



Research Article

GAS CHROMATOGRAPHY AND MASS SPECTROSCOPY ANALYSIS OF BIOACTIVE COMPOUNDS OF *ADIANTUM CAUDATUM* L. AN ANTI-DIABETIC FERN.

Gayathiri M¹, Catharin Sara S^{2*}, Sujatha S¹, Ramya Roselin I¹ and Gnana Deepa Ruby R²

¹ Ph. D Scholars, PG and Research Department of Botany, Holy Cross College (Autonomous), Tiruchirappalli, Tamil Nadu, India

² Assistant Professor, PG and Research Department of Botany, Holy Cross College (Autonomous), Tiruchirappalli, Tamil Nadu, India

*Corresponding Author Email: sara.kameelasivaraman.catharin@gmail.com

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ABSTRACT

The present study deals with the GC MS analysis of *Adiantum caudatum* L. to know the presence of phytochemicals present in this plant. The GC MS profile indicated the presence of some very important molecules such as 1. Quinoline-1-carboxaldehyde, 1, 2, 3, 4-tetrahydro-4, 8-dimethyl-2-spirocyclohexane (19.90%), 2. Cis-9-Hexadecenal, 9-Hexadecenal, (Z)-, (21.76%), 3. 4H-Pyran-4-one, 3-acetyl-2-(4-acetyl phenyl amino)-6-methyl, (21.91%) and 4. Retinoic acid, 5, 8-epoxy-5-8-dihydro- 5, 6-Epoxyretinoic acid (32.77%). These molecules have a number of important medicinal roles which could substantiate their participation towards the medicinal activity of *Adiantum caudatum* L. as a medicine proposed by Ayurveda, Siddha and Unani including anti-diabetic property. Further work in this direction is going on to prove the medicinal efficacy of the plant.

Keywords: *Adiantum caudatum* L., ethanolic extracts, Gas Chromatography-Mass Spectrum, Phytochemical compounds,

INTRODUCTION

India has one of the oldest, richest and most diverse cultural traditions called 'folk tradition' associated with the use of medicinal herbs based indigenous belief, knowledge, skill and cultural practices¹. Traditional medicine is used throughout the World as it is heavily dependent on locally available plant species and plant-based products and capitalizes on traditional wisdom-repository of knowledge². Humanity faces all sorts of conditions and the handling of health issues is a real social problem, especially in developing countries with limited resources³. Despite advances in biology and medicine, the majority of people in developing countries do not have access to adequate healthcare⁴. Still today, the majority of the world population, especially rural people in developing countries like Pakistan, Bangladesh, India, or Nepal, partially or entirely rely on herbal medicine⁵.

Pteridophytes existing today represent ancient plant species which appeared about 300 million years ago in the late Devonian period⁶. Traditionally, the biomedical system and Ayurvedic systems of medicine, named Sushruta (ca. 100 AD) and Charka (ca. 100 AD), suggested the use of some ferns in the *Samhita* texts. Pteridophytes are also used by physicians in the Unani system of medicine⁷. In the traditional Chinese system of medicine, several ferns are recommended by native doctors⁸.

Adiantum caudatum L. is known as walking maiden hair, tailed maiden hair and trailing maiden hair fern belonging to the family Adiantaceae. *Adiantum* species are well-known for their significant medicinal efficacies and are being traditionally used in the treatment of various ailments for a long period. The whole plant parts of *Adiantum caudatum* L. are medicinal⁹. Ayurveda

describes that it would be useful to treat Prameha (diabetes), cough, skin diseases, and fever¹⁰. The present experimental fern generally prefer Humus-rich, moist, well-drained sites, ranging from bottom and soils to vertical rock walls. The present investigation towards the bioactive compounds using GC-MS was the maiden work in this plant and helps to trace the phytochemicals present in this plant apart from folklore references with highest degree of specificity.

MATERIAL AND METHODS

Collection of plants materials

The experimental *Adiantum caudatum* L. was collected from Western Ghats, Palode (Kerala), India. The plant sample was identified by Dr. S. John Britto, The Director, the Rapinat Herbarium and Center for Molecular Systematics, St. Joseph's College, Tiruchirappalli, Tamil Nadu, India (Voucher No.001). The voucher specimens (Voucher No. 001) were deposited in the Department of Botany, Holy Cross College (Autonomous), Tiruchirappalli District, Tamil Nadu for future references.

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

The sporophytic plant *Adiantum caudatum* L. was collected and the fronds were initially separated from the main plant parts (Frond and Rhizome) and rinsed with distilled water and dried under shade on paper towel in laboratory, then homogenized into fine particles and stored in airtight bottles. 10 gm of the powdered whole plant sample was soaked with 20 ml Ethanol for 3 days. The extract was then filtered through Whatman filter paper. From

these extract 1 ml of sample was extracted with ethanol and analyzed in GC-MS for identification of different compounds.

Methodology

GC-MS analysis was carried out on a GC Clarus 500 Perkin Elmer system and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: Column Elite-5MS fused silica capillary column (30 mm × 0.25 mm ID × 1 μm, composed of 5% Diphenyl/95% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; Helium (99.999%) was used as carrier gas at a constant flow of 1 ml/min and an injection volume of 2 μl was employed (split ratio of 10:1); Injector temperature 250°C; Ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min.), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9 min. isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a Turbo Mass Ver 5.2.0.

Identification of Components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) 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. The Name, Molecular weight and Structure of the components of the test materials were ascertained.

RESULTS

The studies on the bioactive components in the ethanolic extract of whole plant of *Adiantum caudatum* L. by GC-MS analysis clearly showed the presence of four bioactive compounds. The active principles with their Retention Time (RT), Molecular Formula (MF), Molecular Weight (MW) and concentration (peak area %) were presented in (Table 1). There were four active phytoconstituents identified by the mass spectroscopy.

The GC-MS chromatogram of the four peaks of bioactive compounds detected were shown in (Figure 1). The total number of compound identified in ethanolic extracts were

1. Quinoline-1-carboxaldehyde, 1, 2, 3, 4-tetrahydro-4, 8-dimethyl-2-spirocyclohexane (19.90%), (Figure 2)
2. Cis-9-Hexadecenal, 9-Hexadecenal, (Z)-, (21.76%), (Figure 3)
3. 4H-Pyran-4-one, 3-acetyl-2-(4-acetyl phenyl amino)-6-methyl, (21.91%), (Figure 4)
4. Retinoic acid, 5, 8-epoxy-5-8-dhydro- 5, 6-Epoxyretinoic acid (32.77%). (Figure 5)

The listed out major phytochemicals and its biological activities obtained through the GC-MS study of the whole plant of *Adiantum caudatum* L. (Table 1) showed antioxidant, antimicrobial, anti-inflammatory, hypo cholesterolemic, nematocidal, haemolytic -5 alpha reductase inhibitor, chemo preventive and chemotherapeutic activities. Among the four compounds there is no indication of activity for one compound (4H-Pyran-4-one, 3-acetyl-2-(4-acetyl phenyl amino)-6-methyl), and maybe it is a new compound in this study.

Table 1: Bioactive of Components detected Bioactivity referred in whole plant ethanolic extract of *Adiantum caudatum* L. through GC-MS Analysis

Name of the compound	Molecular formula	Molecular weight	Peak area	Retention time	Bioactivity
Quinoline-1-carboxaldehyde, 1,2,3,4-tetrahydro-4,8-dimethyl-2-spirocyclohexane-	C ₁₇ H ₂₃ NO	257	19.90	19.80-19.96	Antioxidant, antimicrobial, anti-inflammatory.
cis-9-Hexadecenal, 9-Hexadecenal, (Z)-	C ₁₆ H ₃₀ O	238	21.76	21.71-21.81	Antioxidant, hypocholesterolemic, nematocidal, haemolytic, 5-alpha reductase inhibitor
4H-Pyran-4-one, 3-acetyl-2-(4-acetyl phenyl amino)-6-methyl	C ₁₆ H ₁₅ NO ₄	285	21.91	21.87-21.95	Activity not found
Retinoic acid, 5,8-epoxy-5-8-dhydro- 5, 6-Epoxyretinoic acid	C ₂₀ H ₂₈ O ₃	316	32.77	32.74-32.80	Chemopreventive and chemotherapeutic agents

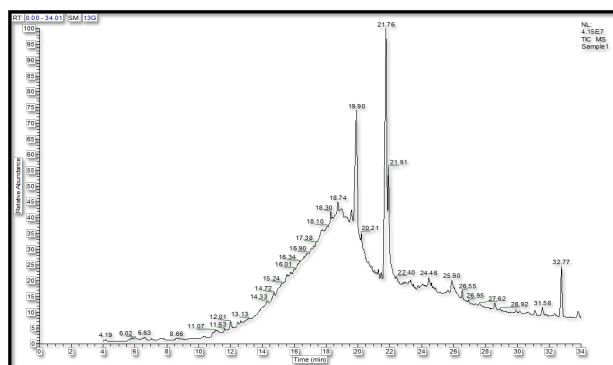


Figure 1: GC-MS Chromatogram obtained from the whole plant ethanolic extract of *A. caudatum* L.

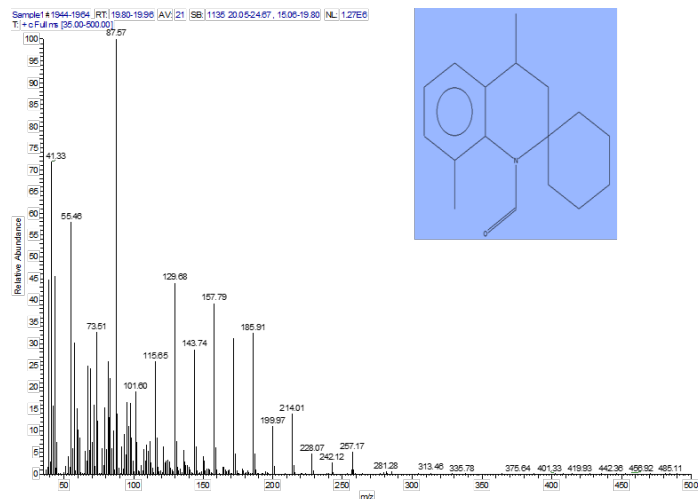


Figure 2: Mass Spectrum of Quinoline-1-carboxaldehyde, 1, 2, 3, 4-tetrahydro-4, 8-dimethyl-2-spirocyclohexane

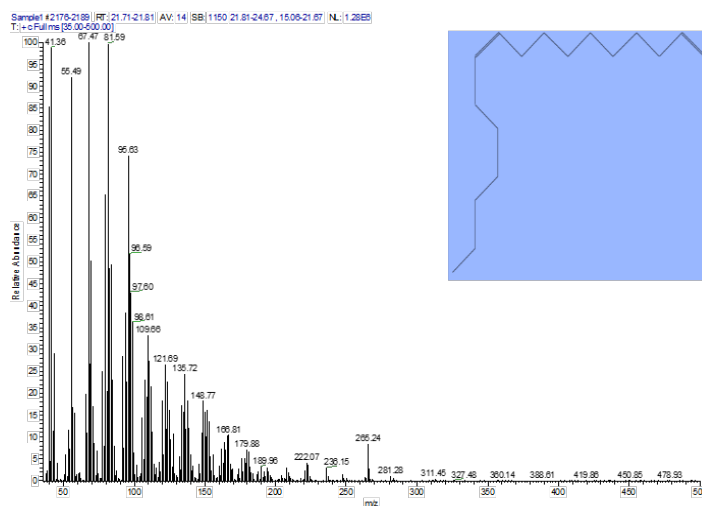


Figure 3: Mass Spectrum of Cis-9-Hexadecenal, 9-Hexadecenal

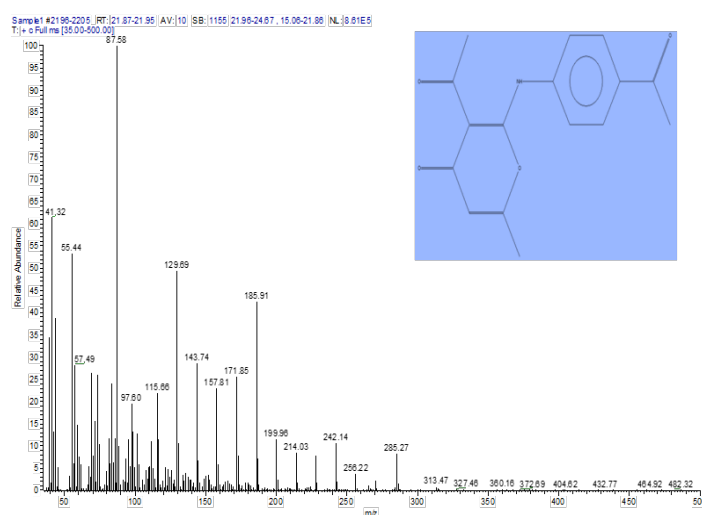


Figure 4: Mass Spectrum of 4H-Pyran-4-one, 3-acetyl-2-[(4-acetylphenyl)amino]-6-methyl-

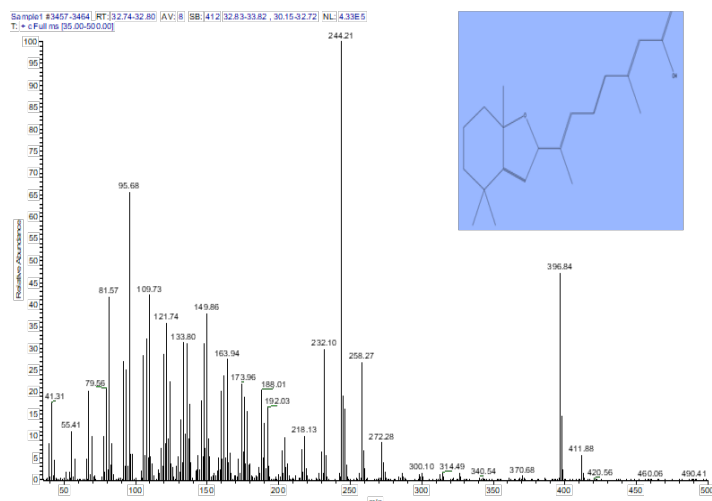


Figure 5: Mass Spectrum of Retinoic acid, 5,8-epoxy-5,8-dihydro- 5, 6-Epoxyretinoic acid

DISCUSSION

In India, many plants are widely used by all section of people either directly as folk remedies or indirectly in pharmaceutical preparations of modern medicine and India is one of the richest countries in the World providing medicinal plants. The limitations associated with synthetic pharmaceutical products have opened avenues for 'Green Medicine' that is considered to be safe, more accessible and affordable too.

GC-MS is one of the best techniques to identify the constituents of volatile matter, long chain, branched chain hydrocarbons, alcohols, acids, esters etc. The GC-MS identification of the chemical constituents was based on comparison of their mass spectra with NIST and WILEY libraries. Structures were defined by percentage similarity values.

In the present study, the potent chemical constituents present in *Adiantum caudatum* L. showed various biological activities like Antioxidant, antimicrobial, anti-inflammatory, hypo cholesterolemic, nematocidal, haemolytic, 5- α reductase inhibitor, Chemo preventive and chemotherapeutic agents. References like Panzella¹¹ and NCBI pub chem bi assays also confirmed the anti-inflammatory, anti-mutagenic, and cancer chemo preventive action. Retinoic acid, 5, 8-epoxy-5,8-dihydro-5, 6-Epoxyretinoic acid 4H-Pyran-4-one, 3-acetyl-2-(4-acetyl phenyl amino)-6-methyl a novel compound which was identified in our work also.

GC-MS analysis carried out by Sivagurunathan and Innocent¹². In the aquatic fern *Marsilea quadrifolia* showed the presence of major components hexadecanoic acid, ethyl ester (26.88%), phytol (16.97%), 9,12-octadecadienoic acid (12.46%), 1,2-benzenedicarboxylic acid, diisooctyl ester (8.62%), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (6.71%) and 2(3H)-furanone, dihydro-3-hydroxy-4,4-dimethyl (6.56%). The phytochemicals possess the same properties as like that of our experimental plants. Hence our experimental plant *A. caudatum* L. proves to be a very important plant related to anti diabetic and antioxidant qualities.

CONCLUSION

The chemical profile of *Adiantum caudatum* L. using GC-MS analysis provides information and individual molecules that characterize the species of ethanolic extract. The results of the

present study may be useful in metabolomics research, nutraceuticals and phyto pharmaceuticals to evaluate their quality and used as a drug in future with immense medicinal properties.

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