Journal of Applied Pharmaceutical Science Vol. 7 (06), pp. 182-189, June, 2017
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2017.70626
ISSN 2231-3354

Antioxidant activities and chemical composition of various crude extracts of *Lepidagathis keralensis*

Leena Palakkal*, N. H. Zeinul Hukuman, Jisha Mullappally

Department of Post Gradaute Studies and Research in Chemistry, Sir Syed College, Taliparamba, Kannur, Kerala, India.

ARTICLE INFO

Article history:
Received on: 03/05/2017
Accepted on: 15/06/2017
Available online: 30/06/2017

Key words:
Lepidagathis keralensis, antioxidant activity, phenolics, flavonoids, GC-MS.

ABSTRACT

Lepidagathis keralensis (Acanthaceae), a plant mainly used as a preventive medicine against malnutrition was studied for the evaluation of *in vitro* antioxidant activity. Petroleum ether, acetone, methanol and aqueous extract of the stem and leaves of the plant were analyzed. The Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) were quantitatively estimated for correlation studies. DPPH radical scavenging assay, Reducing power assay and Phosphomolybdenum method were employed for the evaluation of antioxidant activity. Chemical components of the extracts showing higher antioxidant properties were subjected to Gas chromatography-mass spectrometry (GC-MS) analysis. Potent antioxidant activities were shown by the methanol extract of leaf and acetone extract of the stem in all the assays, with the methanol extract of leaf showing highest DPPH scavenging activity(94.78%), reducing power(1.226) and total antioxidant activity(656.89±1.68 mg/g AAE). Highest TPC and TFC were obtained for the methanol extract of leaf (139.76±0.41 and 258.33±1.47) and acetone extract of stem (102.00±1.40 and 240.00±2.42). Positive correlations were obtained for TPC and TFC with the observed antioxidant activity. GC-MS analysis of the extracts revealed the presence of 13 phytocomponents in the methanol extract of leaf. Cyclopentaneundecanoic acid methyl ester (27.4%), Benzene, (ethenyloxy)-(17.3%), n-Hexadecanoic acid (palmitic acid) (13.93%) and 10-Undecynoic acid, methyl ester (11.67%) were the major components identified in the extract. 25 components were identified in the acetone extract of stem with Cyclopentaneundecanoic acid (29.6%), 1,6-Octadiene,3,7-dimethyl-(17.17%), 10-Undecyn-1-ol(9.54%) and 3-Hydroxy-4-methoxybenzoic acid(6.79%) as the major components. The study confirms that the plant is rich source of natural antioxidants.

INTRODUCTION

Antioxidants are compounds that may protect the cells from oxidative damage caused by free radicals (Pavithra *et al*., 2013; Farida *et al*., 2014). Living organisms generate free radicals during metabolism of aerobic cells (Kandasamy and Aradhya, 2014). These free radicals lead to various chronic diseases such as atherosclerosis, cardio vascular diseases, cancer, ageing, diabetes, gastric ulcer and DNA damage (Shanta *et al*., 2013; Singh *et al*., 2013). Even though the human body possess antioxidant defense mechanisms, under certain conditions it may be inadequate and hence dietary intake of antioxidants are recommended (Padmanabhan and Jangle, 2012).

* *Corresponding Author*
Leena Palakkal, Department of Post Gradaute Studies and Research in Chemistry, Sir Syed College, Taliparamba, Kannur, Kerala, India.
Email: leenasajithp @ gmail.com

Recent research findings have confirmed that synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), which are used in food industry, are associated with several toxic effects. Hence there is an increased attention towards plant based natural antioxidants (Hemalatha *et al*., 2016; Meechai *et al*., 2016). There exists an inverse relation between human diseases and dietary intake of food rich in antioxidants (Yildirim *et al*., 2001). The antioxidant potential of the plant is mainly considered to be due to the presence of phenolics and flavonoids (Galvez, 2015). *Lepidagathis keralensis*, an endemic plant of Kerala belonging to the family Acanthaceae is found near seacoast in exposed lateritic rocks. It is a rigid prostrate under shrub with woody rootstock (Madhusoodanan and Singh, 1992).

The plant possesses several medicinal properties. It is used by the paniya tribes for treating bronchial asthma in children (Divakar *et al*., 2010).

The gruel prepared by cooking the spines of the plant with rice is used as a preventive medicine for malnutrition, malabsorption and digestive disorders. Decoction of the whole plant is recommended for kidney stone and albumin in urine. The plant is also used as a blood purifier and increases blood. Whole plant decoction with cumin seeds is given for chest pain (Prasad S., 2012). The antimicrobial efficiency of the petroleum ether extracts of the plant were studied previously in our laboratory. The chemical constituents in the extract were also determined by GC-MS analysis (Leena *et al*., 2016).

Lepidagathis keralensis is a less explored plant for chemical studies and antioxidant studies in this plant are not known to be reported so far. Thus this study deals with the evaluation of antioxidant potency of the different extracts of the leaves and stem of the plant. Correlations of the observed activity were done with total phenolic content (TPC) and total flavonoid content (TFC). The extracts showing higher antioxidant efficiency were further examined by gas chromatography-mass spectrometry (GC-MS) to identify the bioactive constituents present.

MATERIALS AND METHODS

Collection and preparation of plant extracts

The plant, *Lepidagathis keralensis* was collected from the lateritic rocks of Madayipara, Kerala during September 2015 and was authenticated from the Department of Botany, Govt. Brennen College, Thalassery, Kerala. A voucher specimen (TALI 1174) was deposited at the herbarium, Dept. of PG studies and Research in Botany Sir Syed College, Taliparamba, Kerala. The leaves and stem of the plant were separated, washed well, and dried in shade for three weeks. The shade dried plant parts were then powdered well and extracted with petroleum ether, acetone and methanol sequentially in a soxhlet apparatus. The aqueous extracts were prepared by cold maceration method in a mechanical shaker. The extracts were concentrated under reduced pressure and stored in refrigerator for further studies.

In vitro antioxidant assays

The antioxidant assays of the petroleum ether, acetone, methanol and aqueous extracts of the stem and leaves of the plant were carried out by the following methods:

DPPH radical scavenging assay

Based on the scavenging ability of the stable 1, 1-diphenyl-2- picryl hydrazyl (DPPH) free radical, the antioxidant activity of the extracts were determined by the method described by Dasgupta *et al*.(2004).0.1 ml of Plant extracts (200,300,400,500 and 600 µg/ml in methanol) was added to 3 ml of a 0.004 % of DPPH solution in methanol. After 30 min, absorbance was determined at 517 nm against methanol as blank. The percentage of inhibition activity was calculated as follows:

Percentage inhibition of DPPH activity =[(Ac-As)/Ac]*100

Ac is the absorbance of the control (DPPH solution) and As is the absorbance of the extract/standard. The inhibition curves were prepared and IC_{50} values calculated. Ascorbic acid was used as the standard.

Reducing power assay

The reducing power of the extracts was assayed according to the method described by Alam *et al* (2013). Different concentrations of extracts (50, 100, 150,200 and 250µg/ml) in 1 ml of distilled water were mixed with 2.5 ml of 0.2M phosphate buffer (pH 6.6). 2.5 ml of 1% $K_3Fe(CN)_6$ was added to this solution and the resulting mixture was incubated at 50 °C for 20 min. 2.5 ml of 10%Trichloro acetic acid was then added to the solution and mixture centrifuged at 3000 rpm for 10 min.2.5 ml of the upper layer was collected and mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% $FeCl_3$ was then added. Absorbance of the mixture was then measured at 700nm. Increased absorbance of the reaction mixture indicates increased antioxidant ability. Ascorbic acid was used as the standard.

Total antioxidant activity

The total antioxidant activity of the sample was analyzed by Phosphomolybdenum method (Kumaran and Karunakaran, 2007). 0.3 ml of the sample was taken and mixed with 3 ml of reagent solution (0.6 M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The solution was incubated at 95^{o}C for 90 min. and cooled to room temperature. The absorbance of the solution was measured at 695 nm using Labtronics single beam UV-Visible LT-290 spectrophotometer against methanol as blank. Standard calibration curve was prepared using ascorbic acid and the total antioxidant activity was expressed as ascorbic acid equivalents.

Determination of total phenolic content

The total phenolic content (TPC) of plant extracts were determined using Folin–Ciocalteu reagent (Yu *et al*., 2002). 100 µl plant extract (prepared by dissolving 100 mg extract in 100 ml distilled water) was mixed with 500 µl of the Folin–Ciocalteu reagent and 1.5 ml of 20 % sodium carbonate. The mixture was shaken thoroughly and made up to 10 ml using distilled water. After 2 hours, the absorbance of the solution was determined at 765 nm using Labtronics single beam UV-Visible LT-290 spectrophotometer. From the data obtained, TPC was determined using standard calibration curve of Gallic acid and results were expressed as Gallic acid equivalents. Triplicate measurements were taken for all extracts.

Determination of total flavonoid content

The total flavonoid content (TFC) of each plant extract was estimated by Aluminium chloride colorimetric method (Deori *et al*., 2014). Based on this method, 1ml of the sample was dissolved in 4 ml of distilled water. 0.3 ml of a 5% $NaNO_2$ solution was then added. After 5 min, 0.3 ml of 10% $AlCl_3$ solution was added and allowed to stand for 6 min. 2 ml of 1M NaOH solution was then added to the mixture. The solution was diluted to 10 ml, thoroughly mixed and allowed to stand for

another 15 min. The absorbance of the mixture was then determined at 510 nm using Labtronics single beam UV-Visible LT-290 spectrophotometer. Water was used as blank. TFC was determined using standard calibration curve of Rutin and results were expressed as Rutin equivalents. Triplicate measurements were recorded for all extracts.

Gas chromatography-mass spectroscopic analysis

GC-MS analyses of the extracts showing better antioxidant activity were carried out from Sir Syed College, Taliparamba, Kerala. The analysis were done 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 µm df).GC-MS detection were done with an electron ionization system with an ionizing energy of 70ev. Helium gas (99.99%) was used as the carrier gas at constant flow rate 1ml/min. Injection volume was 1 µl with split ratio 1:8. Injection port temperature of 280^0C and an ion-source temperature of 200^0C were set. The analysis of the extracts were done by programming the oven temperature initially at 70^0C (kept isothermal for 3 min.) and then increased to 180^0C at a rate of 6^0C/min. with a hold time of 3 min. The temperature was then increased at a rate of 5^0C/min. to 240^0C and held isothermally for 5 min. Total GC running time was 40 min. The components in the extracts were identified based on the comparison of mass spectra of the unknown component with those of known components stored in the NIST library.

Statistical Analysis

Measurements were recorded in triplicates for all the analysis. Results were calculated as the mean (n=3) ± SD (standard deviation) for each sample. One-way ANOVA followed by student's t- test was performed using GraphPad Prism version 7.02 for Windows (GraphPad Software, La Jolla California, USA). $p < 0.05$ were considered significant. The Pearson correlation coefficient (r) was used to study correlations of phenolics and flavonoids with observed antioxidant activity.IC_{50} values were determined using nonlinear regression method.

RESULTS AND DISCUSSION

Antioxidant activity

DPPH assay

DPPH is a stable free radical, turning color from red to yellow when scavenged. Antioxidants reduce DPPH by donating a proton and thereby decreasing the absorbance of the solution in methanol at 517nm. The scavenging potential of the extracts is therefore determined by the discoloration of DPPH in methanol. A decrease in absorbance value thus indicates an increase in antioxidant activity. (Thambiraj and Paulsamy, 2012) The radical scavenging activity of the different extracts of leaves and stem are depicted in Figure 1 and 2 respectively. Among the different extracts tested, the methanol extract of leaf showed a maximum inhibition activity of 94.78% which was comparable with that of standard ascorbic acid (96.38%). The extract showed an IC_{50} value of 122.46±0.85. (Table 1) The analysis of stem extracts revealed that the acetone extract of stem was more potential antioxidant showing maximum inhibition activity of 86.46%. The results show that the plant possesses potential free radical scavenging activities.

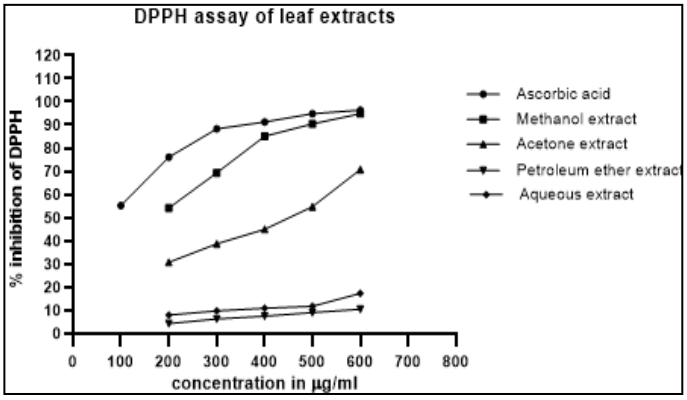

Fig. 1: DPPH radical scavenging activity of the extracts of *Lepidagathis keralensis* leaf and standard ascorbic acid. The values are expressed as mean of three replicate values.

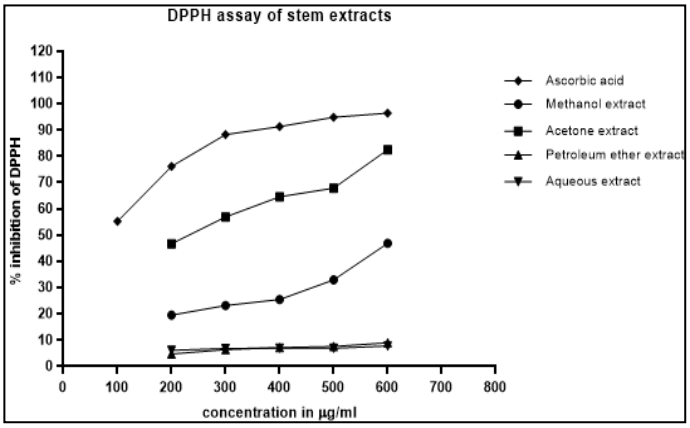

Fig. 2: DPPH radical scavenging activity of the extracts of *Lepidagathis keralensis* stem and standard ascorbic acid. The values are expressed as mean of three replicate values.

Table 1: IC_{50} values obtained for DPPH assay of extracts of leaves and stem of *Lepidagathis keralensis* and standard ascorbic acid.

Extract	IC_{50}	
	Leaf extract	Stem extract
Petroleum ether	ns*	ns*
Acetone	449.27±1.23	231.87±1.69
Methanol	122.46±0.85	618.37±1.12
Aqueous	875±1.04	ns*
Ascorbic acid	76.18±0.68	76.18±0.68

* ns – not significant values are expressed as mean±SD of three replicate values.

Reducing power

This method is based on the principle that antioxidant compounds reduces Fe^{3+}/ferricyanide complex to the ferrous form, changing the yellow color of the solution to shades of green and blue. The colored complex formed (Perl's Prussian blue), shows

an absorbance at 700nm. Thus an increase in absorbance indicates an increase in antioxidant potential (Ferreira *et al*., 2007). All the extracts analyzed, showed an increase in absorbance with increase in concentration which suggests that the plant is a good antioxidant. The results are depicted in Figure 3 and 4. The methanol extract of leaf showed the highest absorbance (1.226) which was close to the absorbance shown by standard ascorbic acid (1.462). Among the extracts of stem, the acetone extract showed highest antioxidant potential with an absorbance value of 0.824.

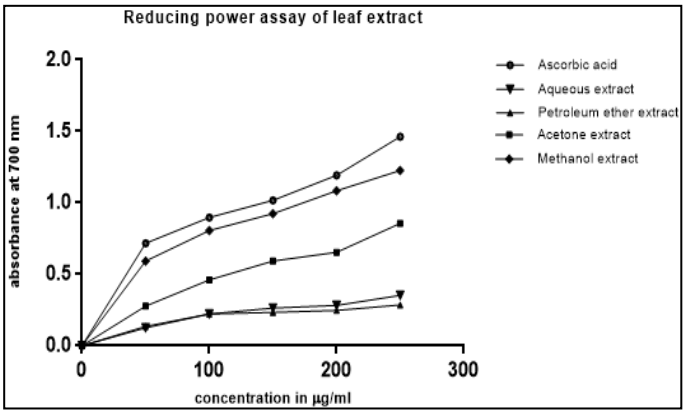

Fig. 3: Reducing power (absorbance at 700nm) of extracts of *Lepidagathis keralensis* leaf and standard ascorbic acid at different concentrations. The values are expressed as mean of three replicate values.

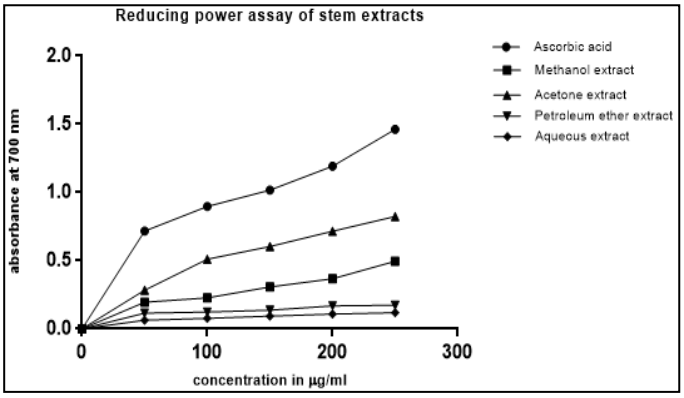

Fig. 4: Reducing power (absorbance at 700nm) of extracts of *Lepidagathis keralensis* stem and standard ascorbic acid at different concentrations. The values are expressed as mean of three replicate values.

Total antioxidant activity

The total antioxidant activities of the extracts as determined by Phosphomolybdenum method are shown in Table 2. The method is based on the reduction of Mo (VI) to Mo (V) to form a green phosphate Mo (V) complex at acidic pH by the analyzed sample. Among the extracts analyzed, highest total antioxidant activity was shown by the methanol extract of leaf (656.89±1.68), followed by the acetone extract of stem (532.00±2.0). A comparative evaluation of total antioxidant activities are depicted in Figure 5.

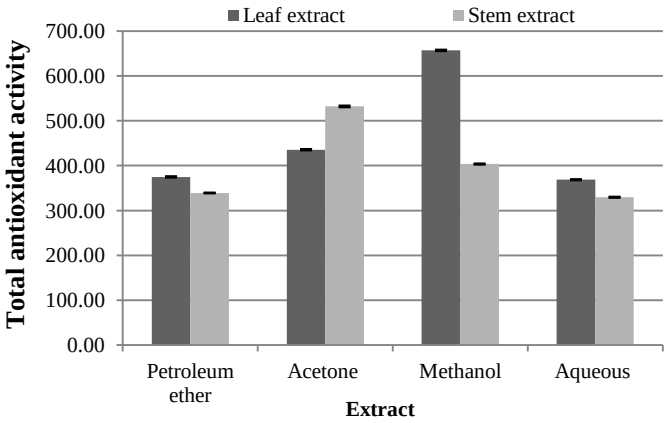

Fig. 5: Total antioxidant activity (mg/g AAE) determined by phosphomolybdenum method of different extracts of leaves and stem of *Lepidagathis keralensis*. The values are expressed as mean±SD of three replicate values.

Table 2: Total antioxidant capacity in mg/g AAE of extracts of leaves and stem of *Lepidagathis keralensis*

Extract	Total antioxidant activity in mg/g AAE	
	Leaf extract	Stem extract
Petroleum ether	374.89±1.68	339.11±1.02
Acetone	435.56±1.64	532.00±2.0
Methanol	656.89±1.68	403.56±1.33
Aqueous	368.67±1.33	329.56±1.39

values are expressed as mean±SD of three replicate values.
AAE-Ascorbic Acid Equivalent

Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

Presence of phenolics and flavonoids are considered to be the main reason for antioxidant capacity of a plant. Polyphenolic compounds show antioxidant activity owing to their redox properties, which provide them the ability to scavenge free radicals by H- atom or e- transfer process, inhibit redox active enzymes, decompose peroxides, quench singlet and triplet oxygen, chelate metal catalysts etc. (Valentova *et al*., 2003; Gil and Couto, 2013). In the present study, TPC and TFC were quantitatively estimated by Folin–Ciocalteu method and Aluminium chloride method respectively.

The results obtained are given in Table 3.It was observed that the methanol extract of leaf contained highest amount of TPC (139.76±0.41 mg GAE/g) and TFC (258.33±1.47 mg RE/g). Among the stem extracts analyzed, the acetone extract showed maximum amount of TPC (102.00±1.40 mg GAE/g) and TFC (240.00±2.42 mg RE/g). The results obtained were in accordance with the studies that plants with higher TPC and TFC show higher antioxidant potential (Lima *et al*., 2014). Even though the petroleum ether extract of stem showed a high TFC content (138.33±1.92 mg RE/g), the observed antioxidant activities for the extract were not so high. This might be due to the presence of large number of glycosidic moieties associated with flavonol

glycosides. Antioxidant activities of flavonoids are influenced by the position, structure and total number of sugar units (Kumar and Pandey, 2013).

Table 3: Quantitative analysis of phenolics and flavonoids in the extracts of leaves and stem of *Lepidagathis keralensis*

	Extract	TPC mg GAE/g	TFC mg RE/g
Leaf	Petroleum ether	14.19±0.25	3.33±0.96
	Acetone	104.73±0.94	91.67±2.42
	Methanol	139.76±0.41	258.33±1.47
	Aqueous	9.81±0.27	6.66±1.47
Stem	Petroleum ether	4.79±0.14	138.33±1.92
	Acetone	102.00±1.40	240.00±2.42
	Methanol	66.98±0.42	61.67±2.00
	Aqueous	3.56±0.26	8.33±2.42

values are expressed as mean±SD of three replicate values.
TPC- Total Phenolic Content expressed as mg/g Gallic Acid Equivalents.
TFC- Total Flavonoid Content expressed as mg/g Rutin Equivalents.

Table 4: Pearson correlation coefficient (r) values for correlation between TPC, TFC and antioxidant activities of *Lepidagathis keralensis*:

		DPPH	Reducing power	Total antioxidant activity
Leaf	TPC	0.995*	0.988*	0.873
	TFC	0.933	0.966*	0.992*
Stem	TPC	0.993*	0.990*	0.955*
	TFC	0.709	0.738	0.796

* Significant correlation at $p < 0.05$ levels

The results of Pearson correlation analysis (r value) of the observed TPC and TFC with the antioxidant activities are tabulated in Table 4. Strong positive correlations were obtained between TPC of leaf extracts and antioxidant activity by DPPH assay (r = 0.995). Results of reducing power method also showed good correlation with TPC (r = 0.988) and TFC (r = 0.966) of leaf extracts. The total antioxidant activity of the leaf was found to be in good correlation with TFC (r = 0.992), while no significant correlations were found with TPC. Antioxidant activities of the stem extracts showed significant correlation ($p<0.05$) with TPC for all the assays, while TFC showed less correlation.

GC-MS analysis

Highest antioxidant efficiencies were shown by the methanol extract of leaf and acetone extract of stem. Hence these extracts were subjected to GC-MS analysis, which revealed the presence of 13 phytocomponents in the methanol extract of leaf and 25 components in the acetone extract of stem (Table 5 and Table 6). Cyclopentaneundecanoic acid methyl ester (27.4%), Benzene, (ethenyloxy)-(17.3%), n-Hexadecanoic acid (palmitic acid) (13.93%) and 10-Undecynoic acid, methyl ester (11.67%) were the major components identified in the leaf extract. Of the several components identified, many possess varied potential bioactivities. Components identified to possess antioxidant properties were Benzene, (ethenyloxy)-, 2-Methoxy-4-vinylphenol and n-Hexadecanoic acid (Flora and Rani, 2010; Gopalakrishnan and Vadivel, 2011; Vijayakumar and Sumathi, 2016). The major components identified in the acetone extract of stem were Cyclopentaneundecanoic acid (29.6%), 1, 6-Octadiene, 3, 7-dimethyl-(17.17%), 10-Undecyn-1-ol (9.54%) and 3-Hydroxy-4-methoxybenzoic acid (6.79%). Among the 25 components identified, 3-Hydroxy-4-methoxybenzoic acid, 2-Methoxy-4-vinylphenol, vanillic acid and 2-Piperidinone, N-[4-bromo-n-butyl] - were found to possess antioxidant activities (Khadem and Marles, 2010; Kumar *et al*., 2011; Meenakshi *et al*., 2012; Hussein *et al*., 2016). The GC-MS chromatograms of the extracts are shown in Figure 6 and 7. Although no reported data are available on the GC-MS studies in this plant, n-Hexadecanoic acid , Vanillic acid, (E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol and 1,6-Octadiene,3,7-dimethyl were previously reported to be present in different plant extracts belonging to the family Acanthaceae(Kalaiselvan *et al*., 2012; Awan *et al*., 2014; Kumar *et al*., 2016). Several other phytocomponents identified in the extracts were found to possess antibacterial, antifungal, anticancer, anti inflammatory and diuretic properties (Kumari *et al*., 2012; Shettima *et al*., 2013). The study thus highlights the medicinal importance of the plant.

Table 5: Compounds identified in the methanolic extract of *Lepidagathis keralensis* leaf by GC-MS analysis.

Sl. No.	RT	Name of the Compound	Molecular formula	Molecular weight	Peak area %
1	5.28	Tiglic acid	$C_5H_8O_2$	100	0.43
2	9.25	Heptanoic acid, 6-oxo-	$C_7H_{12}O_3$	144	3.48
3	12.89	Benzene, (ethenyloxy)-	C_8H_8O	254	17.30
4	13.14	4-Hexen-3-one, 4,5-dimethyl-	$C_8H_{14}O$	126	5.4
5	15.46	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	0.23
6	25.30	2,3,4,5-Tetramethoxybenzaldehyde	$C_{11}H_{14}O_5$	226	0.36
7	29.42	Cyclopentaneundecanoic acid, methyl ester	$C_{17}H_{32}O_2$	268	27.4
8	30.20	n-Hexadecanoic acid(palmitic acid)	$C_{16}H_{32}O_2$	256	13.93
9	31.03	4,5-Dimethoxy-6-[2-nitroethenyl]-2H-1,3-benzodioxole	$C_{11}H_{11}NO_6$	253	1.88
10	31.70	10-Undecyn-1-ol	$C_{11}H_{20}O$	168	8.61
11	31.75	10-Undecynoic acid, methyl ester	$C_{12}H_{20}O_2$	196	11.67
12	31.84	9-Octadecen-12-ynoic acid, methyl ester	$C_{19}H_{32}O_2$	292	1.82
13	31.92	Cyclopropanepentanoic acid, 2-undecyl-, methyl ester, trans-	$C_{20}H_{38}O_2$	310	7.5

Table 6: Compounds identified in the acetone extract of *Lepidagathis keralensis* stem by GC-MS analysis.

Sl. no	RT	Name of the Compound	Molecular formula	Molecular weight	Peak area %
1	3.78	2-Pentanone, 4-hydroxy-4-methyl-	$C_6H_{12}O_2$	116	2.64
2	5.60	(R)-(-)-2,2-Dimethyl-1,3-dioxolane-4-methanol	$C_6H_{12}O_3$	132	3.32
3	6.22	Ethanol, 2,2'-oxybis-	$C_4H_{10}O_3$	106	0.47
4	6.36	(R)-(-)-2,2-Dimethyl-1,3-dioxolane-4-methanol/Solketal	$C_6H_{12}O_3$	132	1.13
5	7.22	2-Butanone, 4-hydroxy-3-methyl-	$C_5H_{10}O_2$	102	1.54
6	15.46	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	0.75
7	17.68	Benzaldehyde, 4-hydroxy-3-methoxy-	$C_8H_8O_3$	152	1.12
8	21.70	4-Methyloctanoic acid	$C_9H_{18}O_2$	158	1.35
9	21.92	Vanillic acid	C8H8O4	168	3.71
10	22.07	3-Hydroxy-4-methoxybenzoic acid	C8H8O4	168	6.79
11	24.84	3,7-Cyclodecadien-1-one, 10-(1-methylethenyl)-, (E,E)-	$C_{13}H_{18}O$	190	0.91
12	25.67	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol	$C_{10}H_{12}O_3$	180	5.55
13	26.11	2,3-Epoxyhexanol	$C_6H_{12}O_2$	116	1.80
14	26.33	(6,7-Dimethoxy-2H-1,3-benzodioxol-5-yl)methanol	$C_{10}H_{12}O_5$	212	0.53
15	27.65	1-Octadecyne	$C_{18}H_{34}$	250	2.86
16	28.15	2-Decen-1-ol	$C_{10}H_{20}O$	156	0.62
17	28.52	1-Dodecyne	$C_{12}H_{22}$	166	0.32
18	29.26	1,1-Dodecanediol, diacetate	$C_{16}H_{30}O_4$	286	0.37
19	30.37	Cyclopentaneundecanoic acid	$C_{16}H_{30}O_2$	254	29.60
20	31.86	(2S,3S)-(-)-3-Propyloxiranemethanol	$C_6H_{12}O_2$	116	0.94
21	32.49	2-Piperidinone, N-[4-bromo-n-butyl]-	$C_9H_{16}BrNO$	233	0.41
22	32.81	1,6-Octadiene,3,7-dimethyl (Dihydromyrcene)	$C_{10}H_{18}$	138	17.17
23	32.87	10-Undecyn-1-ol	$C_{11}H_{20}O$	168	9.54
24	33.07	3-Ethylheptanoic acid	$C_9H_{18}O_2$	158	2.77
25	39.72	1-Heptatriacotanol	$C_{37}H_{76}O$	536	1.99

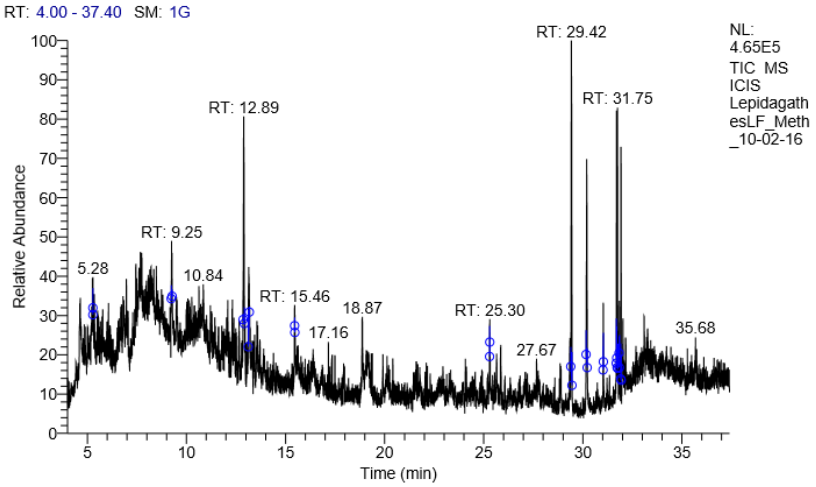

Fig.6: GC-MS chromatogram of methanolic extract of *Lepidagathis keralensis* leaf.

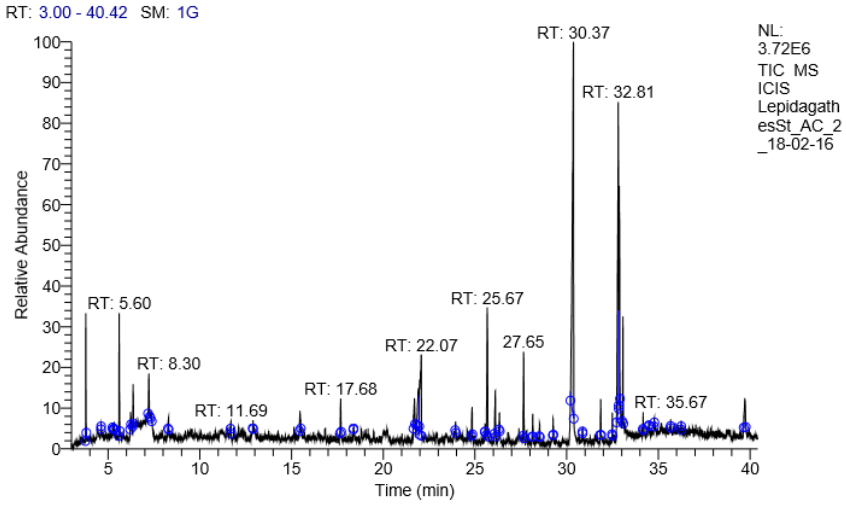

Fig.7: GC-MS chromatogram of acetone extract of *Lepidagathis keralensis* stem.

CONCLUSION

The present study reveals that the methanol extract of leaf and acetone extract of stem of the plant, *Lepidagathis keralensis* possess good antioxidant activities. These activities can be attributed to the presence of high TFC and TPC in the extracts, which were confirmed by correlation studies. The extracts also showed the presence of various biologically active phytocomponents in GC-MS analysis. The presence of these phytocomponents also contributes to the observed medicinal property in addition to the antioxidant activity of the plant. The present study highlights the importance of the plant as a source of natural antioxidant. The plant can thus be further explored for isolation and characterization of bioactive components in the extracts.

ABBREVIATIONS

Gas Chromatography-Mass Spectrometry: GC-MS, Total Phenolic Content: TPC, Total Flavonoid Content: TFC, 1, 1-diphenyl-2- picryl hydrazyl: DPPH, Gallic Acid Equivalents: GAE, Rutin Equivalents: RE.

ACKNOWLEDGEMENTS

The authors are also thankful to Sir Syed College, Kannur, India for providing necessary facilities.

Financial support and sponsorship: The corresponding author is grateful to UGC, India, for providing financial assistance by awarding research fellowship under Faculty Development Program (FDP).

Conflict of Interests: There are no conflicts of interest.

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How to cite this article:

Palakkal L, Hukuman NHZ, Mullappally J. Antioxidant activities and chemical composition of various crude extracts of *Lepidagathis keralensis*. J App Pharm Sci, 2017; 7 (06): 182-189.