to Kerala. The present study deals with the evaluation of antibacterial and antifungal activity of the petroleum ether extracts of the stem and leaves of the plant. The study also investigates the bioactive components responsible for the antimicrobial activity of the extracts. Antimicrobial studies were done by agar diffusion method. For antibacterial evaluation gram negative (*Pseudomonas aeroginosa* & *Klebsiella pneumonia*) and gram positive(*Streptococcus mutans* & *Staphylococcus aureus*) bacterial strains were used. The antifungal efficacy was tested against *Candida albicans* .Identification of bioactive compounds in the extracts were done by GC-MS analysis using Thermo Scientific Trace 1300 Gas chromatograph equipped with ISQ- QD Mass spectrometer. GC-MS analysis showed the presence of 38 phytocomponents in the leaf extract and 20 components in the stem extract. n-Hexadecanoic acid(14.53%), 2-Decanynoic acid(16.71%), 1,6-Octadiene, 3,7-dimethyl-(20.34%) & 1,5-Heptadiene, 3,3-dimethyl-, (E)-(12.77%) were the major components in the leaf while Cyclopentaneundecanoic acid(25.06), Oxalic acid, allyl hexyl ester (10.41) ,1-Iodo-2-methylnonane (9.91) & n-Hexadecanoic acid(9.96%) were the major components in the stem. The analysis revealed the presence of several bioactive phytocomponents which could be responsible for the antimicrobial activity of the extracts.

KEYWORDS: *Lepidagathis keralensis*, antimicrobial, GC-MS, extract.

1. INTRODUCTION

The extensive use of antibiotics has led to bacterial resistance and has decreased the development of synthetic antimicrobial drugs. Hence researches focused on finding out new antimicrobials from alternate sources are gaining importance (Amghalia et al., 2009; Lakshmi et al., 2014). Plants are rich source of biologically active secondary metabolites of which many possess antimicrobial properties (Jadhav et al., 2014; Sasikala & Mohan, 2014).

Lepidagathis keralensis is a plant endemic to Kerala found in Lateritic hills near seacoast. The plant belongs to the family Acanthaceae. It is a rigid prostrate under shrub with woody rootstock usually occurring in exposed lateritic rocks (Madhu. & Singh, 1992). The plant possesses various medicinal properties. The spines of the plant tied in a cloth are cooked with rice and resulting gruel is given to children as a preventive medicine for digestive disorders. Whole plant decoction is recommended for kidney stone. It is a blood purifier and increases blood. (Prasad S., 2012). The fresh roots of *Lepidagathis keralaensis* is used for treating bronchial asthma in children by the Paniya tribes.(Divakar et al, 2010). The other species of the plant *Lepidagathis cristata* has been widely studied for its phytochemical and pharmacological activities and is proved to possess anti-inflammatory and wound healing properties (Reddy & Rao, 2013).No studies on antimicrobial properties of *Lepidagathis keralensis* has so far been known to be reported. Hence the present study aims at the evaluation of antibacterial and antifungal potencies of the petroleum ether extracts of the stem and leaf of the plant.

2. MATERIALS AND METHODS

2.1. Collection of plant material

Lepidagathes keralensis was collected from Madayippara, Kannur,Kerala and authenticated from the Dept. of Botany, Govt. Brennen College, Dharmadam,Kerala. The leaves and stems of the plant were separated, washed well and shade dried for two weeks. The dried plant parts were powdered well and stored for further use.

2.2. Preparation of extracts

The powdered plant parts were extracted with petroleum ether in a soxhlet extractor for 48 hours. The extracts were evaporated to obtain crude dry extracts of the leaf and stem which were stored under refrigeration for further use.

2.3. Evaluation of Antimicrobial activity

2.3.1.Test Organisms

Antimicrobial activity of the petroleum ether extracts were studied against gram negative (*Pseudomonas aeroginosa* ATCC 27853 & Klebsiella *pneumonia* ATCC 13883) and gram positive (*Streptococcus mutans* MTCC 890 & *Staphylococcus aureus* ATCC 25923) bacterial strains and *Candida albicans* ATCC 10231 fungal strain.

2.3.2.Preparation of reagents

a) Muller Hinton Agar Medium (1 L)

The medium was prepared by dissolving 33.8 g of the commercially available Muller Hinton Agar Medium (HiMedia) in 1000ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petriplates (25-30ml/plate) while still molten.

b) Nutrient broth (1L)

One litre of nutrient broth was prepared by dissolving 13 g of commercially available nutrient medium (HiMedia) in 1000ml distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 minutes.

2.3.3. Antibacterial assay

Antibacterial activities of the extracts were determined using agar well diffusion method. 20ml Muller Hinton Agar Medium was added to petriplates. The plates were then seeded with bacterial culture .The growth of the culture was adjusted according to McFards Standard, 0.5%. Wells of approximately 10mm was bored in the plates using a well cutter and different concentrations of the sample (25 µg, 50 µg and 100 µg) were added to the well from a stock concentration of 1g/mL. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993). Streptomycin(20 µg) was used as a positive control.

2.3.4.Antifungal assay

The antifungal activities of the extracts were determined by Agar well diffusion method. Potato Dextrose agar plates were prepared and overnight grown different species of fungus Candida albicans was swabbed. Wells of approximately 10mm was bored using a well cutter and samples of different concentrations (25 µg, 50 µg and 100 µg) were added; the zone of

inhibition was measured after overnight incubation and compared with that of standard antimycotic ,Clotrimazole(20 µg).

2.4. Gas chromatography-mass spectrometry analysis

GC-MS analysis of the petroleum ether extracts of the stem and leaf were carried out at the Research centre, Sir Syed College, Taliparamba, Kerala. The analysis was performed using a Thermoscientific Trace 1300 Gas chromatograph equipped with ISQ- QD Mass spectrometer with TG-5MS non polar column (30 m × 0.25 mm ID ×0.25 µmdf). Electron ionization system with ionizing energy of 70ev was used for GC-MS detection. Helium gas (99.99%) was used as the carrier gas at constant flow rate 1ml/minute and an injection volume of 1µl was employed (Split ratio 1:8). An injection port temperature of 280ºC and an ion-source temperature of 200ºC were set.

For the analysis of leaf extract the oven temperature was programmed from 70^0C(isothermal for 3 minutes) with an increase of 5^0C / minute to 180^0C with a hold time of 3 minutes. Then temperature was increased at a rate of 5^0C/min till 240^0C with a hold time of 5 minutes. Total GC running time was 45 minutes.

For the analysis of stem extract the oven temperature was programmed from 80^0C (isothermal for 3 minutes) with an increase of 15^0C / minute to 180^0C with a hold time of 2 minutes. Then temperature was increased at a rate of 5^0C/min till 240^0C with a hold time of 5 minutes. Total GC running time was 25 minutes. The components in the extract were identified based on the mass spectra of latest NIST library data having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.

3. RESULTS AND DISCUSSIONS

The petroleum ether extracts of the leaf and stem of *Lepidagathis keralensis* were subjected to antimicrobial screening against two Gram-negative bacterial strains (*Pseudomonas aeroginosa & Klebsiella pneumonia*), two Gram-positive bacterial strains (*Streptococcus mutans & Staphylococcus aureus*) and one fungal strain (*Candida albicans*). The zone of inhibition produced by the extracts against fungal strain and bacterial strains are shown in Table 1and Table 2 respectively. It was observed that the stem extracts showed better antimicrobial activities when compared to the leaf. Both the extracts showed higher activity

against the bacteria, *Klebsiella pneumonia*. The leaf extract did not show any activity against the fungus *Candida albicans*, while the stem extract showed good activity.

Inorder to assess the bioactive components responsible for the exhibited antimicrobial activity, GC-MS analysis of the extracts were carried out. The GC-MS chromatograms of the petroleum ether extracts of the leaf and stem are depicted in Figure 3 and Figure 4 respectively. The various phytocomponents identified by comparison of the observed spectra with that of the spectra of known components from NIST library are tabulated in Table 3 and Table 4.

GC-MS analysis showed the presence of 38 phytocomponents in the leaf extract and 20 components in the stem extract. n-Hexadecanoic acid (14.53%), 2-Decanynoic acid (16.71%), 1,6-Octadiene, 3,7-dimethyl-(20.34%) & 1,5-Heptadiene, 3,3-dimethyl-, (E)- (12.77%) were the major components in the leaf while Cyclopentaneundecanoic acid (25.06), Oxalic acid, allyl hexyl ester (10.41) ,1-Iodo-2-methylnonane (9.91) and n-Hexadecanoic acid (9.96%) were the major components in the stem. The bioactivities of some of the phytocomponents identified from the extracts are shown in Table 5 and Table 6. The presence of several bioactive phytocomponents can thus be attributed for the antimicrobial activities of the extracts.

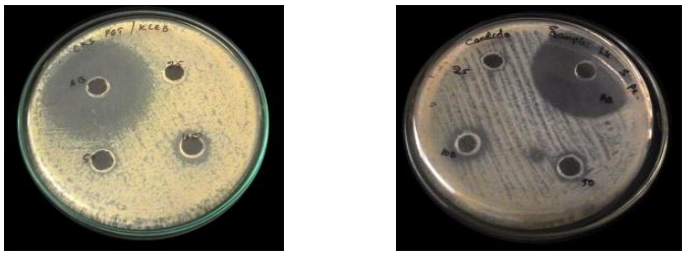
Table 1: Antifungal activity of the petroleum ether extracts of leaf and stem of *Lepidagathes keralensis*

Organism	Concentration in µg/ml	Zone of inhibition in mm			
		Petroleum ether extract of leaf		Petroleum ether extract of stem	
		Clotrimazole	Leaf extract	Clotrimazole	stem extract
Candida albicans	25	39	Nil	39	10
	50		Nil		12
	100		Nil		15

Table 2: Antibacterial activity of the petroleum ether extracts of leaf and stem of *Lepidagathes keralenis*

Organism	Concentration in µg/ml	Zone of inhibition in mm			
		Petroleum ether extract of leaf		Petroleum ether extract of stem	
		Streptomycin	Leaf extract	Streptomycin	stem extract
Klebsiella pneumonia	25	38	10	37	10
	50		13		12
	100		19		15
Pseudomonas	25		Nil		10

aeroginosa	50	39	Nil	40	10
	100		13		14
Streptococcus mutans	25	40	Nil	36	10
	50		Nil		10
	100		11		11
Staphylococcus aureus	25	40	Nil	40	Nil
	50		10		Nil
	100		13		11



A B

Figure 1: Zone of inhibition exhibited by petroleum ether extract of stem against A) *Klebsiella pneumonia* and B) *Candida albicans*

C D

Figure 2: Zone of inhibition exhibited by petroleum ether extract of leaf against C) *Staphylococcus aureus* and D) *Klebsiella pneumonia*

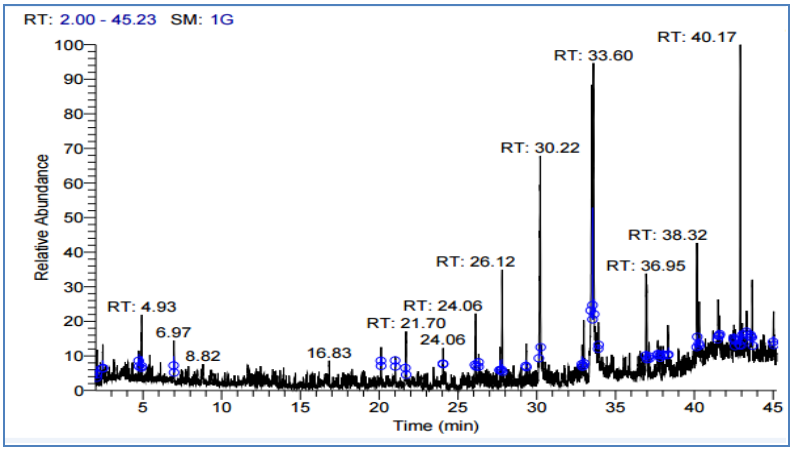


Figure 1: GC MS chromatogram of petroleum ether extract of *lepidagathes keralensis* leaf

Table 3: Compounds identified in the petroleum ether extract of *lepidagathes keralensis* leaf

Sl. no	RT	Name of the Compound	Molecular formula	Molecular weight	Peak area %
1	2.08	2,3-Butanediol, [S-(R*,R*)]-	$C_4H_{10}O_2$	90	0.31
2	2.46	1-(Methoxymethoxy)-3-methyl-3-hydroxybutane	$C_7H_{16}O_3$	148	0.58
3	4.74	2,4,6,8-Tetramethyl-1-undecene	$C_{15}H_{30}$	210	0.09
4	4.93	4-Methyl-1,6-heptadien-4-ol	$C_8H_{14}O$	126	2.3
5	6.97	Hydroxylamine, O-(2-methylpropyl)-	$C_4H_{11}NO$	89	0.68
6	20.12	1,3-Dioxolane, 2-butyl-	$C_7H_{14}O_2$	130	0.2
7	21.02	1b,5,5,6a-Tetramethyl-octahydro-1-oxa-cyclopropa[a]inden-6-one	$C_{13}H_{20}O_2$	208	0.07
8	21.7	4-Methyloctanoic acid	$C_9H_{18}O_2$	158	1.85
9	24.06	(+)-Prostaglandin F2à, 4TMS derivative(pharma chem. Alagarsamy p 756)	$C_{32}H_{66}O_5Si_4$	642	0.14
10	26.12	Cyclopentaneundecanoic acid	$C_{16}H_{30}O_2$	254	2.21
11	26.32	3,6-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	290	0.07
12	27.67	1,7-Nonadien-4-ol, 4,8-dimethyl-	$C_{11}H_{20}O$	168	0.59
13	27.80	Hexane, 1-nitro-	$C_6H_{13}NO_2$	131	3.99
14	29.34	9-Decen-2-one, 5-methylene-	$C_{11}H_{18}O$	166	0.69
15	30.22	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	14.53
16	32.89	1-Nonene	C_9H_{18}	126	0.61
17	32.99	2-Piperidinone, N-[4-bromo-n-butyl]-	$C_9H_{16}BrNO$	233	1.73
18	33.51	2-Decanynoic acid	$C_{10}H_{16}O_2$	168	16.71
19	33.60	1,6-Octadiene, 3,7-dimethyl-	$C_{10}H_{18}$	138	20.34
20	33.91	10-Azido-1-decanethiol	$C_{10}H_{21}N_3S$	215	0.7
21	36.95	1,7-Dimethyl-4-(1-methylethyl)cyclodecane	$C_{15}H_{30}$	210	6.54
22	37.14	2(3H)-Furanone, dihydro-4,4-dimethyl-	$C_6H_{10}O_2$	114	0.34
23	37.69	(2S,3S)-(-)-3-Propyloxiranemethanol	$C_6H_{12}O_2$	116	0.33
24	37.84	8,11,14-Eicosatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{21}H_{36}O_2$	320	0.11
25	38.02	Benzene, 2,4-dinitro-1-(phenylsulfonyl)-	$C_{12}H_8N_2O_6S$	308	0.08
26	38.32	Cyclopropanetetradecanoic acid, 2-octyl-, methyl ester	$C_{26}H_{50}O_2$	394	1.44
27	40.17	2,4,6-Trimethyl-1-nonene	$C_{12}H_{24}$	168	2.95
28	40.32	Malonic acid, bis(2-trimethylsilylethyl ester	$C_{13}H_{28}O_4Si_2$	304	1.09
29	41.51	1-Iodo-2-methylundecane	$C_{12}H_{25}I$	296	0.91
30	41.62	1,2,5-Oxadiazole-3,4-dicarboxamide, 4TMS derivative	$C_{16}H_{36}N_4O_3Si_4$	444	0.54
31	42.52	Heptadecane, 9-hexyl-	$C_{23}H_{48}$	324	0.27
32	42.60	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	0.08
33	42.93	1,5-Heptadiene, 3,3-dimethyl-, (E)-	C_9H_{16}	124	12.77
34	43.05	1,2,5-Oxadiazole-3,4-dicarboxamide,	$C_{16}H_{36}N_4O_3Si_4$	444	0.12

		4TMS derivative			
35	43.33	9-Octadecen-12-ynoic acid, methyl ester	$C_{19}H_{32}O_2$	292	0.18
36	43.58	2-Trifluoroacetoxytridecane	$C_{15}H_{27}F_3O_2$	296	0.03
37	43.68	Oxalic acid, allyl nonyl ester	$C_{14}H_{24}O_4$	256	2.64
38	45.04	1-Iodo-2-methylnonane	$C_{10}H_{21}I$	268	1.30

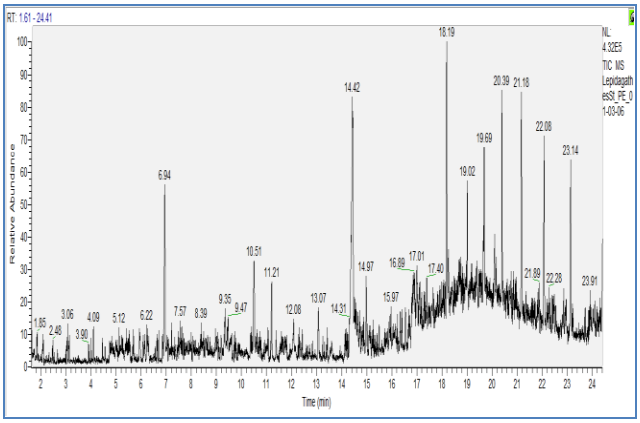


Figure 2. GC MS chromatogram of petroleum ether extract of *Lepidagathes keralensis* stem

Table 4: Compounds identified in the petroleum ether extract of *Lepidagathes keralensis* stem

Sl. No.	RT	Name of the Compound	Molecular formula	Molecular weight	Peak area %
1	6.94	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	9.96
2	10.51	Octanoic acid	$C_8H_{16}O_2$	144	4.88
3	11.21	Hydroxylamine, O-decyl-	$C_{10}H_{23}NO$	173	1.67
4	14.42	Cyclopentaneundecanoic acid	$C_{16}H_{30}O_2$	254	25.06
5	14.97	Dodecane, 5,8-diethyl-	$C_{16}H_{34}$	226	1.67
6	16.86	Cyclododecanone, thiosemicarbazone	$C_{13}H_{25}N_3S$	255	0.11
7	17.01	1-Methylpropylhydroxylamine	$C_4H_{11}NO$	89	0.27
8	18.19	Oxalic acid, allyl hexyl ester	$C_{11}H_{18}O_4$	214	10.41
9	18.26	1',1'-Dicarboethoxy-1á,2á-dihydro-3'H-cycloprop[1,2]cholesta-1,4, 6-trien-3-one	$C_{34}H_{50}O_5$	538	0.37
10	18.71	Astaxanthin	$C_{40}H_{52}O_4$	596	0.16
11	18.75	2-Butanone, 3,4-epoxy-3-ethyl-	$C_6H_{10}O_2$	114	0.24
12	19.02	9-Octadecen-12-ynoic acid, methyl ester	$C_{19}H_{32}O_2$	292	3.21
13	19.69	3,6-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	290	4.18
14	20.11	Oxalic acid, allyl heptyl ester	$C_{12}H_{20}O_4$	228	0.88
15	20.39	Hydroxylamine, O-(3-methylbutyl)-	$C_5H_{13}NO$	103	7.67
16	20.86	L-Proline, 1-[O-(1-oxohexyl)-N-[N-[N6-(1-oxohexyl)-N2-[N-(1-oxohexyl)-L-valy l]-L-lysyl]-L-valyl]-L-tyrosyl]-, methyl ester	$C_{49}H_{80}N_6O_{10}$	912	0.26
17	21.18	1-Iodo-2-methylnonane	$C_{10}H_{21}I$	268	9.91
18	22.08	Dodecane, 2-methyl-	$C_{13}H_{28}$	184	7.40
19	23.14	Oxalic acid, allyl nonyl ester	$C_{14}H_{24}O_4$	256	7.84
20	24.42	Pentane, 2,4-dimethyl-	C_7H_{16}	100	4.82

Table 5: Activity of some of the phytocomponents identified from gc-ms analysis of leaf extract of *Lepidagathes keralensis*

Sl. no.	Name of the component	RT	Activity	Nature of the compound
1	2,3-Butanediol, [S-(R*,R*)]-	2.08	Antimicrobial(Keerthiga & Anand , 2015)	Alcoholic Compound
2	2,4,6,8-Tetramethyl-1-undecene	4.74	Antibiotic(Shakeel et al.,2016)	alkene
3	Cyclopentaneundecanoic acid	26.12	Antimicrobial(Das et al., 2014)	Fatty acid
4	n-Hexadecanoic acid/palmitic acid	30.22	Antioxidant, hypocholesterolemi, nematicides, pesticide(Shettima et al.,2013; Flora& Rani,2013)	Fatty acid
5	2-Piperidinone, N-[4-bromo-n-butyl]-	32.99	Antimicrobial. antioxidant, anti-inflammatory (Meenakshi et al.,2012)	Alkaloid
6	1,7-Dimethyl-4-(1-methylethyl)cyclodecane /germacrene	36.95	Antibacterial activity(Thenmozhi & Rajan ,2015)	Germacrane Sesquiterpenoids
7	Cyclopropanetetradecanoic acid, 2-octyl-, methyl ester	38.32	Antimicrobial(Srivastava et al.,2015)	Fatty acid ester
8	1-Iodo-2-methylundecane	41.51	Antimicrobial, Enhance reproductive activities(Amudha & Rani,2014)	Iodo alkane
9	Ethyl iso-allocholate	42.60	Antimicrobial, Diuretic, Anti-inflammatory, Antiasthma(Muthulakshmi et al.,2012)	Steroid
10	9-Octadecen-12-ynoic acid, methyl ester	43.33	Immunotoxicity effects, and Antioxidant activity(Srivastava et al.,2015)	Unsaturated fatty acid ester
11	2-Trifluoroacetoxytridecane	43.58	Antimicrobial(Jessica et al.,2016)	Fatty acid ester
12	1-Iodo-2-methylnonane	45.04	Antimicrobial(kumar et al.,2014)	Iodo alkane

Table 6: Activity of some of the phytocomponents identified from GC-MS analysis of stem extract of *Lepidagathes keralensis*

Sl. no.	Name of the component	RT	Activity	Nature of the compound
1	n-Hexadecanoic acid/palmitic acid	6.94	Antibiotic, Antioxidant, hypocholesterolemi, nematicides, pesticide(Shettima et al.,2013; Flora& Rani,2013)	saturated fatty acid
2	Octanoic acid	10.51	Pesticide, fungicide*	saturated fatty acid
3	Cyclopentaneundecanoic acid	14.42	Antimicrobial(Das et al., 2014)	
4	Dodecane, 5,8-diethyl-	14.97	Used for Tetany, Pulmonary edema, Muscle weakness(Babu et al,2014)	alkane

5	Astaxanthin	18.71	Anti-oxidant, Anti-ageing, Anti-cancer,protection against Cardiovascular diseases(Lakshmi et al.,2014)	terpene
6	1-Iodo-2-methylnonane	21.18	Antimicrobial(kumar et al.,2014)	Iodo alkane

* Source: Dr. Duke's phytochemical and ethnobotanical databases [Online database].

4. CONCLUSION

The GC-MS analysis of the petroleum ether extracts of the leaf and stem of *Lepidagathis keralensis* showed the presence of various bioactive phytochemicals. This finding supports the antimicrobial activity of the plant against selected pathogens. This plant can therefore be explored for futher research studies based on isolation of bioactive components.

5. ACKNOWLEDGEMENTS

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6. REFERENCE

1. Amghalia E. (2009). Multiplex PCR Assays for the Detection of Clinically Relevant Antibiotic Resistance Genes in *Staphylococcus aureus* isolated from Malaysian Hospitals. *Research Journal of Biological Sciences,* 4(4): 444-448.
2. Amudha M., Rani S.(2014) GC-MS Analysis of Bioactive components of *Cordia retusa* (Boraginaceae). *Hygeia.J.D.Med.,* 6 (1) : 12-19.
3. Babu, Johnson M., Raja D.P., Arockiaraj A.A., Vinnarasi J.(2014). Chemical constituents and their biological activity of *ulva lactuca linn*. *Int.J.Pharm. Drug Anal.,* 2(7): 595-600.
4. Das M.N., Sundari S.S., Karuppusamy S., Mohan V.R., Parthipan B.(2014).GC - MS analysis of leaf and stem bark of *Cleidion nitidum* (Muell. – Arg.) Thw. Ex Kurz. (Euphorbiaceae) .*Asian J.Pharm. Clin .Res,* 7(2): 41-47.
5. Divakar M.C., Sandhya S., Vinod K.R., Razik.A.P, Ranjimol.K.K, & Mohan S. (2010). Traditional knowledge and techniques of the iritty hill tribals of kannur district, kerala.: A review. *International Journal of Drug Formulation & Research,* 1(1): 12-53.
6. Flora G., Rani S.M.V.(2013).GC-MS analysis of *Acanthophora spicifera.Int. J. Pharm. Bio. Sci.,* 4(1): 649 – 653

7. Jadhav M. D., Kedar P., Deobhankar, Rathod L.R. (2014). Preliminary phytochemical screening and in- vitro antibacterial activity of some medicinal plants against *Escherichia coli* .*World Journal of Pharmaceutical Research*, 3(3): 4965-4974.
8. Jessica A., Rao M. R. K. , Anthony J., Prabhu K., , Johnson W.M.S. , Balasubramanian B.S.,Sundaram L. , Dinakar,S.(2016). The GC-MS Study of One Ayurvedic Preparation Katakakhadiradi Kashayam. *Int. J. Pharm. Sci. Rev. Res*., 39(2): 216-224.
9. Keerthiga M Anand S. P.(2015).Bioactive Compound Evaluation of Ethanol Extract from *Geodorum densiflorum* (Lam.) Schltr. by GC-MS analysis. *International Journal of Pharmacological Research, 5* (6): 139-144.
10. Kumar R.N., Vasantha K. and Mohan V.R. (2014).GC-MS Analysis of Bioactive Components of Tubers of *Ruellia tuberosa* L. (Acanthaceae). *American Journal of Phytomedicine and Clinical Therapeutics*, 2(2): 209-216.
11. Lakshmi Ch. S., Uma A., Lakshminarasu M., Venkanna B.(2014). Evaluation of Antimicrobial Property of *Thespesia Populnea* Root Extracts against Genitourinary Tract Infectious Pathogens. *International Journal for Pharmaceutical Research Scholars*, 3(1): 505-519.
12. Madhusoodanan P.V., Singh.N.P(1992). Lepidagathis keralensis, Kew Bull. 47(2): 301
13. Meenakshi V.K. , Gomathy S., Senthamarai S., Paripooranaselvi M., Chamundeswari K.P.(2012). GC-MS determination of the bioactive components of *Microcosmus exasperatus* heller, 1878.*J. Curr. Chem. Pharm. Sc*,. 2(4): 271-276.
14. Muthulakshmi A., Margret J.R., & Mohan V.R. (2012). GC-MS Analysis of Bioactive Components of *Feronia elephantum Correa* (Rutaceae). *Journal of Applied Pharmaceutical Science*, 02 (02: 69-74.
15. National Committee for Clinical Laboratory Standards. (1993). Performance Standards for Antimicrobial Disk Susceptibility Tests—Fifth Edition: Approved Standard M2-A5. NCCLS, Villanova, PA.
16. Prasad S. K. (2012). Exploration and elucidation of traditional medicinal plants of erstwhile Tulunadu and surrounding area of kerala and Karnataka (doctoral thesis). Kannur University, Kerala:660-661.http://hdl.handle.net/10603/41951.
17. Reddy P. A., Rao J.V. (2013). A review of *Lepidagathis cristata*..*Int.Res.J.Pharm*. 4(11): 6-8.
18. Sasikala K., Mohan S.C. (2014). Total phenolic, flavanoid contents and GC-MS analysis of *Canthium coromandelicum* leaves extract. *International Journal of Pharmacy and Pharmaceutical Sciences*, 6(8): 379-381.

19. Shakeel A.A., Thimmappa S.C., Kaliwal B.B. (2016). Phytochemical analysis and antimicrobial activity of *Chorella vulgaris* isolated from Unkal Lake.*Journal of Coastal Life Medicine* ., 4(5): 368-373
20. Shettima A.Y., Karumi Y., Sodipo O.A, Usman H., Tijjani M.A.(2013).Gas Chromatography–Mass Spectrometry (GC-MS) Analysis of Bioactive Components of Ethyl acetate Root Extract of *Guiera senegalensis* J.F. Gmel. *Journal of Applied Pharmaceutical Science*, 3(03): 146-150.
21. Srivastava R., Mukerjee A. & Verma A.(2015). GC-MS Analysis of Phytocomponents in, Pet Ether Fraction of *Wrightia tinctoria* Seed .*Phcog J.*, 7(4): 249-253.
22. Thenmozhi S. & Rajan S.(2015). GC-MS analysis of bioactive compounds in *Psidium guajava* leaves. *Journal of Pharmacognosy and Phytochemistry*, 3(5): 162-166.