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Bioactive constituents of *Actephila excelsa* (dalz.)muell.arg(euphorbiaceae)using GC MS: Traditional medicinal plant

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ABSTRACT

The present phytochemical components have been reported from the species of *Actephila excelsa*, Dalz. (Euphorbiceae). So far there are no reports exist on the phytoconstitutiets except two noble aromatic terpenoids, viz.Actephilol A and epiactephilol A of *Actephila excelsa*. The present study was discussed to determine the bioactive components in aerial parts of the methanol extract of *A.excelsa.* GC-MS analysis of aerial parts of *A.excelsa* was performed using GC-MS equipment, (Thermo scientific Co.) Thermo GC TRACE ultra ver, :5.0 Thermo M S DSQ II The investigation was carried out to determine the chemical constituents from *A.excelsa* by GC-MS technique. The analysis revealed that the seven components in methanolic extract of *A.excelsa, mainly* 4,7-Methano-1H-indene derivatives (35.90%),Methanone(1-hydroxycyclohexyl)phenyl(19.11%), 2,9-bis(2',6'dimethoxyphenyl)-1, 10-phenanthroline, Hexadecanoicacid,methylester; 1,2-Benzenedicarboxylicacid, diisooctylester; Cyclohexasiloxane, dodecamethyl ;Benzene acetic acid, à, 4-bis [(trimethylsilyl) oxy] - trimethylsilyl ester .This is the first report of the identification of active constituents from aerial parts of *A.excelsa* by GC-MS. All identified compounds were generally reported having antioxidant, cancer preventive, hypocholesterolemic, 5-alpha reductase inhibitor, hepato-protective and plasticizer No activity was reported in Methano-1H-indene derivative. From the results, it can be concluded that the presence of various bioactive compounds confirms the application of *A.excelsa* for several ailments by traditional practitioners. Nevertheless the isolation of individual phyto constituents may carry on predicting a novel drug.

Keywords: *Actephila excelsa,* Euphorbiaceae, GC-MS, Traditional medicinal plant.

1. INTRODUCTION

With a high value of traditional medicinal coordination, plants have greater contribution in health care [1-2]. There are number of sources of potentially useful compounds for the expansion of chemotherapeutic agents. One among the important sources is Traditional Medicine [3].According to the WHO, medicinal plants would be the best source to obtain a variety of drugs. And hence, for the better understanding of such plants, there is a need to investigate their properties, safety and efficacy [4]. Herbal medicines are safer than synthetic medicines because the phytochemicals in plant extract target the biochemical pathway .Medicinal plants have been used all over the world for the treatment and prevention of various ailments, particularly in developing countries where infectious disease are endemic and modern health facilities and services are inadequate[5]At present in India there are 20,000 medicinal plants but more than 500 traditional communities use about 800 plant species for curing different diseases. For human alleviation 80% of the world population depends on plant-derived medicine because of its smaller number side effects. Medicinal based drugs with added advantages (simple, effective) offer a broad spectrum of activity through greater emphasis on preventive action [6]. In USA and Canada, as a minimum of 25% issued prescription drugs have bioactive compounds that are derived from or modelled after plant natural products [7]. The WHO has also recommended the evaluation of the plant's effectiveness in order to lack safe modern drugs [8]. The family Euphorbiaceae consisting of one of the largest families of several important medicinal plants are very common and have about 300 genera and 7500 species. They have wide range of biolological activities and interesting number of phytochemical constituents.

Actephila excelsa (Dalz) Muller.Arg (Euphorbiaceac) is a shrub. It grows in temperate and tropical areas in the world. It is distributed in Western Peninsular India, ascending to an altitude of about 1200 m on rocky limestone hills, China, Bangladesh, Myanmar, Indonesia and Malaysia. In India it is commonly known as 'Lambonan'[9]. Leaves are obeveate or elliptic oblong, characterous petioles and leaf blades oblong lanceolate. Flowers are pinkish brown. Fruits are woody capsule subglobose 3 lobed. Leaves of the plant were used traditionally as internal digestive disorders of G.I tract, respiratory tract system disorders, heart-blood circulatory system disorders, urinary tract system disorders and skin disorders. The two novel epimeric aromatic terpenoids were reported from leaf and stems of *Actephila excelsa*[10].

In the recent years, Gas chromatography mass spectrometry GC-MS) has become firmly establish as a key technology platform for secondary metabolite profiling in both plant and non-plant species [11, 12 and 13]. A detailed literature review on the plant in investigation has shown that so far there is no publish reports worldwide related to phytoconstitutients of A.*excelsa* Dalz. So the present research study is aimed to recognize the phytoconstituents present in *A.excelsa* using GC-MS as there is a lack of elaborate published work by first preparing the methanolic extract of the compounds by subjecting it GC-Ms analysis of *A.excelsa*.

2. MATERIALS AND METHODS

2.1. Collection and Preparation of Plant Material

The fresh, healthy and disease free plant of *Actephila excelsa* (Dulz.) Muell. Arg was collected from the natural habitats of Tirunelveli District, Tamil Nadu, and India in the month of March, 2012, and authenticated by Dr.V.Chelladurai (Retired Research Officer), Botany (C.C.R.A.S) Govt. of India, Tamil Nadu, India. The samples were washed thoroughly in running tap water to remove soil particles and adhered debris and finally with sterile distilled water. The whole plants were shade dried and ground into fine powder. A voucher specimen (EM 600-2012-2013) has been preserved in the Department of Pharmacy,Annamalai Nagar, A.U. The samples were stored in air tight container for further use.

2.2. Preparation of plant extract

A portion of shade dried aerial parts about 120 g of *Actephila excelsa* Dalz. was placed in a soxhlet apparatus. The extraction was performed with 800ml of methanol for 48 hr at a temperature not exceeding the boiling point of the solvent. Extract was filtered through a 45µm filter.[14] The Resulting extract was concentrated in a vacuum to dryness in a rotary evaporator to give the methanolic extract (7.2g) The extract was stored in a refrigerator at 4^0C for further use.

2.3. Preliminary phytochemical screening

The methanolic extract was tested for alkaloid, phenols, tannins, flavonoids, terpenoids,[15]phlobatannin[16], reducing sugar[17] ,volatile oil[18], were present. And steroid, saponin, were absent (Table 1).

Table -1: Preliminary phytochemical screening of *A. excelsa.*

Phytoconstituents	Methanol extract of *Actphila excelsa*
Alkaloids	+
Tannins	+
Terpenoids	+
Carbohydrades	+
Steroids	-
Reducing sugars	+
Saponins	-
Flavonoids	+
Phlobatannin	+
Phenolic compounds	+

(+)= indicates presence; (-)= indicates absence

2.4. GC-MS Analysis

The phytochemical investigation of methanolic extract was employed on GC-MS equipment (Thermo Scientific Co.) Thermo GC-TRACE ultra ver:, 5.0 , Thermo MS DSQ II . The experimental conditions were employed of GC- MS system were as follows: TR 5 MS capillary standard non polar column with dimensions of the column 30 m× 0.25mm ID 0.25µm df .The oven temperature was programmed from 40^0 C raised to 250^0 C at 5 ^{0}C / min, at a constant flow rate of mobile phase (Carrier gas : Helium) was set aside for 1ml/min, and injection volume was 1µ l .

For Ms detection, Thermo MS DSQ II electron ionization mode with ionization energy 70 $_e$V and the sample was dissolved in chloroform and 1µl was injected at mass range of m/z 50-650 was employed and the results were compared by using Wiley Spectral library search programme.

3. RESULTS AND DISCUSSION

The phytochemical test showed the presence of alkaloid, phenols, tannins, flavonoids, terpenoids, reducing sugar, phlobatannin and volatile oil in methanolic extract of *A.excelsa* are illustrated in Table 1,The results pertaining to GC-MS analysis of methanol extract of *A. excelsa* (Dalz.) led to the identification of number of compounds. In the present study, the GC-MS analysis of methanol extract of *A. excelsa* showed the presence of seven compounds representing (83.78%). These compounds were identified through mass spectrometry attached with GC. The

results of the seven phytoconstituents were characterized and identified are tabulated in Table 2. The mass spectrometer analysis the compounds eluted at different retention time to identify the nature and structure of the compounds. The large compound fragments into small compounds giving raise to appearance of peaks at different m/z ratios. The results reveal the presence of seven major compounds representing (83.78%)viz. Methano-1H-indene,3a,4,5,6,7,7a-hexahydro-5-2-propenyloxy (35.90%), Methanone (1hydroxycyclohexyl), Phenyl(19.11%)2,9-bis(2',6'-dimethoxyphenyl)-1,10-phenanthroline(10.52%)Hexadecanoic acid,methylester(5.85.%),1,2benzenedicarboxylic acid,diisooctylester,(4.98%),Cyclohexasiloxane, dodecamethyl-(3.45%) Benzene acetic acid, à, 4-bis [(trimethylsilyl) oxy] - trimethylsilyl ester (3.97%).The GC-MS spectrum confirmed the presence of 7major components with their retention time 9.33, 14.33, 17.71, 20.56, 22.99, 29.01, and 37.96, respectively.(Figure 1).

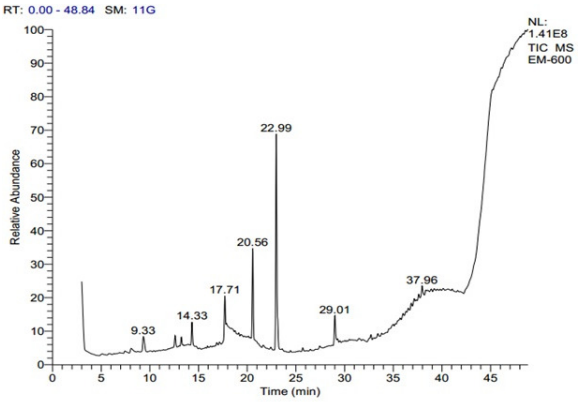

Figure - 1: GC-MS Chromatogram of methanolic extract of *A. excelsa.*

Table – 2: Compound identified in the methanolic extract of *A. excelsa* by GC-MS

RT	Name of the Compound	Molecular formula	Molecular weight	peak area %	Nature of compound
9.33	Benzeneacetic acid, à,4-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester (CAS)	C_1-$H_{32}O_4Si_3$	384	3.97	Ester
14.33	Cyclohexasiloxane, dodecamethyl-	$C_{12}H_{36}O_6Si_6$	444	3.45	essential oil
17.71	2,9-bis(2',6'-dimethoxyphenyl)-1,10-phenanthroline	$C_{16}H_{14}O$	150	10.52	essential oil
20.56	Methanone, (1-hydroxycyclohexyl)phenyl	$C_{13}H_{16}O_2$	204	19.11	ketone
22.99	Methano-1H-indene,3a,4,5,6,7,7a-hexahydro-5-2-propenyloxy	$C_{16}H_{22}O_3$	262	35.9	acrylic monomers
29.01	2-Ethylidene[1,3]dithiane	$C_6H_{10}S_2$	146	5.82	essential oil
37.96	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	4.98	DEHP,Phthalate derivative

4. CONCLUSION

The presence of various bioactive components detected after GC-MS analysis using methanol extract of *A.excelsa* justifies the make use of the aerial parts of plant for an assort of ailments by traditional practitioner. However, the individual bioactive components will be categorically give the fruitful results and will release a new area for pharmacological activity. The GC-MS analysis and detailed review of the plant in investigation has shown that so far there are no publish report worldwide related to the possible bioactive components of *A.excelsa*, Further investigation may help to determine the elucidation of structures and may lead to new chemical entity. From the results, it could be concluded that *A.excelsa* contain various potential bioactive compounds .Therefore, it is recommended for the present study may help for prospect of researcher.

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