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Research Article

GC-MS Analysis of Phytocomponents in the Leaves of *Actinodaphne madraspatana* Bedd

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ABSTRACT

This study was designed to determine the phytocomponents in the methanol and ethanol extracts of leaves of *Actinodaphne madraspatana* Bedd by Perkin Elmer gas chromatography-mass spectrometry (GC-MS). This investigation was carried out to determine the possible phytocomponents from the methanol and ethanol extracts of leaves of *Actinodaphne madraspatana* by GC-MS. This analysis revealed that the methanol extract contained six compounds that have been identified mainly as sesquitriterpene lactone compounds and fatty acid ester compounds and ethanol extract contain twenty compounds that have been identified mainly as fatty acid ester, sesquiterpenes, triterpene and steroid. From the results, it is evident that methanol and ethanol extract of leaves of *Actinodaphne madraspatana* contains various bioactive compounds and is recommended as a plant of phytopharmaceutical importance.

Keywords: GC-MS analysis; Phytocomponents; *Actinodaphne madraspatana*; Leaves; Methanol and ethanol extracts.

INTRODUCTION

Actinodaphne madraspatana Bedd is belonging to the family Lauraceae. It is commonly known as 'Putta thali' in Tamil, 'Ray Laurel' in English, 'Irolimarom, Mungali' in Malayalam, 'Kovangutti' in Telugu (Gupta AK., 2004; Pullaiah, 2006; Wealth of India, 1985; Ram P Rastogi *et al*., 2001; Indian Journal of Chemistry, 1976). It is a medium-sized evergreen tree and Shrub, widely distributed common on the Rock Hill slopes at higher elevations, Aruku valley, Vishakhapatnam District, Talakona, Dharmagiri, Microwave station, on the way to Thumburu Theertham (Saldanha CJ *et al*., 1976; Gamble JS, 1967; Jain SK et al., 1976). Leaves are 4-6 in a whorl, 10-30 cm long, coriaceous, lanceolate, oblanceolate or elliptic. Flowers are small, dioecious, yellowish, the males in clusters of about 8, the females umbellate or sub-racemose on very stout peduncles. Berry 8 mm across, ellipsoid, red when ripe. The plant flowering and fruiting during January to July (Madhava Chetty K., 2008). The leaves of the plant are used traditionally to cure wounds, cure mania, fickle minded behavior and used for the treatment of diabetes (Pullaiah T *et al*., 2003; Pullaiah T., 2002). The present investigation was carried out to identify the possible phytocomponents present in the methanol and ethanol extracts of leaves of *Actinodaphne madraspatana* by GC-MS.

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MATERIALS AND METHODS

Collection and authentication of plant material

The leaves of *Actinodaphne madraspatana* were collected from Talakona forest near to Tirupathi and were authenticated by Dr. K. Madavachetty, S. V. University, Tirupati, Andhra Pradesh. A voucher specimen (ACD) has been kept in the Herbarium of the Department of Pharmaceutical Chemistry, Ratnam Institute of Pharmacy, Pidathapolur, Nellore, Andhra Pradesh, India-524346.

Preparation of sample

The leaves of plant samples were washed with tap water and dried under room temperature for three days. Approximately, about 140 gms of leaves were pulverized to powder in a mechanical grinder.

Extraction procedure

The dry powder of the leaves of *Actinodaphne madraspatana* (70 gms) were separately extracted with methanol and ethanol (500 ml, 46 hours) at temperature between 60-65°C by using Soxhlet extractor. The extracts were filtered in hot condition and concentrated in the vacuum under reduced pressure and dried in desiccators. The extracts contain both polar and non-polar components of the material and 0.2ul sample of the solution was employed in GC-MS for analysis of different compounds.

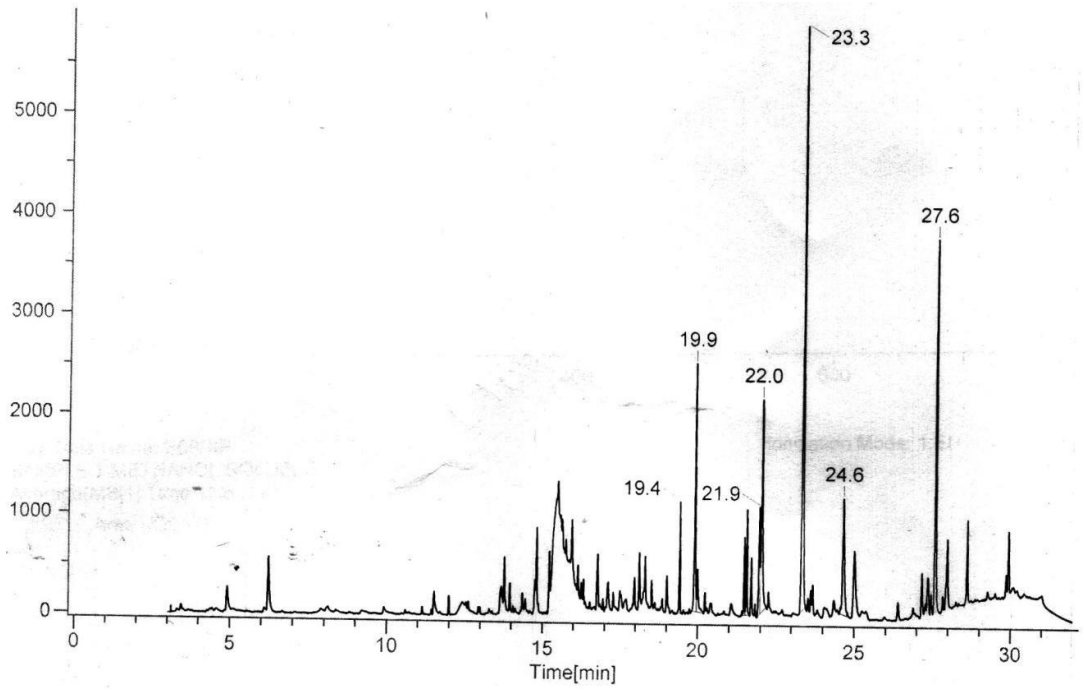

Figure 1: GC-MS chromatogram of methanol extract of leaves of *Actinodaphne madraspatana*

Table 1: Phytocomponents in methanol extract of *Actinodaphne madraspatana* by GC-MS

S.No	RT	Name of the compounds	Molecular formula	Molecular weight	% Peak area
01	19.4	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	2.50
02	19.9	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	6.99
03	21.6	9,12,15-Octadecatrienoic acid, methyl ester	$C_{19}H_{32}O_2$	292	2.74
04	23.3	Arglabin	$C_{15}H_{18}O_3$	246	20.04
05	24.6	α-Santonin	$C_{15}H_{18}O_3$	246	56.99
06	27.6	Estafiatin	$C_{15}H_{18}O_3$	246	10.73

Table 2: Phytochemical compounds, their nature and their biological activities of methanol extract of leaves of *Actinodaphne madraspatana*

S.No	RT	Name of the compounds	Compound Nature	Activity	Reference No's
01	19.4	Hexadecanoic acid, methyl ester	Fatty acid ester	Antioxidant, Flavor, Hypocholesterolemic, 5-α-reductase inhibitor	Sermakkani M *et al*., 2012; Kalpana Devi V *et al*., 2012
02	19.9	n-Hexadecanoic acid	Palmitic acid	Antibacterial, Antioxidant, Pesticide, Antitumor, Cancer preventive, Immunostimulant, Chemopreventive, Lipoxygenase inhibitor	Sermakkani M *et al*., 2012; Kalpana Devi V *et al*., 2012
03	21.6	9,12,15-Octadecatrienoic acid, methyl ester	Fatty acid ester	Anti-inflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Antiarthritic, Antihistaminic, 5-α-reductase inhibitor	Sermakkani M *et al*., 2012; Kalpana Devi V *et al*., 2012
04	23.3	Arglabin	Sesquiterpene lactone	Antitumor	Csuk R *et al*., 2012
05	24.6	α-Santonin	Sesquiterpene lactone	Antipyretic	Martin ML *et al*., 1988
06	27.6	Estafiatin	Sesquiterpene lactone	Anti-inflammatory, Antibacterial, Antihelmintic	Michael Heinrich., 2002

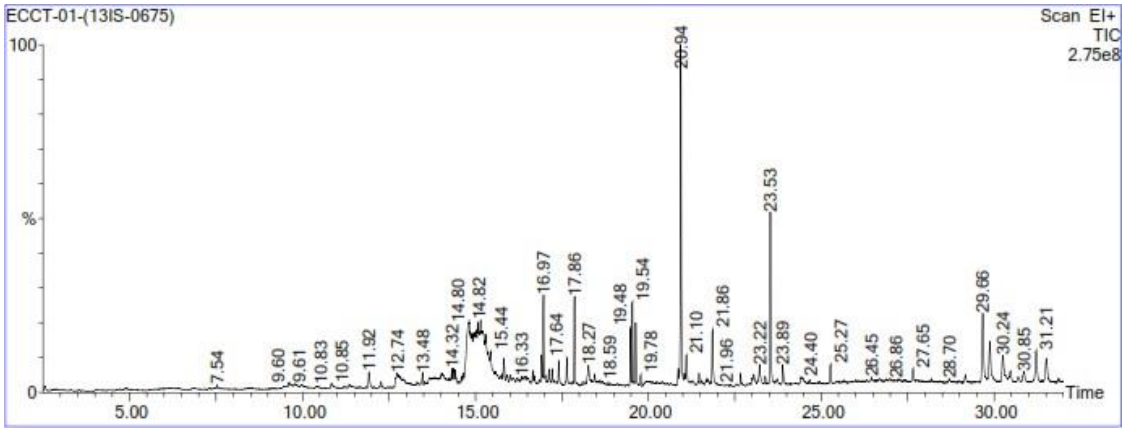

Figure 2: GC-MS chromatogram of ethanol extract of leaves of *Actinodaphne madraspatana*

Table 3: Phytocomponents in Ethanol extract of Actinodaphne madraspatana by GC-MS

S.No	RT	Name of the compounds	M.Formula	M. Wt	% Peak area
01	11.922	(2-methyl-prop-2-enyloxy)trimethyl silane	$C_7H_{16}OSi$	144	0.815
02	14.818	Dichloroacetic acid-2-ethylhexyl ester	$C_{10}H_{18}O_2Cl_2$	240	11.323
03	16.969	Z,Z-6,28-Heptatriactontadien-2-one	$C_{37}H_{70}O$	530	2.031
04	17.414	Butanoic acid-3-methyl-3,7-dimethyl-6-octenyl ester	$C_{15}H_{28}O_2$	240	0.783
05	17.650	Azulene-1,2,3,3A,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-[1R-(1α-3α, β-4ALP)]	$C_{15}H_{24}$	204	0.790
06	17.865	Tetradecanoic acid-10,13-dimethyl-methyl ester	$C_{17}H_{34}O_2$	270	2.303
07	19.48	Methyl-11,14-octadecadienoate	$C_{19}H_{34}O_2$	294	1.465
08	19.540	Methyl-11,14,17-eicosatrienoate	$C_{21}H_{36}O_2$	320	2.313
09	19.640	Z,Z-6,28-Heptatriactontadien-2-one	$C_{37}H_{70}O$	530	1.776
10	20.941	Chloranthalactone A	$C_{15}H_{16}O_2$	228	11.786
11	21.856	Shizukanolide	$C_{15}H_{18}O_2$	230	2.083
12	23.222	1,4-Cyclohexadiene-1-propanoic acid-3-(dichloromethyl)-3-methyl-6-oxo ethyl ester	$C_{13}H_{16}O_3Cl_2$	290	0.854
13	23.532	Boron-diethyl[3-imino-2-(1-iminoethyl)butanenitrilato-N_2,N_3]-(T-4)-	$C_{10}H_{18}N_3B$	191	5.896
14	25.268	2,6,10,14,18,22-Tetracosahexaene-2,6,10,15,19,23-hexamethyl-,(All-E)-	$C_{30}H_{50}$	410	0.580
15	27.649	2(3H)-Benzothiazolimine-3-methyl	$C_8H_8N_2S$	164	0.595
16	29.669	Pregnenolone	$C_{21}H_{32}O_2$	316	3.959
17	29.875	12-Oleanen-3-yl-acetate-(3-alpha.)-	$C_{32}H_{52}O_2$	468	2.330
18	30.250	4,4,6A,6B,8A,11,11,14B-Octamethyl-1,4,4A,5,6,6A,6B,7,8,8A,9,10,11,12,12A,14,14A,14B-octadecahydro-2H-picen-3-one	$C_{30}H_{48}O$	424	1.901
19	31.210	2R-Acetoxymethyl-1,3,3-trimethyl-4T-(3-methyl-2-buten-1-yl)-1T-cyclohexanol	$C_{17}H_{30}O_3$	282	2.008
20	31.505	Glaucyl alcohol	$C_{15}H_{24}O$	220	1.637

GC-MS analysis

The GC-MS analyses of extracts were performed using a Perkin Elmer GC-MS (Model Perkin Elmer Clarus 600, USA) equipped with a VF-5 MS fused silica capillary column (30 m x 0.25 mm i.d, film thickness 0.25µm). GC-MS spectroscopic detection, an electron ionization system with ionization energy of 70 eV was used. Pure helium gas was used as a carrier gas at a constant flow rate of 1 mL/min. Mass transfer line and injector temperatures were set at 250 °C. The oven temperature was programmed at 60 °C for 2 min then increased to 300 °C for 6.0 min at the rate of 10 °C/min. The samples were injected in split mode as 10:1.

Identification of phytocomponents

Interpretation on Mass-Spectrum GC-MS was carried out with reference to the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library.

Table 4: Phytocomponents, their nature and their biological activities of ethanol extract of leaves of *Actinodaphne madraspatana*

S.No	RT	Name of the compounds	Compound Nature	Activity	Reference No's
01	17.414	Butanoic acid-3-methyl-3,7-dimethyl-6-octenyl ester	Fatty acid ester	Antimicrobial	Thanga Krishna Kumari S *et al*., 2012
02	19.640	Z,Z-6,28-Heptatriactontadien-2-one	Fatty acid ester	Vasodilator	Mallika devi T *et al*., 2012
03	20.941	Chloranthalactone A	sesquiterpenes	anti-leishmanial	Acebey L *et al*., 2010
04	25.268	2,6,10,14,18,22-Tetracosahexaene-2,6,10,15,19,23-hexamethyl-,(All-E)-	Triterpene	Antibacterial, Antioxidant, Anti-tumer, Cancer preventive	Sudha T *et al*., 2013
05	29.669	Pregnenolone	Steroid	Anxiolytic	Melchior CL *et al*., 1994
06	29.875	12-Oleanen-3-yl-acetate-(3-alpha.)-	Triterpene	Antidiabetic, anti-inflammatory, Antioxidant	Oluwatoyin *et al*., 2012
07	30.250	4,4,6A,6B,8A,11,11,14B-Octamethyl-1,4,4A,5,6,6A,6B,7,8,8A,9,10,11,12,12A,14,14A,14B-octadecahydro-2H-picen-3-one	Triterpene	Antibacterial, Antioxidant, Anti-tumer, Cancer preventive	Duan DD *et al*., 2011

RESULTS AND DISCUSSION

The GC-MS analysis of phytocomponents in the leaves of *Actinodaphne madraspatana* revealed the presence of six phytocomponents in methanol extract and twenty phytocomponents in ethanol extract. The identification of the phytocomponents was confirmed based on the peak area, retention time, and molecular formula. The major phytocomponents in methanol extract found were shown in **Table 1** and **Figure 1** as Hexadecanoic acid, Methyl ester, n-Hexadecanoic acid, 9,12,15-Octadecatrienoic acid methyl ester, Arglabin, α-Santonin and Estafiatin. These phytocomponents are responsible for various pharmacological actions like anti-inflammatory, antibacterial, anti-helmintic activities for sesquiterpene lactones and anti-arthritic, pesticide, antitumor, cancer preventive, anti-histaminic, hepatoprotective, hypocholesterolemic and anti-inflammation activities for fatty acids ester. The results are presented in **Table 2**.

The major phytocomponets in ethanol extract found were shown in **Table 3** and **Figure 2** as (2-methyl-prop-2-enyloxy)trimethyl silane, Dichloroacetic acid-2-ethylhexyl ester, Z,Z-6,28-Heptatriactontadien-2-one, Butanoic acid-3-methyl-3,7-dimethyl-6-octenyl ester, Azulene-1,2,3,3A,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-[1R-(1α-3α, β-4ALP)], Tetradecanoic acid-10,13-dimethyl-methyl ester, Methyl-11,14-octadecadienoate, Methyl-11,14,17-eicosatrienoate, Z,Z-6,28-Heptatriactontadien-2-one, Chloranthalactone A, Shizukanolide, 1,4-Cyclohexadiene-1-propanoic acid-3-(dichloromethyl)-3-methyl-6-oxo ethyl ester, Boron-diethyl[3-imino-2-(1-iminoethyl) butanenitrilato-N_2,N_3]-(T-4)-, 2,6,10,14,18,22-Tetracosahexaene-2, 6, 10, 15,19,23 -hexamethyl-,(All-E)-, 2(3H)-benzothiazolimine-3-methyl, Pregnenolone, 12-Oleanen-3-yl-acetate-(3-alpha.)-, 4,4,6A,6B,8A,11,11,14B-Octamethyl-1,4,4A,5,6,6A,6B,7,8,8A,9,10, 11,12,12A, 14,14A,14B-octadecahydro-2H-picen-3-one, 2R-Acetoxymethyl-1,3,3-trimethyl-4T-(3-methyl-2-buten-1-yl)-1T-cyclohexanol and Glaucyl alcohol. These phytocomponents are responsible for various pharmacological actions like antibacterial, anti-inflammatory, antioxidant activities for triterpene and anti-microbial, vasodilator activities for fatty acids ester. The results are presented in **Table 4**.

CONCLUSION

GC-MS analysis of phytoconstituents in the methanol and ethanol extracts of leaves of *Actinodaphne madraspatana* was carried out. The methanol extract showed the presence six phytoconstituents and ethanol extract showed the presence of twenty phytoconstituents. The presence of various bioactive compounds justifies the use of the leaves for various ailments by traditional practitioners. However, isolation of individual phytochemical constituents and subjecting it to biological activity will definitely give fruitful results. It could be concluded that *Actinodaphne madraspatana* contains various bioactive compounds. So it is recommended as a plant of phytopharmaceutical importance.

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