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CHEMICAL PROFILING OF ENDEMIC PLANT *Osbeckia wynaadensis* CLARKE. BY GAS CHROMATOGRAPHY –MASS SPECTROMETRIC METHOD

M.S. SIMI[a1] AND C.N. SUNIL[b]

[ab]Research Department of Botany, S.N.M. College Maliankara, Moothakunnam P O, India

ABSTRACT

Plant kingdom is a treasure house of potential drugs. The medicinal value of these plants lie in some chemically active substances, which may help in the protection against incurable diseases. However only very few of these plant species have found to be used in medicine and little or no literature exit on their chemical and biological activities. *Osbeckia wynaadensis* is underutilized herbal medicinal plant, restricted in Southern Western Ghats. This study revealed the presence of 21 phytocomponents. Among these Methyl 3β-hydroxyolean-18-en-28-oate is major bioactive compound. Isolation of individual components would however, help to find new drugs.

KEYWORDS: GC-MS, Methyl 3β-hydroxyolean-18-en-28-oate, NIST, *Osbeckia wynaadensis.*

In recent years several new diseases has been arised due to our undisciplined life styles and activities. Scientific world are focusing about its remedies. The plants have valuable source of natural products for maintaining human health. The medicinal properties of plants are due to the accumulation of complex bioactive compounds in different plant parts. Screening of active compounds from the plants has leads to the invention of new medicinal drugs, which has efficient property for the treatment of various diseases. GC-MS is the best technique to identify the bioactive constituents present in the plants and it is the powerful tool for screening, identification, quantification of many phytocompounds present in plant extracts. It is the valuable method for non-polar and volatile components.

However only very few of these plant species have found to be used in medicine and little or no literature exit on their chemical and biological activities. Traditionally different species of *Osbeckia* used in different medicinal purpose while the species *Osbeckia wynaadensis* Clarke.(Melastomataceae) is an underutilized herbal medicinal plant, rare, endemic to Southern Western Ghats. It is a wild plant usually seen marshy area at about 4500ft above the sea level. The plant is used to cure vitiated pitta, inflammation, urinary tract infection, hemorrhage, menorrhagia, hemorrhoids and leucorrhea. Antioxidant, cytotoxicity and antimicrobial activity of plant extracts was reported by Mujeeb et al., 2014 and Sujina et al.,2012. Tribal peoples in Nilagiries Kurumbas and Paniyas are consuming its raw fruits (Ramanchandran et al., 2012). Flowers and fruits are used for the preparation of dye by Kani tribals of Ponmudi hills (Bosco et al., 2015).There is no previous report for the chemical characterization of bioactive components present in plant. To consider its medicinal importance, the ethanol extract of *Osbeckia wynaadensis* were analyzed in this study by GC-MS analysis.

MATERIALS AND METHODS

Collection of Plant Material

The plant material *Osbeckia wynaadensis* was collected from Mayiladumpara Malakkapara (N-10º15.700'.E-076º52.958') in the month of March 2015. Plant was Taxonomically identified and authenticated.

Preparation of Plant Extract

The whole plant was cleaned, shade dried and powered in a mechanical grinder.100gm of powered sample were extracted with ethanol (500ml,24 h) at temperature between 60ºC by using Soxhlet apparatus. The extracts were collected and the solvent was evaporated by using rotary evaporator and stored in refrigerator for further study. The required quantity of final residues was then subjected to GC-MS analysis.

GC-MS Analysis

The experiment was carried out in the sophisticated instrumentation facility, CARe KERALAM, Koratty Thrissur. GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: The column (HP5) was fused silica 50 m×0.25mm I.D. Analysis conditions were 20 minutes at 100ºC, 3 minutes at 235ºC for injector temperature, helium was the carrier gas and split ratio was 5:4. The sample (1µl) was evaporated in a split less injector at 300ºC.Run time was 22 minutes. Interpretation on mass spectrum GC-MS was done by using the data base of National Institute of Standard and Technology (NIST).

[1]Corresponding Author

The spectrum of unknown component was compared with the spectrum of the known components stored in the NIST library.The name, molecular weight and structure of the components of the test material were identified and recorded.

RESULTS AND DISCUSSION

The GC-MS chromatogram of ethanol extract of *Osbeckia wynaadensis* showed the presence of the Twenty one active principle compounds are given in Figure 1. Major phytocomponents present along with molecular formula, molecular weight (MW),retention time (R T),and concentration (peak area%) were presented in the table 1. Methyl 3β-hydroxyolean-18-en-28-oate (61.99%), Urs-12-en-28-oic acid 3-hydroxy-methylester, (3β) (13.17%), Acetic acid, 17 (1, 5 dimthylhexyl) 4, 4, 10, 13, 14, penta methyl 1, 2, 3, 4, 5, 8, 10, 12, 13, 14, 15, 16, 17 dodecahydro-1H- (5.106%), β sitosterol (3.61%), Ethane, 1, 1-diethoxy (3.65%), Chenodiol (3.16%), Hexadecanoic acid ethyl ester (3.13%) are major phytochemical components. Major phytocomponents and their biological activities are listed in Table 2. The biological activities listed are based on Dr. Duke's phytochemical and ehanobotanical Databases created by Dr.Jim Duke of the Agricultural Research service/USDA.

Methyl 3β-hydroxyolean-18-en-28-oate (61.99%) (Methyl morolate) is most abundant compound and is morolic acid ester derivative. It is simple precursor of morolic acid has various pharmacological properties such as cytotoxic, anti-HIV, Anti- diabetogenic, Anti- tumoral, Antiulcer and Anti-inflamatory activities.(Ito et al 2001;Rios et al 2001). This compound was first identified from outer bark of Ecalyptus grandis χ globules.(Patinha 2011;Patinha et al., 2013).

Urs-13-en-28-oic acid, 3-hydroxy-methyl ester,(3β)(methyl ursolate) is Ursolic acid ester derivatives; found in apple peels, have several biological activities such as antibacterial, anti HIV(Kashiwada et al.,2000), anti diabetic (Zhang et al., 2006; Jang et al., 2010) and anti inflammatory activity (Tatiana et al., 2014). It has cytotoxic activity in several cell lines (Ana, 2012; Lin et al., 1988) Similar compound (13.17%) is observed in ethanol leaf extract of *Osbeckia wynnadensis*. Sujana et al., 2012 reported that *Osbeckia wynaadensis* showed activity against human cervical cancer cell line (HeLa),mouse embryonic fibroblasts cell line (NIH 3T3) and murine embryonic fibroblast cell line. Antioxidant and antimicrobial activity of this plant was also reported by Mujeeb et al., 2014. But there is a gap in chemical characterization of *Osbeckia wynnadensis* species till the present study. Present report correlates along with the above biological activities. Aceticacid, 17-(1,5-dimethyl-hexyl)-4, 4, 10, 13, 14-pentamethyl-2, 3, 4, 5, 8, 10, 12, 13, 14, 15, 16, 17-dodecahydro-1H-is a steroid (5.1%) observed and has Anti- inflammatory, anti asthmatics, Antipsoriatics, antioxidants properties also reported in Vilvam plant (Ariharan,2015).

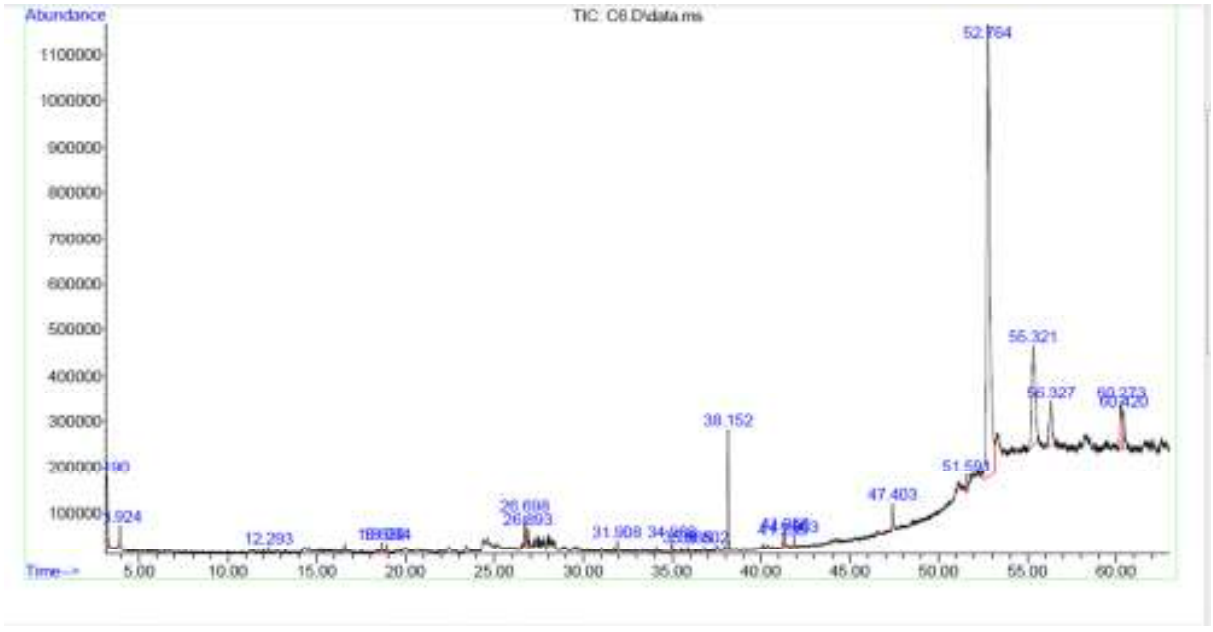

Figure 1: GC –MS Chromatogram of ethanol leaf extract of *Osbeckia wynaadensis*

Table 1: Components detected in the leaf extract of *Osbeckia wynaadensis*

No	Name of the compound	RT	Molecular formula	MW (gm/mol)	Peak area%
1	Ethane,1,1-diethoxy	3.19	$C_6H_{14}O_2$	118.18	3.65
2	Silane,diethoxydimethyl	3.92	$C_6H_{16}O_2Si$	148.27	1.37
3	Carbonic acid, decyl propyl ester	12.29	$C_{14}H_{28}O_3$	244.37	0.19
4	Azulene	18.63	$C_{10}H_8$	128.17	0.29
5	1-Dodecanol	18.93	$C_{12}H_{26}O$	186.34	0.22
6	Benzeneethanamine,2,5dimethoxy-α methyl-	26.69	$C_{11}H_{17}NO_2$	195.25	0.29
7	2-Hydroxy-4-methoxy-7-methyl-7,8,9,10,11,12,13,14-octahydro-6-oxabenzocyclododecen-5-one	26.89	$C_{17}H_{24}O_4$	324.50	0.47
8	Dodecyl acrylate	31.90	$C_{15}H_{28}O_2$	240.387	0.33
9	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	34.98	$C_{20}H_{40}O$	296.53	0.33
10	Hexadecanoic acid,methyl ester	36.80	$C_{17}H_{34}O_2$	270.45	0.11
11	Hexadecanoic acid,ethyl ester	38.15	$C_{18}H_{36}O_3$	284	3.13
12	Methyl6,9-octadecadienoate	41.23	$C_{19}H_{34}O_2$	294.47	0.28
13	Ethyl Oleate	41.35	$C_{20}H_{38}O_4$	310.52	0.37
14	Octadecanoic acid,ethyl ester	41.86	$C_{20}H_{40}O_2$	312.53	0.34
15	1,2-Benzenedicarboxylic acid,diisooctyl ester	47.40	$C_{24}H_{38}O_4$	390.55	0.41
16	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carbohxaldehyde	51.59	$C_{23}H_{32}$	324.50	0.89
17	Methyl 3β-hydroxyolean-18-en-28-oate	52.76	$C_{33}H_{50}O_3$	470.72	61.9
18	Urs-12-en-28-oic acid,3-hydroxy-,methyl ester,(3β)	55.32	$C_{31}H_{50}O_3$	470.72	13.17
19	Aceticacid,17-(1,5-dimethyl-hexyl)-4,4,10,13,14-pentamethyl-2,3,4,5,8,10,12,13,14,15,16,17-dodecahydro-1H-	56.32	$C_{24}H_{42}O_4$	430.61	5.10
20	β-sitosterol	60.27	$C_{29}H_{50}O$	414.71	3.612
21	Chenodiol	60.42	$C_{24}H_{40}O_4$	392.57	3.164

Table 2: Major compounds and its Biological activities of Ethanol leaf extracts of *Osbeckia wynaadensis*

No	Compound	Peak area%	Compound nature	Biological activity
1	Methyl3β-hydroxyolean-18-en-28-oate	61.99	Triterpene	Antidiabetogenic activity, cytotoxic activity, Antitumoral, Antiulcer, Anti-inflammatory.
2	Urs-12-en-28-oic acid,3-hydroxy-methyl ester,(3β)	13.17	Triterpene	Cytotoxic activity, Antiulcer, Anti-inflammatory, Antiviral
3	Aceticacid,17-(1,5-dimethyl-hexyl)-4,4,10,13,14-pentamethyl-2,3,4,5,8,10,12,13,14,15,16,17-dodecahydro-1H-	5.10	Steroid	Anti- inflammatory, anti asthmatics, Antipsoriatics, For dermatological disorders, antioxidants
4	Ethane,1,1-diethoxy	3.65	Stearyl alcohol	Flavoring agent
5	β-Sitosterol	3.61	Steroid	Anti-inflammatory, Anti-pyretic, Analgesic, Anti-tumoral, Antiulcer, used for Tuberculosis treatment, Cholesterol treatment.
6	Chenodiol	3.16	Steroid	Dissolving Gallstones
7	Hexadecanoic acid, ethyl ester	3.13	Palmitic acid ester	Antioxidant, Hypocholesterolemic, Nematicide, pesticide, Lubricant, Antiandrogenic,Flavour,Hemolytic,5-Alpha reductase inhibitor

β-sitosterol is the most common phytosterol found in wood and higher plants; used as an antioxidant and antidiabetic agent (Karan et al.,2012). It is also considered to be highly effective in the treatment of prostate enlargement (Dufoour, 1984), to boost the function of T cells and primes the immune system functions (Bouic et al., 1996). Human liver microsomes studies show that β-sitosterol inhibits the cholesterol absorption (shefer et al., 1988). It also shown antifertility (Malini and Vanithakumari, 1991; Nema et al., 2011), anti-inflammatory and antipyretic activity (Gupta et al., 1980). Similar compound was observed in various plants,bsuch as leaves of *Ocimum sanctum* (Rahman et al., 2009), rhizomes of the *Stylochiton lancifolius* (Pateh et al.,2009),fruits of *Corylus colurna* Linn (Akhtar et al.,2010) *Solanum xanthocarpum* (Khanam and Sultana,2012) and from *Momordica charantia* (Amit et al., 2012). This compound 3.61% is identified in *Osbeckia wynaadensis*. Hexadecanoic acid, (3.13%) found showing antioxidant, antiandrogenic, hypochloesterolemic activities and used as a nematicide and pesticide. This compound was reported as a component in *Cassia italic* leaf (Sermakkani, 2011) and *Neolamarckia cadamba*, leaf (Mohamed 2014).

One of the interesting compound observed (3.16%) is Chenodeoxycholic acid (Chenodiol), is reported as a bile acid and it reduces cholesterol secretion in bile. It has anticholelithic action. Chenodiol reduce gall stones size and number (Johnson et al., 1973).

GC MS analysis is the direct and fast analytical method for the identification of bioactive component present in plant extract and it is the first step towards understanding the nature of active principles in the medicinal plants. This study will helpful for further study. Further studies on biological activities of the species are going on in our laboratory. In the present study 21 bioactive compounds are identified, most of them are high value terpenoids. The presence of such compounds justified that plant,*Osbeckia wynaadensis* is pharmaceutically important. Further studies are needed to isolate compounds from plants; it may create a new way to treat many incurable diseases.

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