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Research Article

ANALYSIS OF BIOACTIVE COMPOUNDS OF *HEMIONITIS ARIFOLIA* (BURM.) MOORE. AN ANTI-DIABETIC FERN USING GAS CHROMATOGRAPHY AND MASS SPECTROSCOPY

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ABSTRACT

Hemionitis arifolia (Burm.) Moore. is an important medicinal plant belongs to the family Hemionitidaceae. In Asian folklore *Hemionitis arifolia* is used to treat diabetes. The fern has been medically evaluated for its anti-diabetic properties and for intestinal worms. The present work is an important exploration and aims at analysing ethanolic extract of the plant using Gas Chromatography-Mass Spectrometry. The mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technologies (NIST) library. Gas chromatography mass spectrometry (GC-MS) analysis revealed the presence of nine compounds and the phyto constituents screened were 1. 4-(chloromethyl)-1-azabicydo-(2.2.2)octane (18.26%), 2. 4-Nitrobenzoic acid, 2,4,6-trichlorophenyl ester (18.70), 3. Decanoic acid, 2-hexyl-,methyl ester(19.91), 4. N-(4-(-Chlorophenyl)isothiazol-5-yl)-2-methylpiperidin-2-imine (21.73), 5. Pregnane-3,20-dione, 17,21-{(methylborylene)bis(oxy)}-,5a- (23.76), 6. Retinoic acid, 5,8-epoxy-5,8-dihydro (24.80), 7. Androst-5-en-3-one, 19-acetoxy-4, 4-dimethyl-, oxine (26.14), 8. Choestan-26-oic acid, 3,7,12-trihyroxy-, (3a,5a,7a, 12a) (28.07), 9. Benzamide, N-(4-chlorotetrahydro-3-thinyl)-3, 5- dimethoxy, s-dioxide (31.52). The nine compounds identified provide positive light upon anti-diabetic works and drug could be formulated in future for diabetic condition. Hence *Hemionitis arifolia* is an important medicinal material for further research leading to possible drug development.

Keywords: Gas Chromatography-Mass spectrum, *Hemionitis arifolia* (Burm.) Moore. Bioactive compounds. Decanoic acid, Retinoic acid

INTRODUCTION

Medicinal plants have been used by human being since ages in traditional medicine due to their therapeutic potential and the search on medicinal plants led to the discovery of novel drug candidates used against diverse diseases. According to the World Health Organisation (WHO) in 2008, more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. The Pteridophytes from Western Ghats and Eastern Ghats were used by tribal's for the treatment of various ailments like stomach disorders, poisonous bites, rheumatics, cough, asthma, fever, diabetes, etc[1]., Moreover the dominant tribes involved in using Pteridophyte as medicines in the District are Santhal, Kol, Bhumija, Bhuyan, Mahalis, Sounti and Saharas. The plant parts like fronds and rhizomes were used as raw or cooked forms for the treatment of malaria, gonorrhea, leprosy and rheumatism, the mostly used genera are *Adiantum*, *Asplenium*, *Lygodium* and *Pteris*[2,3].

Bioactive compounds continue to play a dominant role in the maintenance of human health. Reports available on green plants represent a reservoir of effective chemo therapeutants, these are non phytotoxic, more systematic and easily biodegradable[4-6]. Medicinal value of Pteridophytes lists against bacteria, fungi, virus, cancer, rheumatism, diabetes, inflammation, fertility, diuretic, pesticides, hepatoprotective and sedative. Besides sugar, starch, proteins and amino acids, ferns contain a variety of alkaloids, glycosides, flavonoids, terpenoids, sterols, phenols etc. as potential components used in various industries[7].

Hemionitis arifolia is an attractive fern belonging to the family Hemionitidaceae. It is both an epiphyte as well as a terrestrial plant. In Asian folklore *Hemionitis arifolia* is used to treat diabetes. According to references the fern has been medically evaluated for its hypoglycemic properties and for intestinal worms. It is commonly called 'Heart or Rabbit ear' fern. This attractive fern has dark green cordate (heart shaped) fronds which grow on blackish stems with fronds that reach 5 to 7.5 cm (2" to 3") in length.

Since the experimental plant is used by tribals, people may explore the plants more within short time for its medicinal value. This leads to insufficiency of the plant as well as extinction in future so that the medicinal value should be exploited immediately. References points out anti-diabetic properties in this plant and diabetes is a chronic diseases that kills major population of the World nowadays and hence there is a dire need for drug related to diabetes. Since there is not much work on this plant, the present work aims at screening of bioactive compounds present in this plant by performing GC-MS analysis where the components of long chain hydrocarbons, alcohols, acids and volatile compounds present in the plant could be revealed.

MATERIALS AND METHODS

Collection of plants materials

The whole plant of *Hemionitis arifolia* was collected from Eastern Ghats (Kolli hills) of Tamil Nadu, India. This fern is both an epiphyte and grows on trees as well as a terrestrial plant. It is an attractive and dwarf fern. The plant sample were 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 plant material of *Hemionitis arifolia* 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 1ml of sample was extracted with ethanol and analyzed in GC-MS for identification of different components.

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 µmdf, 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 *H. arifolia* by GC-MS analysis clearly showed the presence of nine bioactive compounds. The active principles with their retention time (RT), molecular formula (MF), molecular weight (MW) and concentration (peak area %) were presented and biological activity of nine peaks were presented in Table 1. The GC-MS chromatogram of the nine peaks of bioactive compounds detected were shown in Figure 1. The total number of compound identified in ethanolic extracts were

1. 4-(Chloromethyl)-1-azabicydo-(2.2.2)octane, 4-(Chloromethyl)quinuclidine (18.26%),
2. 4-Nitrobenzoic acid, 2, 4, 6-trichlorophenyl ester (18.70),
3. Decanoic acid, 2-hexyl-, methyl ester, Methyl 2-hexyldecanoate (19.91),
4. N-(4-(-Chlorophenyl) isothiazol-5-yl)-2-methylpiperidin-2-amine, 4-(4-Chlorophenyl)-N-{(2E)-1-methylpiperidinylidene}-5-isothiazolamine (21.73),
5. Pregnane-3, 20-dione, 17, 21-{(methylborylene)bis(oxy)}-,5a-dihyroxy-, cyclic methaneboronate (23.76),
6. Retinoic acid, 5, 8-epoxy-5, 8-dihydro, 5, 6-Epoxyretinoic acid (24.80),
7. Androst-5-en-3-one, 19-acetoxy-4, 4-dimethyl-, oxine, 3-(Hydroxyimino)-4, 4- dimethylandrost-5-en-19-yl acetate (26.14),
8. Choestan-26-oic acid, 3, 7, 12-trihyroxy-, (3a, 5a, 7a, 12a) – 5a-cholestan-26-oic acid, 3a, 7a-trihydroxy- (28.07),
9. Benzamide, N-(4-chlorotetrahydro-3-thinyl)-3, 5- dimethoxy, s-dioxide (31.52).

The listed out major phytocompounds and its biological activities obtained through the GC-MS study of the whole plant of *Hemionitis arifolia* showed the mass spectrum of seven bioactive constituents. The bioactive compounds find to act as anti-fungal, anti-bacterial, anti-cancerous agents, fluorinating agent, pharmaceuticals, dye, nematicides, pesticides, chemotherapeutic agents, compounds curing fibrosis, obesity and diabetes. There is no bioactivity found for two compounds and these compounds are considered as novel compounds to be explored in future.

DISCUSSION

Phytochemicals identified from medicinal plants present an exciting opportunity for the development of new types of therapeutics. Phytomedicine has been used since ancient times in various parts of the World where access to modern medicine is limited. In recent years, considerable attention has been directed towards identification of plants with anti-diabetic ability that may be used for human consumption[8]. According to an estimation of the International Diabetes Federation, approximately 366 million people are suffering from diabetes and this may double by 2030, In India is 40.9 million now which is expected to grow to 60.9 million by 2025[9].

GC-MS analysis of *Hemionitis arifolia* yielded nine bioactive compounds respectively. All the compounds and its bioactivity are listed in Table 1. Out of nine, seven compounds showed their activity during reference whereas two compounds were found to possess no activity and hence to be possibly new compounds. Studies revealed that the plant can be used as antibacterial, anti-fungal, anti-diabetic and anti-cancerous. Various authors have worked GC-MS in plants like *Nephrolepis cordifolia* and *Adiantum capillus-veneris* and the activities related to the compounds found out coincide with our results[10,11]. The anti-diabetic property of the experimental plant reveals that the plant could be an important source for the investigation related to diabetics. Moreover efforts should be taken to find out the drug present in the plant and also to elucidate and characterize the structure and clinical properties in coming days.

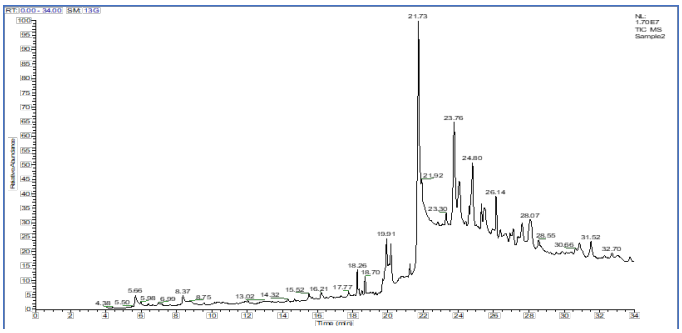

Figure 1: Chromatogram of frond ethanolic extract of GC-MS with the whole plant of *H. Arifolia* (Burm.) Moore

Table 1: Components detected in whole plant ethanolic extract of *Hemionitis arifolia* (Burm.) Moore. through GC-MS Analysis

Name of the compound	Molecular formula	Molecular weight	Peak area	Retention time	Bioactivity
4-(Chloromethyl)-1-azabicydo-(2.2.2)octane , 4-(Chloromethyl)quinuclidine	$C_8H_{14}CIN$	159	18.26	18.22-18.31	Used as Fluorinating agents and therapeutic and diagnostic purpose
4-Nitrobenzoic acid, 2,4,6-trichlorophenyl ester	$C_{13}H_6C_{13}NO_4$	345	18.70	18.67-18.74	Production of pharmaceuticals and dyes
Decanoic acid, 2-hexyl-,methyl ester, Methyl 2-hexyldecanoate	$C_{17}H_{34}O_2$	270	19.91	19.88-19.98	Nematicide and Pesticide
N-(4-(-Chlorophenyl)isothiazol-5-yl)-2-methylpiperidin-2-amine, 4-(4-Chlorophenyl)-N-{(2E)-1-methylpiperidinylidene}-5-isothiazolamine	$C_{15}H_{16}CIN_{38}$	305	21.73	21.69-21.82	Activity not found
Pregnane-3,20-dione, 17,21-{(methylborylene)bis(oxy)}-,5a-dihyroxy-, cyclic methaneboronate	$C_{22}H_{33}BO_4$	372	23.76	23.67-23.87	Anti-bacterial, anti-fungal and anti-cancer activity
Retinoic acid, 5,8-epoxy-5,8-dihydro, 5,6-Epoxyretinoic acid	$C_{20}H_{28}NO_3$	316	24.80	24.76-24.83	chemopreventive and chemotherapeutic agents
Androst-5-en-3-one, 19-acetoxy-4, 4-dimethyl-, oxine, 3-(Hydroxyimino)-4, 4-dimethylandrost-5-en-19-yl acetate	$C_{23}H_{35}O_3$	373	26.14	26.10-26.19	Activity not found
Choestan-26-oic acid, 3,7,12-trihyroxy-, (3a,5a,7a, 12a) – 5a-cholestan-26-oic acid, 3a,7a-trihydroxy-	$C_{27}H_{46}O_5$	450	28.07	28.03-28.14	used to treat inflammation,fibrosis,obesity and diabetes
Benzamide, N-(4-chlorotetrahydro-3-thinyl)-3, 5- dimethoxy, s-dioxide	$C_{27}H_{46}CINO_{55}$	333	31.52	31.49-31.56	Anti-oxidant and anti-diabetic

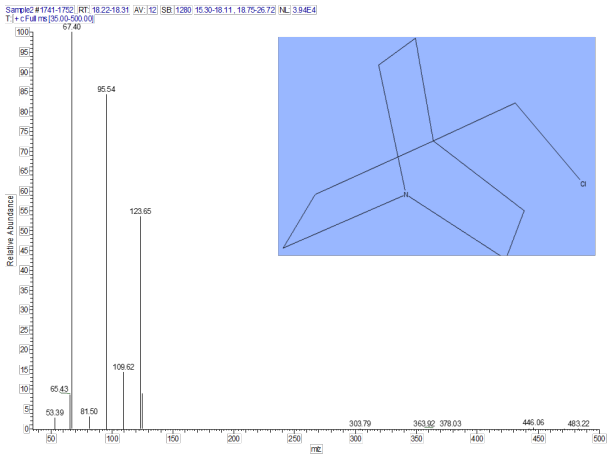

Figure 2: Mass spectrum of 4-(Chloromethyl)-1-azabicyclo[2.2.2]octane, 4-(Chloromethyl) quinuclidine

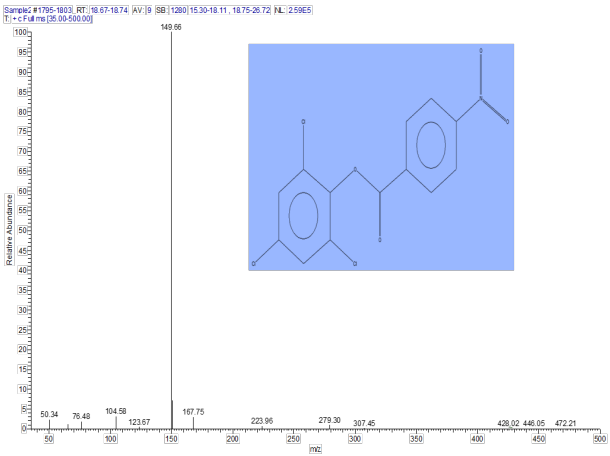

Figure 3: Mass spectrum of 4-Nitrobenzoic acid, 2,4,6-trichlorophenyl ester

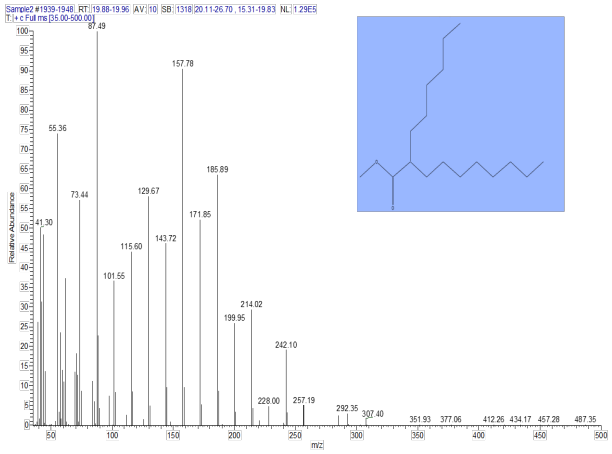

Figure 4: Mass spectrum of Decanoic acid, 2-hexyl-, methyl ester, Methyl 2-hexyldecanoate

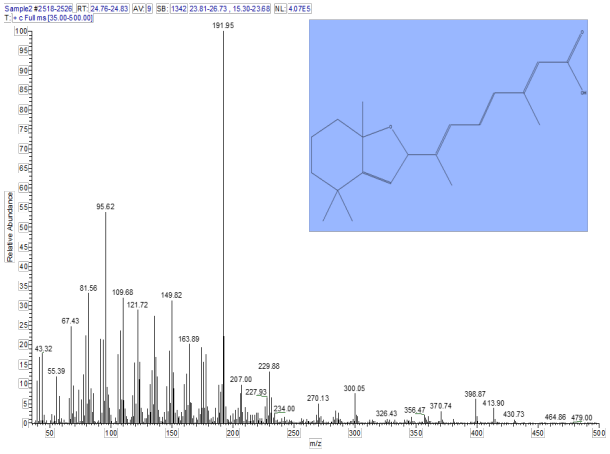

Figure 5: Mass spectrum of Retinoic acid, 5,8-epoxy-5,8-dihydro-5,6-Epoxyretinoic acid

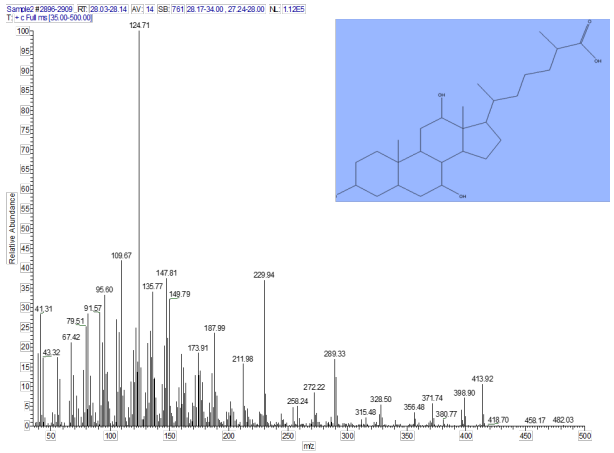

Figure 6: Mass spectrum of Cholestan-26-oic acid, 3,7,12-trihydroxy-,(3å,5å,7å12å)-5å-Cholestan-26-oic acid,3å,7å,12å-trihydroxy

CONCLUSION

The establishment of natural plant product industries increase in domestic employment, the creation of relevant technological capability, an increase in export earnings and the betterment of the rural population have improved in terms of health care provision. The present experimental plant proves to be a promising plant for the exploration of drug towards diabetics and its implications.

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REFERENCES

1. Reddy CS, Reddy KN, Rao KT, Pattanaik C. Ethno botanical studies on medicinal plants used by the Chenchus of Nallamalais in Kurnool District, Andhra Pradesh, India. Research Journal of Medicinal Plant 2007; 1(4): 128-133. https://scialert.net/fulltextmobile/?doi=rjmp.2007.128.133
2. Singh BP, Upadhyay R. Ethno-botanical importance of Pteridophytes used by the tribe of Pachmarhi, Central India. Journal of Medicinal Plants Research. 2012; 6(1): 14-8. https://www.researchgate.net/profile/Ravi_Upadhyay/publication/235256086_Ethno-botanical_importance_of_Pteridophytes_used_by_the_tribe_of_Pachmarhi_Central_India/links/0912f510a9a3f72058000000/Ethno-botanical-importance-of-Pteridophytes-used-by-the-tribe-of-Pachmarhi-Central-India.pdf
3. Rout SD, Panda T, Mishra N. Ethno medicinal studies on some Pteridophytes of similipal biosphere reserve, Orissa, India. International Journal of Medicine and Medical Sciences 2009; 1(5): 192-7. http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.861.4338&rep=rep1&type=pdf
4. Vyas GD. Soil fertility Deterioration in crop land due to pesticide. Journal of the Indian Botanical Society 1999; 78(1-2): 177-8.
5. Kaushik JC, Sanjay A, Tripathi NN, Arya S. Anti-fungal properties of some plant extracts against the damping-off fungi of forest nurseries. Indian Journal of Forestry 2002; 25(3-4): 359-61.
6. Lal C, Verma LR. Use of certain bio-products for insect-pest control. Indian Journal of Traditional knowledge 2006[cited 2017; 5(1): 79-82. http://nopr.niscair.res.in/bitstream/123456789/6804/1/IJTK%205(1)%20(2006)%207982.pdf?utm_source=The_Journal_Database&trk=right_banner&id=1400274308&ref=d80cd4276d9db7d333ca36295011ec7e
7. Kulandairaj D, John DB. Antibacterial and antifungal activity of secondary metabolites from some medicinal and other common plant species. Journal of Economic and Taxonomic Botany 2000; 24: 21.

8. Malviya E, Sanjay J, Sapna M. Anti-diabetic potential of medicinal plants. Acta Poloniae Pharmaceutica Drug Research 2010; 67(2): 113-118.
9. Mitra A, Dewanjee D, Dey B. Mechanistic studies of lifestyle interventions in type 2 diabetes. World Journal of Diabetes 2012; 3(12): 201-207. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3538986/pdf/WJD-3-201.pdf
10. Rukmini K, Devi PS. GC-MS Analysis and Phytochemical Screening of A Rare Pteridophyte *Nephrolepis cardifolia* (L.) Presl. FromTirumala Hills. International Journal of Pharmaceutical Research and Reviews 2014; (12): 13-19. https://pdfs.semanticscholar.org/eff4/b37fd44689fc5ace99b8d8f4c8fdb921d0e9.pdf
11. Kumar S, Samydurai P, Ramakrishnan R, Nagarajan N. Gas chromatography and mass spectrometry analysis of bioactive constituents of *Adiantum capillus-veneris* L. International Journal of Pharmacy and Pharmaceutical Sciences 2014; 6: 60-63.

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