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GC-MS ANALYSIS AND PHYTOCHEMICAL SCREENING OF A FERN *HEMIONITIS ARIFOLIA* (BURM.) MOORE FROM TIRUMALA HILLS

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ABSTRACT

The present paper reveals the phytochemical screening and GC-MS analysis of Hemionitis *arifolia*. Phytochemical studies showed the presence of alkaloids, flavonoids, phenols, tannins and saponins in higher to moderate concentrations and percentage of alkaloid 0.28 %, flavonoid 0.32 %, tannins 0.26% and saponins 0.52%. Butanol extract of the plant was analysed using Gas Chromatography- Mass Spectrometry, while 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 six compounds. The phytoconstituents screened were Mesityl oxide, 2- pentanone, Benzene, Neophytadiene, 2- Hexadecen-1,ol, n-Hexadecanoic acid, Phytol, oleic acid, stearic acid, Stigmast-4-en-3-one. The ten compounds were identified by comparing their retention time and peak area with that of literature and by interpretation of mass spectra. This study scientifically validates the use of plant and further research leading to possible drug development.

KEY WORDS: **GC- MS Analysis, Phytochemical Screening, *Hemionitis arifolia* ,Tirumala Hills**

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INTRODUCTION

Man has been using ferns as a source of food, medicines and many other necessities of life since ancient times. According to world fern statistics by Micheal Hassler, India has 1042 pteridophytes species, out of 10,000-12,000 fern species of the world. The medicinal qualities of fern real or imaginary are mentioned as early as 300 B.C. by the Greek, Philosopher Theophrastus and his Indian Contemporaries Sushrut and Charak [1-3]. Pteridophytes are used in Homeopathic, Ayurvedic, Tribal and Unani medicines and provide insecticides, antibiotics, food and ornamentation but habitat destruction by man has today endangered more than 10% of the fern species. Kirthikar *et al.(1935)* have described 27 species of ferns having varied medicinal uses [4]. Chopra *et al*. (1956) have included 44 species and Nadkarni (1954) recorded 11 species of Pteridophytes having medicinal importance [5,6]. Nayar (1959) recorded 29 medicinal ferns [7]. Medicinal plants represent a rich source of antimicrobial agents [8]. Herbal drugs and natural products have been known to human being for many years and they have been used as a source for different therapy and treatment of many diseases [9]. Still many species of pteridophytes are yet to be explored for their potential applications for future use and to isolate new active principles from them. *Hemionitis arifolia* (Burm.) Moore of family Hemionitidaceae is one of the endemic and widely distributed species on Tirumala hills of Tirupathi, Andhra Pradesh, India. Ethanomedicinally, the genus is important and popularly known as Ramabanum. It has been used in burns, menstrual disorders, antifertility and anti-flatulence. *Hemionitis arifolia* fronds are simple, rhizome short, sub erect, clothed with narrow brown scales. Stipes of sterile fronds 4-9cm long, stipes of fertile frond 15-30cm long, lamina dimorphous, sori continues along the veins, forming a network all over the surface. *Hemionitis arifolia* is widely distributed in eastern ghats of Tirumala hills. *Hemionitis arifolia* frond juice has been used to cure burns and a folklore anti- diabetes fern, was evaluated for its hypoglycaemic and anti-diabetic properties using rats [10, 11]. The medicinal importance of *Hemionitis arifolia* 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 plant viz., roots, leaves, stems etc., Considering all these facts, the present investigation is designed to find out phytochemical screening and GC-MS analysis of *Hemionitis arifolia* which evokes various therapeutic effects.

Figure 1
Hemionitis arifolia (Burm.) Moore

MATERIALS AND METHODS

Collection and Identification of Plant

The total plant of *Hemionitis arifolia* (Burm.) Moore was collected from the Thumburatheertham of Tirumala hills, Tirupati. The plant was identified using a dictionary of the Pteridophytes of India and was authenticated by Dr.K.Madhava Chetty, Assistant Professor, Department of Botany, S.V. University, Tirupati voucher specimen No.2029. The plant was washed 2-3 times with tap water and distilled water to remove the soil and dirt particles then gabbled, pulverized, air dried and subjected to gradient extraction with soxhlet apparatus.

Phytochemical Screening

The Extracts were analyzed for the presence of phytoconstituents such as Alkaloids, Flavonoids, Saponins, Tannins and Phenols. Following standard procedures were used.

Mayer's test for Alkaloids

To the acidic solution, Mayer's reagent (Potassium mercuric iodide solution) was added. Appearance of cream coloured precipitate indicates the presence of alkaloids.

Ferric chloride test for Tannins

Small quantity of extract was mixed with water and heated on a water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green solution indicates the presence of tannins.

Shinoda's test for Flavonoids

To one ml each of alcoholic extract, a small piece of magnesium ribbon or foil was added and 3-4 drops of concentrated HCL. were added, change in colour from red to pink shows the presence of flavonoids.

Froth test for Saponins

About 0.2 g of the extracts was shaken with 5 ml of distilled water and then heated to boil. Frothing (appearance of a creamy mass of small bubbles) shows the presence of saponins.

Ellagic test for Phenols

One ml of each of the various extracts dissolved in alcohol and treated with 2-3 ml of 5% neutral ferric chloride solution. Colour change indicates the presence of phenols.

Quantitative analysis

Determination of Alkaloids

5 g of the sample was weighedinto 200 ml of 20% acetic acid in ethanol was added and covered to stand for 4 h. This was filtered and the extract was concentrated using a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed.

Determination of Flavonoids

To estimate flavonoids quantitatively, 10 g powdered sample of each plant material was extracted twice with 10 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No.1, the filtrate was later transferred into crucibles, evaporated to dryness on a water bath to a constant weight.

Determination of Saponins

Twenty grams of each powdered sample were added to 100 ml of 20% aqueous ethanol and kept in a shaker for 30 min. The samples were heated over a water bath for 4 h at 55°C. The mixture was then filtered and the residue re-extracted with another 200 ml of 20% aqueous ethanol. The combined extracts were reduced to approximately 40 ml over a water bath at 90°C. The concentrate was transferred into a 250 ml separatory funnel, extracted twice with 20 ml diethyl ether. Ether layer was discarded while aqueous layer was retained and 60 ml n-butanol was added to it. Then n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath and after evaporation the samples were dried in oven (40°C) to a constant weight. The saponin content was calculated as percentage of the initial weight of sample taken.

Determination of Tannins

Tannin determination was done with some modifications. Distilled water (50 ml) was added to 500 mg of the sample taken in a 500 ml flask and kept in shaken for 1 h. It was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtrate was pippetted out into a test tube and mixed with 2 ml (10 fold diluted) of 0.1 M FeCl3 in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 605 nm within 10 min.

Table 1
Qualitative Phytochemical Screening of Hemionitis arifolia

S.No	Name of the test	Acetone	Butanol	Methanol	Water
A.	Alkaloids	++	+++	+	-
B.	Flavonoides	-	++	+	-
C.	Tannins	+	-	+++	-
D.	Phenols	++	-	-	+
E.	Saponins	+ +++	++		+

+++ - High Concentration, ++ - Moderate Concentration, + - Low Concentration - Negative

Table 2
Quantitative Phytochemical Constituents of Hemionitis arifolia

Phytochemical study	Results (mg/g)
Alkaloids	0.28
Flavonoid	0.32
Saponin	0.52
Tannin	0.26

Preparation of sample for GC-MS analysis

30 gm of the powdered plant material was soaked in 95 % acetone for 12 hrs. The extract was then filtered through Whatman filter paper No.1 along with 2 gm sodium sulphate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper was made wet with 95 % ethanol along with sodium sulphate. The filtrate was then concentrated by bubbling nitrogen gas into the solution. 2 µl of this solution was employed for GC-MS analysis.

GC-MS analysis

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 capillary column (30 x 0.25mm x 0.25m df) composed of 5% Diphenyl / 95% Dimethyl poly siloxane. Helium (99.999%) was used as the carrier gas at a constant flow of 1ml per min and injection volume of 2µl was adopted (split ratio of 10:1) The injected sample was detected by Turbo mass gold detector (Perkin Elmer) with the aid of Turbomass 5.2 software. During 36 minute GC extraction process, the oven temperature was programmed from of 110° C with an increase of 10°C/min up to 200° C, then 5°C/min up to 280° C (9 minutes hold). The injector temperature was set at 250°C (mass analyzer). Other parameters involved in the operation of Clarus 500MS, was also standardized (Inlet line temperature: 200°C; Source temperature: 200° C). Mass spectra were taken at 70eV and fragments from 45-450 Da. The MS detection was completed in 36 minutes. The detection employed the NIST (National Institute of Standards and Technology).

Compounds identification

Identification was based on the molecular structure, molecular mass and calculated fragments. Interpretation of mass spectrum GC-MS was conducted using the database of

National Institute Standardand Technology [NIST]. The name, molecular weight and structure of the components of the test materials were ascertained. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The spectrum of the unknown component was compared with the spectrum of the component stored in the NIST library.

RESULTS AND DISCUSSION

In the present study, the phytochemical screening of *Hemionitis arifolia* qualitatively and quantitatively shown the presence of Alkaloids, Flavonoids, Tannins, Saponins along with Phenols in all the extracts investigated. Pink colour was observed for Flavonoids (Shinoda's test), cream coloured precipitate for Alkaloids, blue colour was observed for tannins and saponins (Table 1 & Table 2).Some of the flavonoids that favour polar solutes entry bind to the bacteria's structural membrane proteins called porins, causing changes in the tridimensional confirmation exposuring the hydrophilic character of the pore, which lead to an easier passage of other polar bioactive compounds via diffusion [22]. Saponins have the property of precipitating and coagulating human RBC [23]. Flavonoids on the other hand are water soluble antioxidants and free radical scavengers, which are capable of preventing oxidative cell damage and have strong anticancer activity [24, 25].Tannins have astringent property, hasten healing of wounds and inflamed mucous membrane.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. They are confirmed by the study of base peaks, retention time (RT), and molecular weight (MW) of the compounds. By the study of GC-MS we identify ten major chemical constituents. They are identified as 3- penten -2-one (synonym: 4- methyl & Mesityl oxide), 2-pentanone, (snonym: 4- Hydroxy -4- methyl & diaceton), Benzene (synonym: 1,3- dimethyl & m- xylene & m-xylo), Neophytadiene (synonym 2,6,10 – Trimethyl, 14- Ethylene), 2-Hexadecen-1,ol (synonym: 3,7,11,15 – tetramethyl-R), n-Hexadecanoic acid, Phytol, 9-octadecenoic acid(z) (synonym: oleic acid), Octadecenoic acid (synonym: stearic acid) and Stigmast-4-en-3-one (synonym: 4- stigmasten-3-one). (Table 3) (Figure 2 & Figure 3). The first compound identified with less retention (3.765 min) 3- penten -2-one and 4- methyl & Mesityl oxide whereas Stigmast-4-en-3-one was the last compound which took longest retention time (26.425 min) to identify. N-Hexadecanoic acid – Palmitic acid (RT) can be an antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant activities and hemolytic 5-alpha is a reductase inhibitors. Phytol is reported to have antioxidant, antiallergic, antinociceptive and anti-inflammatory activities [26, 27]. Recent studies have revealed that phytol is an excellent immunostimulant, it is superior to a number of commercial adjuvants in terms of long-term memory induction and activation of both innate and acquired immunity [28, 29]. Our results are in agreement with reports that many plant extracts have been as a source of medicinal plant to cure a number of diseases.

Figure 2
GC-MS Chromatogram of Hemionitis arifolia, Butanol extract

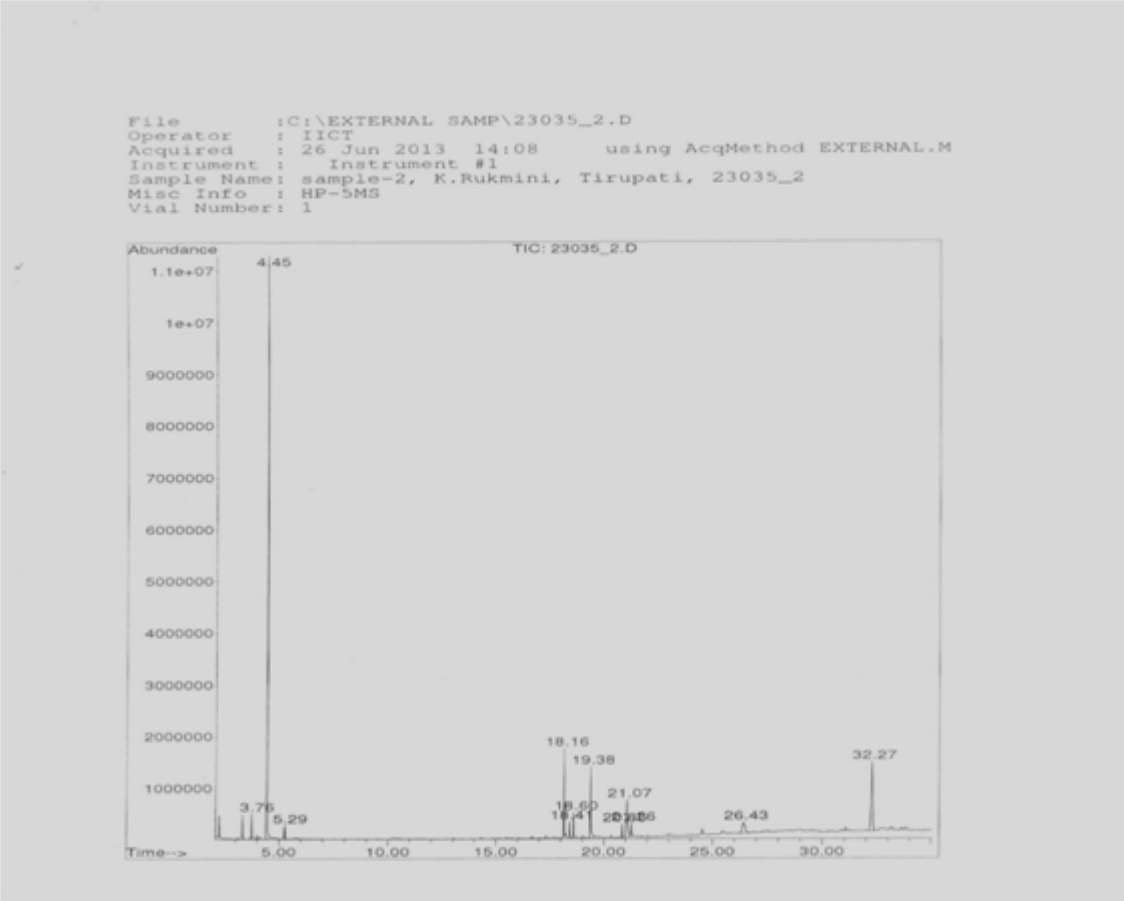
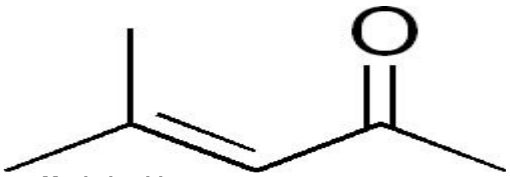


Table 3
Chemical constituents identified in the Butanolic extract of Hemionitis arifolia by GC-MS analysis

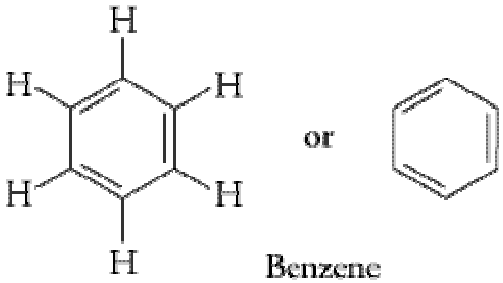
S.NO.	Retention time	Name of the compound	Base peak	Molecular Ion Peak
1	3.750	Mesityl oxide	83	98
2	4.456	2- pentanone	43	101
3	5.288	Benzene	91	106
4	18.165	Neophytadiene	68	278
5	18.594	2- Hexadecen-1,ol	68	278
6	19.376	n-Hexadecenoic acid	43	256
7	20.839	Phytol	71	123
8	21.066	oleic acid	55	264
9	21.267	stearic acid	43	284
10	26.425	Stigmast-4-en-3-	124	412

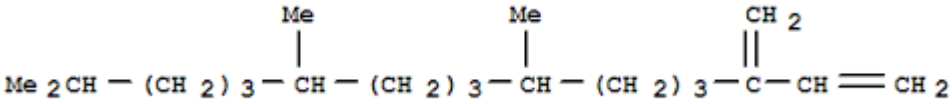
Figure 3
Phytochemical compounds identified in Butanolic extract of Hemionitis arifolia



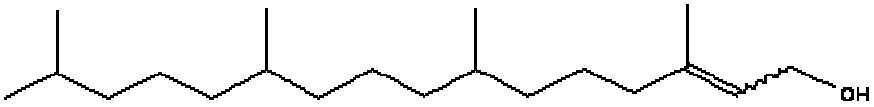
Mesityl oxide

2- pentanone



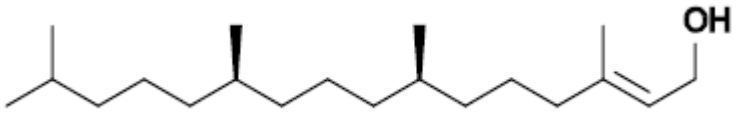


Neophytadiene



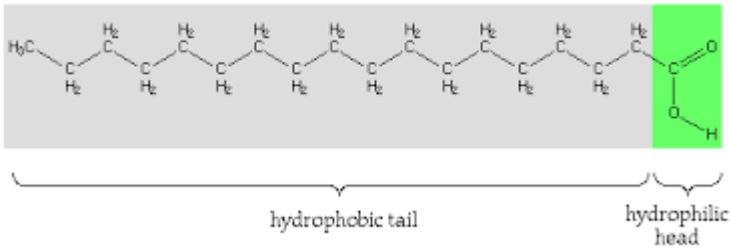
2-Hexadecen-1-ol

n-hexadecanoic acid

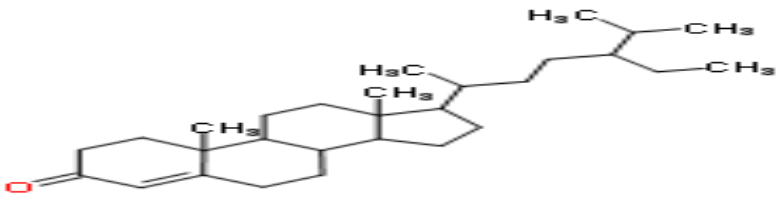


Phytol

Oleic acid



Stearic acid



Stigmast-4-en-3-one

CONCLUSION

It is concluded that the total plant extract of *Hemionitis arifolia* proved to be a reservoir of bioactive compounds, which could be used in various diseases in future. However, isolation of individual compounds and their biological activities needs to be uncovered further to enhance its pharmacological importance and open new avenues in research and may be recommended as a plant of phytopharmaceutical importance.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

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