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Research Article

GAS CHROMATOGRAPHY AND MASS SPECTROSCOPY ANALYSIS OF BIOACTIVE COMPOUNDS OF *ADIANTUM LATIFOLIUM* LAM.

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ABSTRACT

Adiantum latifolium Lam. is an important medicinal fern belongs to the family Adiantaceae. The medicinal property of *Adiantum latifolium* is yet to explore except a few secondary metabolites on quantitative analysis level. The present study is the maiden effort and aims at analyzing Gas Chromatography and Mass Spectroscopy analysis of whole plant Ethanolic extract of *Adiantum latifolium* Lam. The study was carried out with GC-MS instrument of ITQ 900 Model of Thermo Fisher Scientific make. The Ethanolic plants samples revealed fifteen bioactive compounds, through GC-MS such as **1:**Propane, 1,1- diethoxy- (8.30%), **2:**Benzoic acid, 3-diethylamino-, ethylester (14.67%).**3:** Diethylpyridine-3,4-dicarboxylate (15.71%). **4:**(5R,8aR)-5-PropyloctahydroIndolizine(18.26%), **5:**2Hexyldecanoicacid(19.90%), **6:**Ecosen-1-ol,cis-9-(21.24%), **7:**12-hydroxy-14-methyl-oxacyclotetradec6en2one(21.72%),**8:**Pregnane 3, 20dione,17,21[(methylborylene)bis(oxy)]-, (5a) (21.92%), **9:**Stigmastanol (23.64%), **10:**12 Hydroxyabieta-8,11,13-triene6,7-dione(23.14%), **11:** Androst-5-en-3-one, 19-acetoxy4, 4di methyl ,oxime(24.84%), **12:** Pyrrolo[3,2-k]anthracene-4,6-diole,(25.82%), **13:** Isotetrahydrohis trotoxin287a(26.92%),**14:**Cephalotaxin(31.48%), **15:**8,9-Seco-3,19-epoxyandrostane-8,9 dione, 17-acetoxy-3methoxy-4,4-dimethyl-(33.70%). Based on the references the identified compounds were found to be antioxidant, antimicrobial and anti-infective agent. Some of the compounds seem to possess therapeutic agents for primary treatment for HIV/AIDS etc. Anticancer compound is also reported. Moreover, compounds with unknown activity were also reported. Hence the present experimental plant possess compounds with different curative properties. The further analysis for photochemical for therapeutic molecule from this plant is continued.

Keywords: Gas Chromatography, *Adiantum latifolium,* bioactive compounds.

INTRODUCTION

Plants have been an important source of medicine and mainly on traditional remedies. Ferns has been used as popular folk medicine. The medicinal importance of the Pteridophyte is due to the presence of some special compounds like alkaloids, flavonoids, phenols, tannins and saponins. These active principles usually remain concentrated in the storage organs of the plants viz., roots, fronds, rhizome etc. In general these secondary metabolites are an important source of drug with a variety of structural arrangements and properties [1].

Adiantum latifolium Lam. is a terrestrial species with wide spread creeping rhizome, often branched, up to 0.4cm thick, densely scaly all over, apex are acuminate, margin sparsely fimbriate, pale brown hairs and scales densely distributed all over the costa and rachis, texture herbaceous. Sori oblong or reniform, distributed all along the upper margin and unexcised part of the lower margin, sporangia and spores abortive[2]. According to references this plant is, native to tropical America from Mexico to South America, as well as the Greater Antilles, Virgin Islands and Trinidad. In India it is distributed throughout the Kerala, whereas in Tamil Nadu it is reported in Kanyakumari district[3].

The plants are employed in folk medicine Worldwide. The plant is called "Avenca" in Brazil and famous for anti-inflammatory, analgesic, anti infectious and diuretic Properties[4,-7]. In recent years GC-MS studies have been increasingly applied for the analysis of medicinal plants as this technique has proved to be a reliable method for the analysis of non-polar components and volatile essential oil, fatty acids, lipids and alkaloids[8&9]. GC-MS is the best technique to identify the bioactive components of long chain hydrocarbons, alcohols, acids used in the analysis of the herbal medicines, and there are more significant advantages[10]. Thus, GC-MS should be the most preferable tool for the analysis of the volatile chemical compounds in herbal medicines. Considering all these facts, the present investigation is designed to find out the photochemical present in *A. latifolium* through GC-MS technique.

MATERIALS AND METHODS

Collection of plant material

The sporophytic plants of *A. latifolium* Lam. were collected near the Coconut grooves of Kanyakumari District, Tamil Nadu, India. Dr. Raju Antony from Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI) Palode, Western Ghats, Kerala, India, identified the plant as *A. latifolium* Lam. These plant samples were authenticated by Dr. S. John britto, The Director, the Raphinet Herbarium Centre for Molecular Systematic, St. Joseph's College, Tiruchirappalli and a voucher specimen was deposited in the Department of Botany, Holy Cross College Trichy District, Tamil Nadu (Voucher No. 001).

Processing, Preparation and Extraction of sample for GC-MS analysis

The whole plants were washed with sterile distilled water. They were cut into small pieces and dried in shade and made into fine powder, using blender, and stored in air tight containers. 10 gm of the powdered whole plant sample was soaked with 20ml Ethanol for 3 days. The extract was then filtered through Whatman filter paper. From these extract 1ml of samples were extracted with ethanol and analyzed in GC-MS for identification of different components.

Methodology

The extract (1ml) was subjected to the GC-MS analysis on a combined GC-MS instrument (ITQ 900 Model of Thermo Fisher Scientific make) using a HP-5 fused silica gel capillary column. The method to perform the analysis was designed for both GC and MS. 1 µL aliquot of sample was injected into the column using a PTV injector whose temperature was set at 275°C. The GC program was initiated by a column temperature set at 60°C for 5 min, increased to 300°C at a rate of 8 C/min, held for 10 min. Helium was used as the carrier gas (1.5 mL/min). The mass spectrometer was operated in EI mode with mass source as 200°C. The chromatogram and spectrum of the peaks were visualized[11]. The particular compounds present in the samples were identified by matching their mass spectral fragmentation patterns of the respective peaks in the chromatogram with those stored in the National Institute of Standards and Technology Mass Spectral database library[12].

Identification of compound (Data analysis)

The mass spectra of compound in samples were obtained by Electron Ionization (EI) the detector operator in scan mode from 40 to 1000 m/z atomic mass units. Identification of compounds was based on the Molecular weight, Molecular formula, Retention time and peak area percentage[13].

Identification of compounds

Identification was based on the active principle with their Retention time (RT), Molecular weight (MW) and concentration (Peak area %). It is done in order to determine whether this plant species contains any individual compound or group of compounds which may substantiate its current commercial and biological activity, in these compounds[13].

RESULTS AND DISCUSSION

Gas chromatography and mass spectroscopy (GCMS)

The studies on the bioactive components in the Ethanolic extract of whole plant of *A. latifolium* Lam. by GCMS analysis clearly shows the presence of 15 bioactive compounds. The GCMS chromatogram of the peaks of bioactive compounds detected with their retention (RT), molecular formula (MF), molecular weight (MW) and concentration (Peak area %) were presented in Table-1& Fig 1. There were 15 active phytoconstitutents identified by the mass spectroscopy.

The total numbers of compounds and their peak area identified in Ethanolic extracts were **1:**Propane,1,1-diethoxy-(8.30%), **2:**Benzoicacid,3-diethylamino,ethylester (14.67%), **3:**Diethyl Pyridine-3, 4-dicarboxylate (15.71%), **4:** (5R,8aR) -5-PropyloctahydroIndolizine (18.26%), **5:** 2 Hexyldecanoicacid (19.90%), **6:** Ecosen-1-ol,cis-9-(21.24%), **7:** 12-hydroxy-14-methyl-oxa-cyclotetradec6en2one (21.72%), **8:** Pregnane-3,20 dione,17,21 [(methylborylene)bis(oxy)], (5a) (21.92%), **9:** Stigmastanol (23.64%), **10:** 12Hydroxy abieta-8 11, 13-triene 6,7-dione (23.14%), **11:**Androst-5-en-3-one,19-acetoxy-4,4dimethyl, Oxime(24.84%), **12:**Pyrrolo[3,2-k]anthracene-4,6-diole, (25.82%).**13:**IsotetraHydrohistro toxin287a(26.92%), **14:**Cephalotaxin(31.48%), **15:** 8,9-Seco-3,19-epoxyandrostane-8,9-dione,17-acetoxy-3methoxy-4,4-dimethyl-(33.70%) (Table 1).

Table 1 shows the significant biological activity of the active principles studied from *A.latifolium* Lam, Specifically, compounds like Stigmastanol found to be helpful in the primary treatment of HIV/AIDS (Fig.3). Compounds like Androst-5-en-3-one,19-acetoxy-4,4-dimethyl-,oxime and Propane, 1, 1 – diethoxy, Benzoic acid (Fig.2) ,3-diethylamino-,ethyl ester and Pyrrrolo[3,2-k]anthracene-4,6-diol,3-methoxy-4b,5,6,7,8,9,10,11,11a,12-decahydro-11-ethyl- related to Antitumor activity and antioxidant activity was reported in the present investigation. Cephalotaxin is another compound which can cure Leukemia, and it is present in this plant (Fig.4). Two new compounds without any activity were also reported in this work (Table 1).

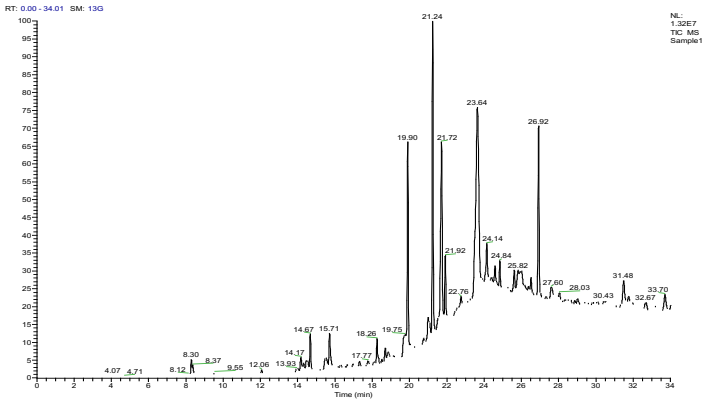


Fig 1: Chromatogram of whole plant Ethanolic extract of *A. latifolium* Lam.

Table 1: Components detected in Ethanolic extract of *Adiantum latifolium* Lam. through GCMS studies

S.No	Name of the compounds	Molecular Formula	Molecular Weight	Peak Area %	Retention Time	Biological activity
1.	Propane,1,1-diethoxy-	C7H16O2	132	8.30	8.27 -8. 34	Antioxidant activity[14]
2.	Benzoic acid,3-diethylamino-,ethyl ester	C13H19NO2	221	14.67	14.62 -14.73	Antioxidant; Hypocholesterolemic Nematicide; Pesticide, Lubricant; Antiandrogenic Flavor; Hemolytic [15].
3.	Diethyl pyridine-3,4-dicarboxylate	C11H13N04	223	15.71	15.67-15.73	Antimicrobial activity[16]
4.	(5R,8aR)-5-Propyloctahydroindolizine	C11H21N	167	18.26	18.22-18.30	Antimicrobial and Antibacterial Activity[17]
5.	2-Hexyldecanoic acid	C16H3202	256	19.90	19.84-19.97	Antimicrobial activity [18]
6.	Eicosen-1-ol,cis-9-	C20H40O	296	21.24	21.20-21.34	Antibacterial[19]
7.	12-hydroxy-14-methyl-oxa-cyclotetradec-6en-2-one	C14H24O3	240	21.72	21.03-21.78	No Activity Reported
8.	Pregnane-3,20-dione,17,21-[(methylborylene)bis(oxy)]-,(5a)-	C22H33BO4	372	21.92	26.85-26.99	Antiglucocorticoids[20]
9.	Stigmastanol	C29H52O	416	23.64	23.55-23.74	Primary treatment for HIV/AIDS[21]
10.	12-Hydroxyabieta-8,11,13-triene-6,7-dione	C20H26O3	314	24.14	24.12-24.19	Anti bacterial activity[22]
11.	Androst-5-en-3-one,19-acetoxy-4,4-dimethyl-,oxime	C23H35NO3	373	24.84	24.80-24.87	antimicrobial, anti-inflammatory and antitumor activity[15]
12.	Pyrrrolo[3,2-k]anthracene-4,6-diol,3-methoxy-4b,5,6,7,8,9,10,11,11a,12-decahydro-11-methyl-	C18H25NO3	303	25.82	25.77-26.06	antioxidant and antibacterial activities[23]
13.	Isotetrahydrohistrionicotoxin287a	C19H29NO	287	26.92	26.86-26.98	Antinociceptive activity (Pumiliotoxins)[24]
14.	Cephalotaxine	C18H21NO4	315	31.48	31.44-31.53	Anti-leukemic activity[25]
15.	8,9-Seco-3,19-epoxyandrostane-8,9-dione,17-acetoxy-3-methoxy-4,4-dimethyl-	C24H36O6	420	33.70	33.65-33.74	No Activity Reported

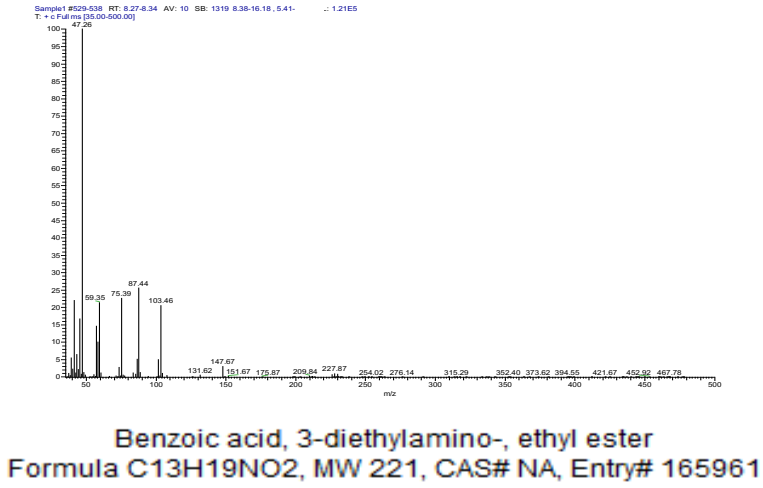
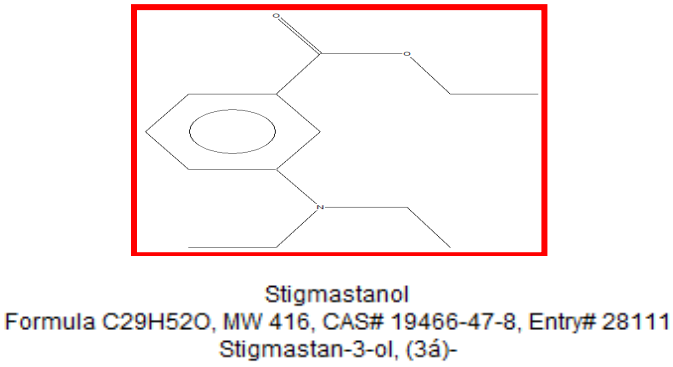


Fig 2: Mass spectrum of Benzoic acid, 3-diethylamino-, ethyl ester



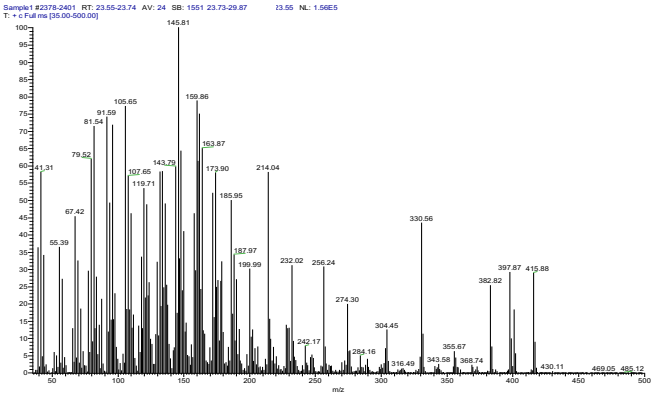
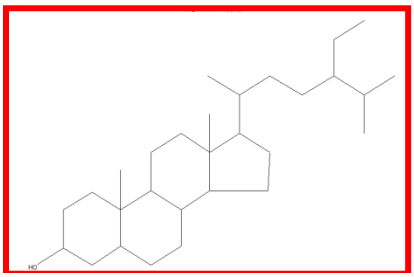


Fig 3: Mass spectrum of Stigmastanol



Cephalotaxine
Formula C18H21NO4, MW 315, CAS# 24316-19-6, Entry# 200702
4H-Cyclopenta[a][1,3]dioxolo[4,5-h]pyrrolo[2,1-b][3]benzazepin-1-ol, 1,5,6,8,9,14b-hexahydro-2-methoxy-

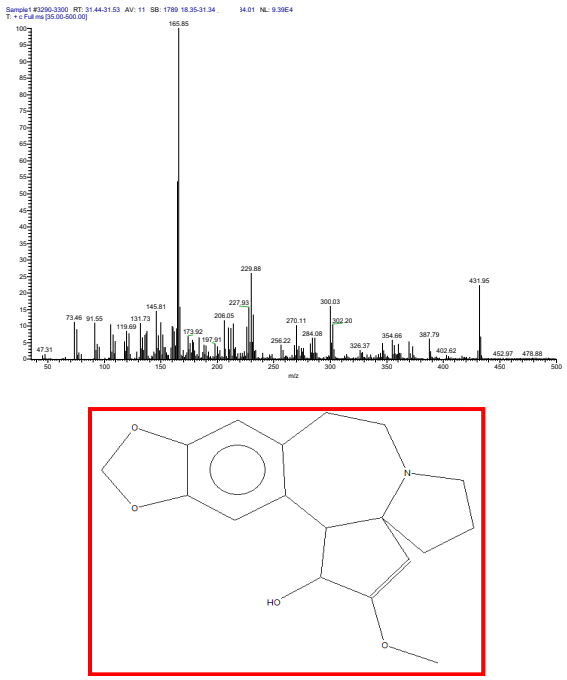


Fig 4: Mass spectrum of Cephalotaxin

The compounds mentioned from our results was also studied from plants like *Polygonum glabrum, Salvia barrelieri, Adiantum capillus-Veneris, Adiantum latifolium, Hypoxis* and *Sutherlandia* species, and *Cephalotaxus harringtonia* . Apart from these activites, it is having more disease curing properties and various works also have been done by using these plants. The antibacterial, antioxidant and antitumor activity of *Salvia barrelieri* plant have also reported. South African S*utherlandia frutescens* contains flavonoid glycosides as marker compound[26]. *Cephalotaxus harringtonia* plant possess same anticancer and anti-leukemic compound which is reported from the present experimental plant[27].

A bioactive compound obtained from our studies is related to the HIV/AIDS activity of *Sutherlandia* species. The *Sutherlandia* plant decoction is also used in the treatment of open wounds, fever, and Chicken pox[28]. The methanol extract also contains the highest concentration of the cycloartane glycosides found exhibiting similar anti-cancer properties[29].

CONCLUSION

In the present study, fifteen chemical constituents have been identified from Ethanolic extract of *Adiantum latifolium* Lam. whole plant material by Gas Chromatogram Mass Spectroscopy (GC-MS) analysis. These results revealed that the presence of various bioactive compounds which may have high medicinal value to cure various diseases. However, further studies are needed on these compounds in order to isolate, identify, characterize and elucidate the structure of these compounds.

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