WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 7, Issue 3, 842-848. Research Article ISSN 2277– 7105

EVALUATION OF PHYTO COMPOUNDS FROM PETROLEUM ETHER EXTRACT OF *ALTRNANTHERA SESSILIS* (L.) DC. BY USING GC-HRMS TECHNIQUES

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Article Received on
06 Dec. 2017,

Revised on 27 Dec. 2017,
Accepted on 17 Jan. 2018

DOI: 10.20959/wjpr20183-10854

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ABSTRACT

Alternanthera sessilis(L.) DC., belong to family Amaranthaceae occupy an important place in Traditional Siddha Medicine literatures. 'Food as Medicine' is one of the basic concepts of Traditional Siddha Indian Medicine. The today's term Nutraceuticals can be used alternative for Siddha medicine. This plant is used to cure various diseases and being widely used for cooking by people of Southern states of India. Oils and fats are the principle stored forms of energy in many plants and contributing essential part of daily diet. Present study deals with evaluation of phyto compounds from petroleum ether extract of *A.sessilis* leaf by using GC-HRMS technique. Result showed that presence of seventeen compounds including Geranylgeraniol (MW. 290), 2-Bromotetradecane (MW. 276), Cholestan-3-ol (MW. 400), Vitamin E (MW. 430), 1-Heptatriacotanol(MW. 536).

KEYWORDS: *Alternanthera sessilis*, GC-HRMS, Nutraceuticals, Traditional Siddha Medicine.

INTRODUCTION

Due to high side effects of modern medications more awareness has been created towards Nutraceuticals and Traditional Indian Medicine for Healthier life. 'Nutraceutical' the term is designed as a food or parts of food that provide medical or health benefits, including the prevention and treatment of disease (De Felice, 1992) this term is similar to concepts of Traditional Siddha Indian Medicine. The Siddha system of medicine is also one among the precious Medical systems, native to Tamilnadu, Siddha Treatment principles for ultimate

cure to both mind and body systems. Diet and lifestyle play a major role not only in maintaining health but also in curing diseases, 'Food as Medicine' is one of the basic concepts of Traditional Siddha Indian Medicine (Walter *et.al.*, 2014). The herb, Alternanthera sessilis(L.) DC., belong to family Amaranthaceae is being widely used for cooking by people of Southern states of India (Chandrika *et.al.*, 2006). It has appreciable amount of Proteins, carbohydrates, fats and moisture content, many phytochemical were found to present in leaves of this plant (Umate and Marathe, 2017). it is used as antidote for snake bite, scorpion sting and also in skin diseases (Nadkarni, 2000). It possess anti-microbial, wound healing, anti-oxidant, hepato-protective, anti- Ulcer, anti-diarrhoeal and anti- inflammatory activity (Johnson *et.al.*, 2010; Jalalpure *et.al.*, 2008; Borah *et.al.*, 2011; Song-Chow Lin *et.al.*, 2006; Roy and Saraf, 2008; Yadav and Das, 2013; Subhashini *et.al.*, 2010). It has been used in different drug formulation in treatment of about 96 eye diseases, tuberculosis, anaemia, leprosy etc. (Murugesa, 1988; Hakim, 2006). *A.sessilis* has significant medical application and used as wild edible hence in approaching its nutraceutical properties, present study has focused on GC-HRMS (Gas Chromatography with High Resolution Mass Spectrometer) analysis of ether extract from leaves of this plant. GC-HRMS analysis is the one of important step towards understanding the nature of active principles in medicinal plants.

MATERIALS AND METHODS

Collection and identification of plant material

The Leaf of *A.sessilis* was collected from nearby areas of Nanded, Maharashtra from July to August 2016, identified at PG Dept. of Botany NES Science College Nanded by using standard flora (Naik, 1998). It was properly shade dried in an airy place, crushed, powdered and stored in sealed dry glass container.

Preparation of extract

Dried powder of leaves extracted with petroleum ether at 50^0C in soxhlet apparatus for about 8 Hrs. The extract was then concentrated and evaporated with rota evaporator. After evaporation the extract is kept in small vials at freezing temperature until its use.

GC-HRMS analysis

Crude petroleum ether extract of *A. sessilis* leaves were used for GC-HRMS investigation. The GC-MS analysis was carried out using a Agilent, 7890, FID detector, Head Space injector Combipal autosampler Gas Chromatograph equipped and coupled to The Jeol mass spectrometer (Model: Accu TOF GCV). Split ratio was 10: 1. Column temperature program:

Initial temperature 120^0C for 3 min. Ramp: 8^0C/min to 270^0C. Again isothermal for 3 min, then ramp at 10^0C/min. to 280^0C, isothermal for 12 min., Column used was HP5, The injector temperature was 200^0C, detector temperature was 280^0C. Helium was used as carrier gas at 1 mL/min. Mass spectral scan range was set at 10 - 2000 amu, Mass resolution – 6000. A gas chromatograph coupled with mass spectrometer (GC-HRMS) is a combined analyzer that has a superior ability in analysing organic compounds qualitatively and quantitatively. It inherits the features of high resolution and accurate mass measurement with sample operation and high sensitivity. 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. (SAIF IITB).

RESULT AND DISCUSSION

Percentage of ether Extract from leaf of *A. sessilis*

Leaves of *A.sessilis* were extracted in petroleum ether and extract percentage found to be 3.53%. Petroleum ether extract contains lipids, fatty acids, sterols, steroid components of plant. Oils and fats are the principle stored forms of energy in many plants and contributing essential part of daily diet also.

GC-MS analysis in ether extract of leaf of *A.sessilis*

Ether extract of *A.sessilis* leaves was investigated by GC-HRMS and results showed the presence of about seventeen different phyto-compounds. Using computer searches on a NIIST MS data library and comparing the spectrum obtained through GC-HRMS (Figure 1 and 2), major compounds present in the plants extract were identified (Table No.1) as Phytol (MW. 296), Oxalic acid allyl nonyl ester (MW. 256), Hexadecane (MW. 226), 3-Eicosene (MW. 280), 1-Docosene (MW.308), Geranylgeraniol (MW. 290), 2-Bromotetradecane (MW. 276), Cholestan-3-ol (MW. 400), Vitamin E (MW. 430), 1- Heptatriacotanol(MW. 536) etc. Many of the compounds identified have medicinal properties like anti-inflammatory, anti-cancerous, hepato-protective activity etc. Based on studies, some of the constituents revealed by GC–HRMS Like Vitamin E, a biologically active nutraceutical compound shows analgesic, anti-diabatic, anti-inflammatory, antioxidant, anti-dermatitic, anti-leukemic, antitumor, anticancer, hepato-protective, antispasmodic activities (Combs 1992), 1-Heptatriacotanol (MW. 536) shows antimicrobial activity (Kumaradevan *et.al*., 2015), Hexadecane is found in allspice and isolated from oil of *Piper longum* (HMDB),

Geranylgeraniol is an intermediate compound in biosynthesis of vitamin A and K, Phytol was proven to exhibit antioxidant and it is also precursor of synthetic vitamin E and vitamin K (Inoue *et.al.,* 2005). It was found to be cytotoxic against breast cancer cell lines -MCF7 (Sheeja *et.al.,* 2016). The presence of various bioactive compounds justified the use of *A.sessilis* for various ailments by traditional practitioners. Thus it is recommended for use as Nutraceutical and for Siddha Traditional medicine. However further studies will need to be undertaken to ascertain its bioactivity fully.

Table 1: GC-HRMS profile for petroleum extract of *A.sessilis.*

Sr.No.	Name of Compound	Molecular Formula	Molecular weight	Estimated non-polar Retension index (iu)
1	Oxalic acid, allyl nonyl ester	$C_{14}H_{24}O_4$	256	1738
2	Hexadecane	$C_{16}H_{34}$	226	1612
3	3- Tetradecane	$C_{14}H_{28}$	196	1421
4	Oxalic acid, allyl tridecyl ester	$C_{18}H_{32}O_4$	312	2135
5	Oxirane, tetradecyl(hexadecane epoxide)	$C_{16}H_{32}O$	240	1702
6	Phytol	$C_{20}H_{40}O$	296	2045
7	Sulfurous acid, hexyl pentadecyl ester	$C_{21}H_{44}O_3S$	376	2732
8	3- Eicosene, [E]	$C_{20}H_{40}$	280	2017
9	Nonadecane, 2-methyl	$C_{20}H_{42}$	282	1945
10	Eicosane, 2- methyl	$C_{21}H_{44}$	296	2045
11	1-Docosene	$C_{22}H_{44}$	308	2198
12	Geranylgeraniol	$C_{20}H_{34}O$	290	2192
13	2- Bromotetradecane	$C_{14}H_{29}Br$	276	1645
14	Cholestan-3-ol, 2-methylene	$C_{28}H_{48}O$	400	2652
15	Vitamin E (a-tocopherol)	$C_{29}H_{50}O_6$	430	3112
16	tert-Hexadecanethiol	$C_{16}H_{34}S$	258	1831
17	1-Heptatriacotanol	$C_{37}H_{76}O$	536	3942

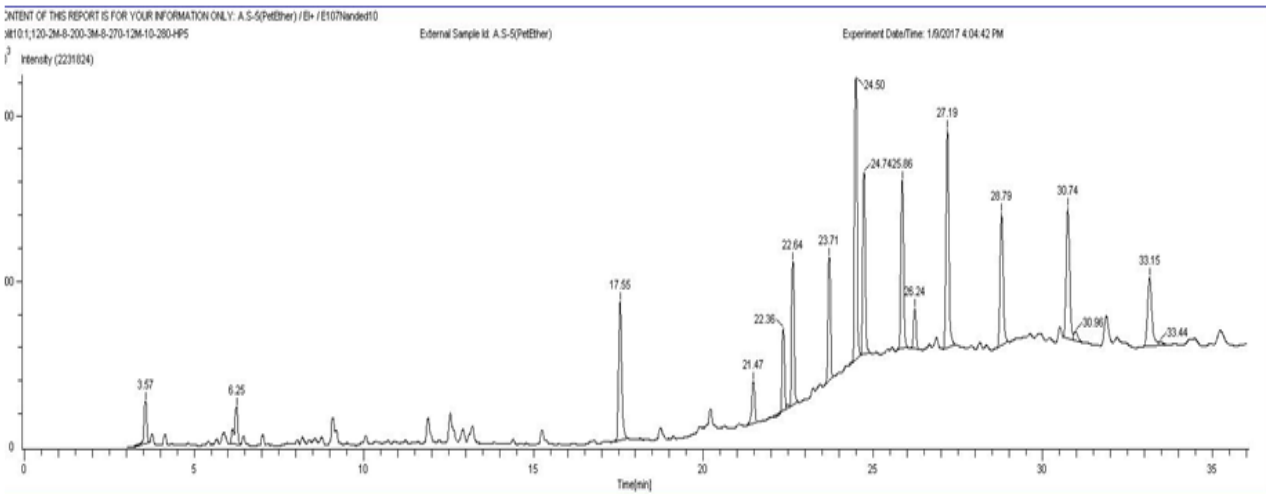

Figure 1: GCMS Chromatogrm.

Peak#	Time [min]	Type	Peak Width(FWHM) [min]	Area [Intens. * sec]	Height	Description	Start Point		End Point	
							Time[min]	Height	Time[min]	Height
1	3.57	BB	0.0882	1471654.01	253939.58		3.23	2737	3.66	27653
2	6.25	BB	0.0896	1679648.99	226166.52		6.06	19822	6.38	14867
3	17.55	BB	0.0986	5350605.04	834738.93		17.42	36835	17.78	52547
4	21.47	BB	0.0943	1598764.78	251949.51		21.26	126936	21.62	152312
5	22.01	BV	0.0751	21156.54	2335.66		21.83	159485	22.09	188522
6	22.36	VV	0.0868	2508512.24	497317.96		22.09	188522	22.44	227235
7	22.50	VB	0.0913	107422.89	20110.28		22.44	227235	22.54	239148
8	22.64	BB	0.0875	4695944.23	869876.65		22.54	239148	22.80	271816
9	23.71	BB	0.0880	4002886.30	741240.89		23.55	369591	23.84	428553
10	24.50	BB	0.0878	9201234.82	1700006.30		24.29	491095	24.64	559402
11	24.74	BB	0.0882	5970161.37	1093895.35		24.64	559402	24.91	570868
12	25.86	BB	0.0901	5702728.38	1012280.77		25.71	587431	26.02	604125
13	26.24	BB	0.0893	1365080.45	247312.34		26.09	597262	26.42	587828
14	27.19	BB	0.0931	7814159.06	1313961.93		27.07	592240	27.40	619700
15	28.79	BB	0.0990	5060227.55	797229.12		28.65	598061	28.97	638486
16	30.74	BV	0.1121	5676512.92	791349.05		30.60	661511	30.90	644756
17	30.96	VB	0.1343	481322.03	58099.66		30.90	644756	31.18	629119

Figure 2: GCMS Chromatogram table for corresponding time and peak areas.

CONCLUSION

In the present study 17 compounds from the ether extract of *A.sessilis* leaves were identified by Gas-chromatography with High Resolution Mass spectrometry (GC-HRMS) analysis. Most of the identified phyto-components used for antimicrobial, antioxidant, anticancerous activities. The research findings have shown that the ether extract is extensively rich in secondary metabolites, terpenes and fat soluble vitamins. These findings have provided scientific basis to the ethno medical usage of the plant. However, isolation of the individual phytochemical constituents, subjecting it to biological activity and toxicity profile will give fruitful results. This plant can therefore be explored for further research studies based on isolation of bioactive components.

ACKNOWLEDGEMENTS

The authors are thankful to SAIF, IIT Bombay for GC-HRMS facility and their cooperation.

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