IJPSR (2015), Vol. 6, Issue 6 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 18 October, 2014; received in revised form, 30 December, 2014; accepted, 11 February, 2015; published 01 June, 2015

GC-MS ANALYSIS OF BIOACTIVE CONSTITUENTS OF *GLOCHIDION ELLIPTICUM* WT.

Indhiramuthu Jayashree*, D. H. Geetha and M. Rajeswari

PG and Research Department of Botany, Vellalar College for Women, Erode-12, Tamil Nadu, India

Keywords:

GC-MS, *Glochidion ellipticum*, Porphyrin, Eicosane, Anti-cancer, Anti-microbial and Phytol.

Correspondence to Author:
Indhiramuthu Jayashree

PG and Research Department of Botany, Vellalar College for Women, (Autonomous), Erode- 638012, Tamil Nadu, India.

E-mail: indramuthujayashree@gmail.com

ABSTRACT: The aim of the present study is to identify the volatile, bioactive phytoconstituents present in the ethanolic extract of the leaves of *Glochidion ellipticum* Wt. (Euphorbiaceae) was carried out by using GC-MS analysis, while mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology and Wiley library. Twenty seven components from leaves of the above said plant were identified. The active principles with their retention time, molecular formula, molecular weight and concentration (%) in the ethanol extract of leaf of *G. ellipticum* are obtained. This is the first report of documentation of active constituents from leaves of *Glochidion ellipticum*. The research reveals the potential of *G. ellipticum* leaves as a good source of bioactive compounds such as esters, ethers, alkaloids, alkenes, fatty alcohols, terpenoids, amines, porphyrin and silicon compounds that justify the use of this plant for its various ailments by traditional practitioners.

INTRODUCTION: Nature is the best combinatorial chemist and possibly has answers to all diseases of humankind. Green plants represent a reservoir of effective chemotherapeutants as they are non- phytotoxic, more systemic and easily biodegradable [1]. Till now, natural compounds discovered from medicinal plants have provided numerous clinically useful drugs. A knowledge of the chemical constituents of plants is desirable not only for the discovery of therapeutic agents, but also because such information may be of great value in disclosing new sources of economic phytocompounds for the synthesis of complex chemical substances and for discovering the actual significance of folkloric remedies [2].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.6(6).2546-50

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.6(6).2546-50

In recent years GC-MS studies have been increasingly applied for the analysis of medicinal plants as this technique has proved to be a valuable method for the analysis of non-polar components and volatile essential oil, fatty acids, lipids and alkaloids [3].

The genus *Glochidion* of the family Euphorbiaceae has many representative species in India and most of them are chemically constituted with constituents like triterpenoid saponins, sesquiterpenoids, glycosides and alkaloids which vary from one plant to another [4]. Therefore it can be considered that these plants may possess a lot of medicinal value which may be in one way or the other beneficial for the human well-being. Much attention can be given in complete exploration of the different species of this genus as they have not yet come in the limelight of the researchers.

MATERIALS AND METHODS: The leaves of *Glochidion ellipticum* Wt. were collected from the

fringes of evergreen forests of Idukki district, Kerala, India and were authenticated and deposited at the PG and Research Department of Botany, Vellalar College for Women, Erode (Tamil Nadu), India. Fresh leaves were collected and air-dried at room temperature and then homogenized to obtain coarse powder. The powdered leaf was extracted with the solvent ethanol by hot extraction using soxhlet apparatus, collected and stored in a vial for further analysis [5].

GC-MS Analysis: Ethanolic extract of leaf of *G.ellipticum* was analyzed for the presence of different volatile compounds by Gas chromatography-Mass spectroscopy (GC-MS) technique. GC-MS analysis of some of the potent volatile constituents present in the extract was performed at The South India Textile Research Association (SITRA), Coimbatore (Tamil Nadu), India. GC analysis of the extracts was performed using a GC-MS (Model; Thermo Trace GC Ultra Ver.5.0) equipped with a DB-35MS fused silica capillary column (30m length X outside diameter 0.25 mm X internal diameter 0.25µm) and gas chromatograph interfaced to a Mass Selective Detector (MS-DSQ-II) with XCALIBUR software. For GC-MS detection, an electron ionization system with ionization energy of -70eV was used.

Helium gas was used as a carrier gas at a constant flow rate of 1ml/min and the sample injected was 1µl; Injector temperature 250oC; Ion source temperature 200^{o}C.

The oven temperature was programmed from 70° to 200°C at the rate of 10°C/min, held isothermal for 1minutes and finally raised to 250°C at 10°C/min. Interface temperature was kept at 250^{o}C. Total GC run time was 37.50 min. The relative percentage of each extract constituent was expressed as percentage with peak area normalization.

Identification of components:
The identity of the components in the extract was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library NIST (Mc Lafferly, 1989) [6], WILEY (Stein, 1990) [7] and also with published literatures.

RESULT: The GC-MS analysis of the ethanolic extract of leaf of *G.ellipticum* revealed the presence of twenty seven bioactive phytochemical constituent that could contribute to the medicinal value of the plant. GC and MS running time was 37.50 minutes. The GC-MS chromatogram of the test plant is presented in **Fig. 1**. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and peak area are presented in **Table 1**. The spectra of the compounds are matched with Wiley 9.0 and NIST libraries.

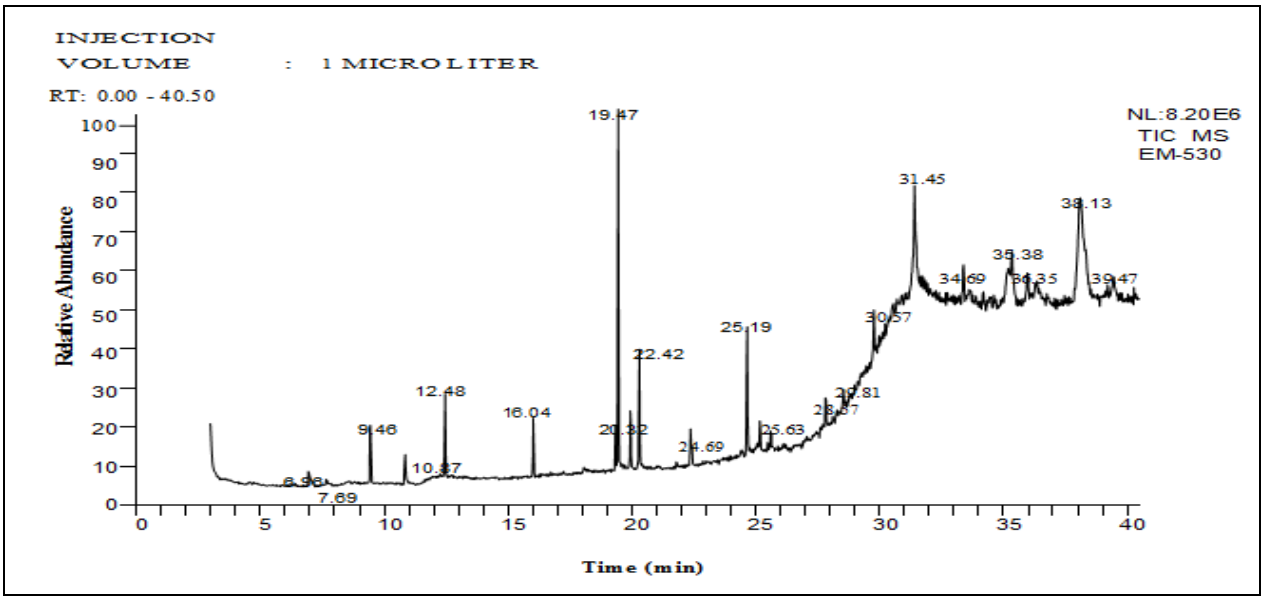

FIG.1: GC-MS CHROMATOGRAM OF THE ETHANOLIC EXTRACT OF LEAF OF *GLOCHIDION ELLIPTICUM*

TABLE 1: BIOACTIVE COMPOUNDS IDENTIFIED IN THE ETHANOLIC EXTRACT OF LEAF OF *GLOCHIDION ELLIPTICUM* BY GC-MS

S. No.	RT	Name of the compound	Molecular formula	Molecular weight	Peak area %
1	6.96	Benzoic acid, 2,3-bis[(trimethylsilyl)oxy]-,trimethylsilyl ester	$C_{16}H_{30}O_4Si_3$	370	1.14
2	7.69	Benzene, 1-methoxy-4-(1-propenyl)-	$C_{10}H_{12}O$	148	0.30
3	9.46	Cyclohexasiloxane, dodecamethyl-	$C_{12}H_{36}O_6Si_6$	444	2.61
4	10.87	4-Methoxy-6-(trimethylsilyl)-1,3-diazabiphenylene	$C_{14}H_{16}N_2O$ Si	256	1.37
5	12.48	6-(4-Chlorophenyl)-2,5,5-triphenyl-5,8-dihydro-6H-azeto[1,2-a][1,3]thiazolo[4,5-d]pyrimidine	$C_{31}H_{22}ClN\ 3_S$	503	2.62
6	16.04	Cyclooctasiloxane, hexadecamethyl-	$C_{16}H_{48}O_8Si_8$	592	1.81
7	19.47	3-Eicosane	$C_{20}H_{38}$	278	12.69
8	19.96	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	2.07
9	20.32	Neophytadiene	$C_{20}H_{38}$	278	3.65
10	22.42	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	$C_{16}H_{50}O_7Si_8$	578	1.90
11	24.69	Phytol	$C_{20}H_{40}O$	296	5.27
12	25.19	N,N-Bis(trimethylsilyl)-2-(2-thienyl)quinolin-4-amine	$C_{19}H_{26}N_2SSi_2$	370	2.33
13	25.63	9-Octadecenoic acid (Z)-, 9-octadecenyl ester, (Z)-	$C_{36}H_{68}O_2$	532	1.05
14	26.15	1,3"-Bis(tert-butyldimethylsilyl)-3,1':3',1"-ter(benzo[c]thiophene)	$C_{36}H_{42}S_3\ Si_2$	626	0.36
15	27.07	Normorphine, bis(trimethylsilyl) ether	$C_{22}H_{33}NO_3\ Si_2$	415	0.32
16	28.57	2-[3,4-Bis(tetradecyloxy)phenyl]-4,4,5,5-tetramethyl-1,3,2-dioxaborolane	$C_{40}H_{73}\ BO_4$	628	0.73
17	29.30	(+)-Cycleatjehine	$C_{36}H_{34}N_2O_6$	590	0.72
18	29.81	Cyclodecasiloxane, eicosamethyl-	$C_{20}H_{60}O_{10}Si_{10}$	740	1.65
19	30.57	6,6'-Bis[4-(triethylsilyl)-1,3-butadiynyl]-2,2'-bipyridine	$C_{30}H_{36}N_2Si_2$	480	2.59
20	31.45	Silicone oil	N/A	0	11.26
21	33.42	Tetracosamethyl-cyclododecasiloxane	$C_{24}H_{72}O_{12}Si_{12}$	888	1.91
22	34.69	1-(4-t-butylphenyl)-2,3,4,5-tetraphenylcyclopenta-2,4-diene	$C_{39}H_{34}$	502	0.41
23	35.38	Glaucine (Rs)-(1) (Racemic)	$C_{21}H_{25}NO_4$	355	9.54
24	36.00	1H-Purin-6-amine, [(2-fluorophenyl)methyl]-	$C_{12}H_{10}FN_5$	243	2.27
25	36.35	Tetraethyl 2,2-(propylidenedithio)ethenediylidenebisphosphonate	$C_{13}H_{26}O_6P_2S_2$	404	3.37
26	38.13	3,5-Pentano-13,17-diethyl-2,7,8,12,18-pentamethylporphyrin	$C_{34}H_{40}N_4$	504	18.63
27	39.47	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	$C_{12}H_{38}O_5Si_6$	430	4.48

The most prevailing major compounds were 3,5-Pentano-13,17-diethyl-2,7,8,12,18- pentamethyl porphyrin (18.63%), 3-Eicosane (12.69%), Silicone oil (11.26%), Glaucene (Rs)-(1) (Racemic)(9.54%), Phytol (5.27%), Neophytadiene (3.65%), Cyclohexasiloxane, Dodecamethyl- (2.61%)and pharmacologically important components like Tetracosamethyl-cyclododecasiloxane (1.91%), Cyclodecasiloxane eicosamethyl - (1.65%),9-Octadecenoic acid (Z)-, 9-octadecenyl ester, (Z)- (1.05%), Benzene, 1 – methoxy – 4 - (1-propenyl)- (0.30%) were also detected in minor amounts in the leaf sample. Most of these compounds have not been reported from this plant so far. However, there may be variation in the chemical composition based on topography.

DISCUSSION: Most of the identified compounds possessed many biological properties. For instance, 3,5-Pentano-13,17-diethyl-2,7,8,12,18-pentamethyl porphyrin (18.63%) is the major volatile constituent in the present study. Porphyrins occur widely in nature and play important roles in various biological processes. For example, they have found applications in virus destruction [8], cancer therapies as it selectively kill the tumor cells and their side effects are limited [9]. It is also known to possess anti- microbial[10] property. Glaucine is an alkaloid found in many species of the family Euphorbiaceae. It has bronchodilatory and anti-inflammatory effects [11] and is used medically as an anti-tussive [12]. Animal studies demonstrate the ability of glaucine to decrease heart rate and lower blood pressure [13], anticonvulsant and antinociceptive properties [14].

Phytol is one among the twenty seven identified compounds in the present study. Phytol is a

diterpene and is widely used as an anti-microbial, antioxidant, anti-tumor, anti-cancer, anti- arthritic, immuno stimulatory, anti-diabetic, chemo preventive, pesticidal and diuretic agent and has sunscreen properties [15-17]. Similarly, the presence of phytol was detected in the leaves of *Mallotus philippensis* [18], *Mimosa pudica* [19], Mohan *et al.* [20] and Sudha *et al.*[21] reported the aerial parts of *Kirganelia reticulata* and *Fluggea leucopyrus*, respectively.

The compound 3-Eicosane is an alkane is noted for its potent anti-tumor activity against the human gastric SGC-7901 cell line [22]. Likewise, Sivasubramanian and Brindha [23] detected the aerial parts of *Centratherum punctatum*. Neophytadiene, a terpenoid compound has antipyretic, anti-inflammatory, anti-microbial and antioxidant activity. Correspondingly, Carretero *et al.*[24] and Mendiola *et al.*[25] identified Neophytadiene in *Bursera simaruba* and red alga *Centroceras clavulatum* which were used as analgesic and vermifugic, including a topical application for sores and inflammation.

Tetracosamethyl-cyclododecasiloxane is one of the biologically active compound possessed hepato protective activity [26] and antispasmodic, anti-rheumatic, anti-soporific baths, insecticides for mosquito control, appetizing agent, to combat indigestion, stomach pain, nausea and infection of the gall bladder [27]. Venugopal *et al.*[28] noticed the leaves of *Hyptis suveolens*.

In the current research, the compound Cyclohexasiloxane, dodecamethyl- is widely used as a conditioning agent, emollient, in personal care products, lubricant and de-foaming agent [28]. Silicone oil is a polymerized siloxane with organic side chains. Consumer products to control flatus (anti- flatulents) often contain silicone oil. Silicone oils have been used as a vitreous fluid substitute to treat difficult cases of retinal detachment, such as those complicated with proliferative vitro retinopathy, giant retinal tears, and penetrating ocular trauma [29]. Mohan *et al.*[20] and Anandhi *et al* [30] reported the presence of 9-octadecenoic acid (Z), in the aerial parts of *Kirganelia reticulate* and leaves of *Tricalysia sphaerocarpa*, respectively. 9-octadecenoic acid (Z)-, 9- octadecenyl ester, (Z)-, an fatty acid ester group has effective hypocholesterolemic, nematicide, anti-arthritic, hepato protective, anti-androgenic, hypocholesterolemic 5-alpha reductase inhibitor, anti-histaminic, anti-coronary, insectifuge, anti-eczemic, anti-acne. Benzene,1-methoxy-4-(1-propenyl)-an ether compound has potent antimicrobial properties [31], anti-helmintic [32] nematicidal activity [33], lowering of body temperature, hypnotic, analgesic, anticonvulsant effects and carcinogenicity but insufficient data [34].

In line with the present study, Geetha *et al.*[35] and Santhosh Kumar *et al.*[36] observed similar results in leaves of *Elaeocarpus serratus*[35] and the whole plant of *Adiantum capillus-veneris*[36] respectively. Therefore, the chemical standardization study revealed that *Glochidion ellipticum* is rich in secondary metabolites which possessed wide range of biological activities.

CONCLUSION: The greatest lacuna existing in herbal-based medicines are lack of standards and validation studies. Terpenoid compounds were the chief constituents in the leaves, followed by alkaloids, silicon compounds, and aromatic hydrocarbons like alkenes, alkanes and fatty acid esters. Terpenoids are vital for life of most organisms and are currently being explored as anticancer agents in clinical trials. Considering the immense bioactive potentials of *G. ellipticum* in the present work, attempts can be made to scientifically validate and chemically standardize formulations for life threatening diseases.

ACKNOWLEDGEMENT: Thanks to the South India Textile Research Association (SITRA), Coimbatore (Tamil Nadu), India for help in screening the GC-MS analysis.

REFERENCES:

1. Chaman Lal and Verma LR: Use of certain bio-products for insect-pest control. Indian Journal of Traditional Knowledge, 2006; 5(1): 79- 82.
2. Milne A: Inhalational and local anesthetics reduce tactile and thermal responses in *Mimosa pudica* Linn. Masui, 1993; 1190-1193.
3. Betz JM, Gay ML, Mossoba MM, Adams S and Portz BS: J AOAC Int., 1997; 80: 303.
4. Sandhya S, Chaintanya RSNAKK, Vinod KR, Rao KNV, David Banji, Sudhakar K and Swetha R: An updated review on the Genus *Glochidion* Plant. Archives of Applied Science Research, 2010; 2(2):309-322.

5. Mukherjee PK: "Quality Control of Herbal Drugs. An approaches to evaluation of botanicals", edition 1st published by Business Horizons, New Delhi. 2002; 390-403.
6. Mc Lafferly F.W: Registry of mass spectral data, ed. 5, Wiley New York. 1989.
7. Stein S.E: National Institute of Standards and Technology (NIST) Mass Spectral Database and Software, Version 3.02, USA. 1990.
8. Li G, Pandey SK, Graham A, Dobhal MP, Mehta R, Chen Y, Gryshuk A, Rittenhouse- Olson K, Oseroff A, Pandey RK, J: Org. Chem., 2003; 69:158.
9. Hao E, Syntheseses and evaluation of porphyrin derivatives for applications in medicine and in material science. Ph.D. Dissertation, Louisiana State University and Agricultural and Mechanical College, 2007.
10. Bozja J, Yi K, Shafer W, Stojiljkovic I: "Porphyrin-based compounds exert antibacterial action against the sexually transmitted pathogens". International Journal of Antimicrobial Agents, 2004; 24(6): 578-584.
11. Cortijo J, Villagrasa V, Pons R, Berto L, Martí-Cabrera M, Martinez-Losa M, Domenech T, Beleta J, Morcillo EJ: "Bronchodilator and anti-inflammatory activities of glaucine: *In vitro* studies in human airway smooth muscle and polymorphonuclear leukocytes". British journal of pharmacology, 1999; 127 (7): 1641-1651.
12. Rühle, K.H., Criscuolo, D., Dieterich, H.A., Köhler, D., Riedel, G: "Objective evaluation of dextromethorphan and glaucine as antitussive agents". British journal of clinical pharmacology, 1984;17 (5): 521-524.
13. Orallo, F., Fernández Alzueta, A., Campos-Toimil, M., Calleja, J.M: "Study of the *in vivo* and *in vitro* cardiovascular effects of (+)-glaucine and N- carbethoxysecoglaucine in rats". British journal of pharmacology, 1995; 114 (7): 1419-1427.
14. Zetler G: "Neuroleptic-like, anticonvulsant and antinociceptive effects of aporphine alkaloids: Bulbocapnine, corytuberine, boldine and glaucine". Archives internationales de pharmacodynamie et de therapie, 1988; 296: 255-281.
15. Ezhilan BP and Neelamegam R: GC-MS analysis of phytocomponents in the ethanol extract of *Polygonum chinense* L . Pharmacog. Res., 2012; 4(1): 11-14.
16. Sermakkani M and Thangapandian V: GC-MS analysis of *Cassia italica* leaf methanol extract. Asian Journal of Pharmaceutical and Clinical Research, 2012; 5(2): 90-94.
17. Venkata Raman B, Samuel La, Pardha Saradhi M, Narashimha Rao B, Naga Vamsi Krishna A, Sudhakar M. and Radhakrishnan TM: Antibacterial, antioxidant activity and GC-MS analysis of *Eupatorium odoratum*. The Useful Plants of India, NISCAIR, New Delhi, 5th ed. 2012: 23.
18. Velanganni J and Kadamban D: Phytoconstituents of ethanol extract of *Mallotus philippensis* (Lam.) Mull. Arg.var. philippensis (Euphorbiaceae). Int.J.of.pharm.re. and Dev., 2011; 3(8):73-76.
19. Sridharan S, Meenaa V, Kavitha V and Agnel Arul John N: GC-MS study and phytochemical profiling of *Mimosa pudica* Linn. J.Pharm. Res., 2011; 4(3): 741-742.
20. Mohan VR, Sudha T and Chidambarampillai S: GC-MS analysis of bioactive components of aerial parts of *Kirganelia reticulata* Poir. (Euphorbiaceae). J. Curr. Chem. Pharm. Sc., 2013; 3(2): 113-122.
21. Sudha T, Chidambarampillai S and Mohan VR: GC-MS Analysis of Bioactive components of aerial parts of *Fluggea leucopyrus* Willd. (Euphorbiaceae). Journal of Applied Pharmaceutical Science, 2013; 3(5): 126-130.
22. Fa-Rong Yu, Xiu-Zhen Lian, Hong-Yun Guo, Peter M, McGuire, Ren-De Li, Rui Wang, Fa-Hong Yu: Isolation and characterization of methyl esters and derivatives from *Euphorbia kansui* (Euphorbiaceae) and their inhibitory effects on the human SGC- 7901 cells. J Pharm Pharmaceut Sci., 2005; 8(3): 528-535.
23. Sivasubramanian R and Brindha P: *In vitro* cytotoxic, antioxidant and GC-MS studies on *Centratherum punctatum* cass. Int. J .Pharm .Pharm. Sci., 2013; 5(3): 364-367.
24. Carretero ME, López-Pérez JL, Abad MJ, Bermejo P, Tillet S, Israel A Noguera-PB: Preliminary study of the anti-inflammatory activity of hexane extract and fractions from *Bursera simaruba* (Linneo) Sarg. (Burseraceae) leaves. J. Ethnopharmacol., 2008; 116:11.
25. Mendiola JA, Santoyo S, Cifuentes A, Reglero G, Ibáñez E and Señoráns FJ: Antimicrobial activity of sub-and supercritical CO_2 extracts of the green alga *Dunaliella salina*, J. Food Prot., 2008; 71: 2138.
26. Babalola OO, Ojo OE, Oloyede FA: Hepato protective activity of aqueous extract of the leaves of *Hyptis suaveolens* (l.) Poit on acetaminophen induced hepato toxicity in rabbits. Research Journal of Chemical Sciences, 2011; 1(7): 85-88.
27. Singh HB and Handique AK: Antifungal activity of the essential oil of *Hyptis suaveolens* and its efficacy in bio control measures in combination with *Trichoderma harzianum*. 2007; 9: 683-687.
28. Venugopal G, Jwala Mounika S, Sowjanya K, Rao T, Krishna Chakravarthy MSR, Allu Prasada Rao R: GC-MS analysis and *in silico* molecular docking studies of mosquito repellent compounds from *Hyptis suaveolens L.* International Journal of Bioassays, 2012; 01 (09): 36-41.
29. http://toxnet.nlm.nih.gov/: 540-97-6.
30. Anandhi G, Pragasam A, Prakash Yoganandam G: Characterization of the methanolic extract of leaves of *Tricalysia sphaerocarpa* (Dalzell Ex Hook.F.) Gamble by GC-MS. International Journal of Phytopharmacy Research, 2014; 5 (1): 53-56.
31. De M, De AK, Sen P, Banerjee AB: "Antimicrobial properties of star anise (*Illicium verum* Hook f)". Phytother Res., 2002; 16 (1): 94-95.
32. Camurça-Vasconcelos AL, Bevilaqua CM, Morais SM, Maciel MV, Costa CT, Macedo IT, Oliveira LM, Braga RR, Silva RA and Vieira LS: "Anthelmintic activity of *Croton zehntneri* and *Lippia sidoides* essential oils". Vet. Parasitol., 2007; 148 (3-4):288-294.
33. Oka Y, Nacar S, Putievsky E, Ravid U, Yaniv Z and Spiegel Y: "Nematicidal activity of essential oils and their components against the root-knot nematode". Phytopathology, 2000; 90 (7): 710-715.
34. WHO, Food Additives Series 28 (717). International Program on Chemical Safety (IPCS) 1998.
35. Geetha DH, Rajeswari M, Indhiramuthu Jayashree: Chemical profiling of *Elaeocarpus serratus* L. by GC-MS. Asian Pacific Journal of Tropical Biomedicine, 2013; 3(12): 985-987.
36. Santhosh Kumar S, Samydurai P, Ramakrishnan R and Nagarajan N: GC-MS analysis of bioactive constituents of *Adiantum capillus-veneris* L. International Journal of Pharmacy and Pharmaceutical Sciences, 2014; 6(4): 60-64.

How to cite this article:
Jayashree I, Geetha DH and Rajeswari M: GC-MS Analysis of Bioactive Constituents of *Glochidion Ellipticum* Wt. Int J Pharm Sci Res 2015; 6(6): 2546-50.doi: 10.13040/IJPSR.0975-8232.6(6).2546-50.