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IDENTIFICATION AND QUANTIFICATION OF PHYTOCHEMICALS IN *HEMIONITIS OPPOSITA* (KAULF.) CHRISTENH, AN UNEXPLORED FERN OF VALPARAI HILLS

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ABSTRACT

Plants protect themselves from biotic stresses with the help of secondary metabolites and researchers over the years demonstrate that they can also protect humans against diseases. Among plant species, the pteridophytes are the least favoured group in exploring phytochemicals to utilize in medicine preparation. Hence, the present study was proposed to investigate the secondary metabolites of *Hemionitis opposita*, a small to medium sized fern which belongs to the family Pteridaceae. The leaves of *Hemionitis opposita* were analyzed for the phytochemicals and inorganic constituents. Preliminary phytochemical screening reported the presence of alkaloids, tannins, terpenoids, glycosides and cardiac glycosides in methanolic extract and flavonoids alone in the chloroform extract. GC-MS analysis of methanol extract revealed the presence of bioactive compounds. Quantification of total alkaloids, tannins and terpenoids in the methanolic extract of selected fern reveals least amount of alkaloids (0.074 mg/ml) and more amounts of terpenoids (0.705 mg/ml). Analysis of inorganic nutrients in the methanolic extract of *H. opposita* contains calcium, chloride, phosphate and nitrate.

KEYWORDS: *Hemionitis opposita*, phytochemicals, GC-MS, inorganic constiuents.

INTRODUCTION

The survival of the human race would be more complicated when no plant species were found on our living planet earth. The dependence of human beings on plants dates back when

the human race starts to evolve (Muhammad *et al.*, 2016). According to the World Health Organization (WHO), 80% of the world's population depends on plant-derived medicines for their healthcare needs.

Ferns and their allies are in a major division of the plant kingdom and they have been around for millions of years. They play a vital role in the earth's biodiversity (Pradnya *et al.*, 2015). There are over 250 different genera and 12, 000 species of ferns reported all over the world. It has been observed that pteridophytes are not infected by microbial pathogens, which may be one of the important factors for the evolutionary success of pteridophytes and the fact that they have survived for more than 350 million years. As per the folk medicine, pteridophytes have been known for more than 2000 years and have also been mentioned in the ancient literature. The tribal communities, ethnic groups and folklore throughout the world are utilizing fern parts like rhizome, stem, fronds, pinnae and spores in various ways for the treatment of various ailments (Kumar and Kaushik, 1999). The Ayurvedic and unani systems of medicine recommended the medicinal use of pteridophytes (Panda *et al.*, 2014).

Generally the ferns contain higher levels of carbohydrates, amino acids, proteins, lipids and secondary metabolites, which are economically useful and biologically active. Phytochemical characterization of ferns is important as it relates to therapeutic actions. It is perhaps obvious that different species of ferns would have different chemical constituents while these plant products protect them from various stresses, they can also protect humans against diseases (Smitha and Vadivel, 2019).

Therefore, the present study has been carried out to investigate phytochemicals and the inorganic constituents of *Hemionitis opposita*, a small to medium sized fern which belongs to the family Pteridaceae and distributed in abundance in the study area.

MATERIALS AND METHODS

Collection and identification of *H. opposita*

Healthy and matured fern leaves were collected from the Valparai hills, Western Ghats, part of Tamil Nadu. The fern was identified and authenticated by Botanical survey of India, Southern Regional Centre, Coimbatore (voucher number BSI/SRC/5/23/2021/Tech/89). The fern samples collected were washed thoroughly 2 to 3 times with running tap water followed by a wash with distilled water. Then the leaves were shade dried, coarsely powdered and stored in air tight container for further analysis.

Figure 1: Habit of *Hemionitis opposita.*

Sample extraction

Organic solvents in the increasing order of polarity (chloroform, methanol) and the powdered leaf extract of the *Hemionitis opposita* were prepared according to the method described by Harbone (1998). The samples were then sequentially extracted using a soxhlet apparatus and were subjected to detect the presence of different phytoconstituents.

PHYTOCHEMICAL ANALYSIS

Qualitative study

The methanolic and chloroform extracts of *H. opposita* were tested using the standard procedures for the presence/absence of phytochemical constituents viz. alkaloids, tannins and cardiac glycosides (Shruti *et al.*, 2013); phenols, saponin, resins, phlobatannins and volatile oils (Shakoor *et al.*, 2013); flavonoids and glycosides (Santhi and Sengottuvel, 2016); terpenoids (Damayanthi *et al*., 2019) and steroids (Edeoga *et al*., 2005).

Quantitative Estimation

Alkaloids, tannins and terpenoids reported in the methanol extracts of *Hemionitis opposita* were quantified in the present study.

Estimation of Alkaloids

The alkaloid content was estimated by Harborne (1973) method. Plant sample (1gm) was added with 10% acetic acid (200ml) and allowed to stand for four hours. Filtered sample was concentrated $1/4^{th}$ of original volume. Precipitation was obtained by adding concentrated NH_4OH and washed with dilute NH_4OH. The residue was dried and weighed.

Estimation of Tannins

Five grams of the fern powder was dissolved in 50mL of methanol and left for 2 hrs on shaking at 200-220 rpm at room temperature. The mixture was then added with ferric chloride drop wise until the green-black colored tannin precipitate was formed. Tannins were separated from the precipitate and dried on filter paper under the shade and weighed for the total plant tannin contents (Hossain and Nagooru, 2011; Shah and Hossian, 2014).

Estimation of Terpenoids

About 10 gm of powdered sample was taken and soaked in alcohol for 24 hrs. It was filtered and the filtrate extracted with petroleum ether was estimated as total terpenoids (Ferguson, 1956).

Tests for inorganic nutrients

The methanolic extract was tested for the presence and absence of the inorganic nutrients viz. chloride, sulphate, phosphate, nitrate, carbonate, iron and calcium (Bharti, 2018).

GC-MS Analysis

Gas Chromatography (GC) analysis was carried at Vellore Institute of Technology (VIT), Chennai for methanol extract of *H. opposita*. It is one of the key techniques generally used for the screening of many groups of the phytochemicals. The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min and the injector temperature (260°C) during the chromatographic run. The 1µL of extract sample injected into the instrument the oven temperature was as follows: 60 °C (2 min); followed by 300 °C at the rate of 10 °C min−1; and 300 °C, where it was held for 6 min. The mass detector conditions were: transfer line temperature 230 °C; ion source temperature 230 °C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The fragments from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library.

RESULTS AND DISCUSSION

The results of the preliminary phytochemical analysis of the methanol and chloroform extracts of *Hemionitis opposita* are given in Table 1. The methanolic extract of *Hemionitis opposita* revealed the presence of five phytochemicals such as alkaloids, terpenoids, tannins,

cardiac glycosides and glycosides, but the chloroform extract reported only the presence of flavonoids.

Table 1: Phytochemical analysis of *Hemionitis opposita* (Kaulf.) Christenh.

Phytoconstituents	Methanol	Chloroform
Flavonoids	-	+
Terpenoids	+	-
Phenols	-	-
Alkaloids	+	-
Saponins	-	-
Tannins	+	-
Cardiac glycosides	+	-
Glycosides	+	-
Steroids	-	-
Resins	-	-
Phlobatannins	-	-
Volatile oils	-	-

+ connotes Present

- connotes Absent

The quantitative phytochemical analysis of the methanolic extract of *Hemionitis opposita* reveals more amount of terpenoids (0.705 mg/ml) and less amount of alkaloids (0.074 mg/ml) and (Fig 1).

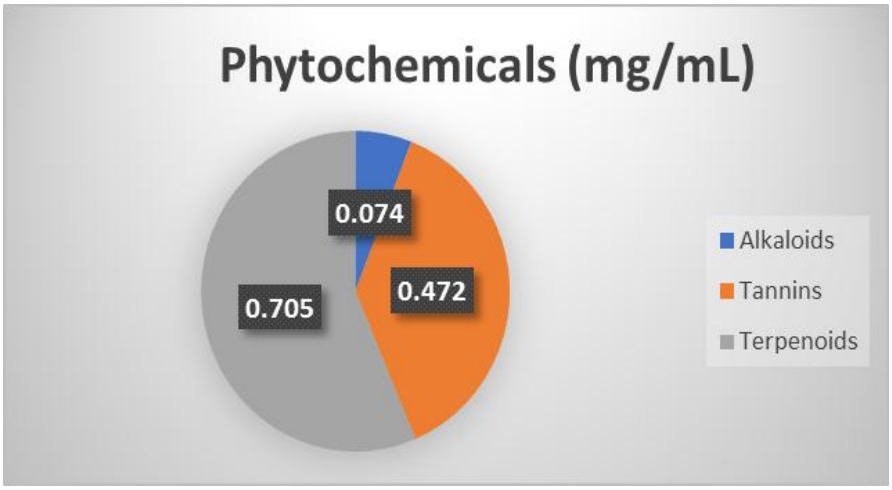

Fig. 1: Quantitative analysis of phytochemicals (methanol extract).

The methanolic extract of *Hemionitis opposita* was subjected to the analysis of inorganic constituents which revealed the presence of four inorganic constituents and the result are given in Table 2.

Table 2: Inorganic nutrients of *Hemionitis opposita* (methanol extract).

Nutrients	Observation
Calcium	+
Chloride	+
Sulphate	-
Phosphate	+
Nitrate	+
Carbonate	-
Iron	-

+ connotes Present

- connotes Absent

The present investigation reported an array of secondary metabolites in the selected fern *Hemionitis opposita*. Panda *et al*., (2014) reported the phytochemical investigation of some native pteridophytes. The result revealed the presence of various secondary active compounds. The methanolic extract demonstrated maximum occurrence of phytocontituents compared to chloroform extract. Similar results were obtained in the present study. The preliminary phytochemical analysis of *Blechnum orientale* L. showed the presence of twelve secondary metabolites (alkaloids, anthocyanin, anthraquinones, cardiac glycosides, coumarin, diterpines, emodins, flavonoids, saponins, steroids, tannins and terpenoids) in methanol extract (Fredrick Raja and John Peter 2019). In the present study also methanol extract of *Hemionitis opposita* also showed positive result for some of the phyto constituents (Table 1). *Hemionitis opposita* showed more amounts of terpenoids followed by tannins and less amounts of alkaloids. Kalpana Devi Rajesh *et al*., (2016) also observed highest amount of terpenoids and considerable amount of tannins in *Dicranopteris linearis*. Bharti (2018) reported the presence of inorganic nutrients in the methanol leaf extract of *Lygodium flexuosum* and *Amylopteris prolifera*. The result revealed the presence of six inorganic constituents (sulphate, phosphate, potassium, iron, chloride and calcium). In the present study, methanolic leaf extract of *Hemionitis opposita* also reported the four inorganic nutrients.

GC-MS Analysis

Methanol leaf extract of *Hemionitis opposita* subjected to the identification of a number of compounds which were identified through mass spectrometry attached with GC (Fig-3). Using the recorded retention time, molecular weight, molecular formula, peak area percentage, CAS number, different compounds were identified (Table-3).

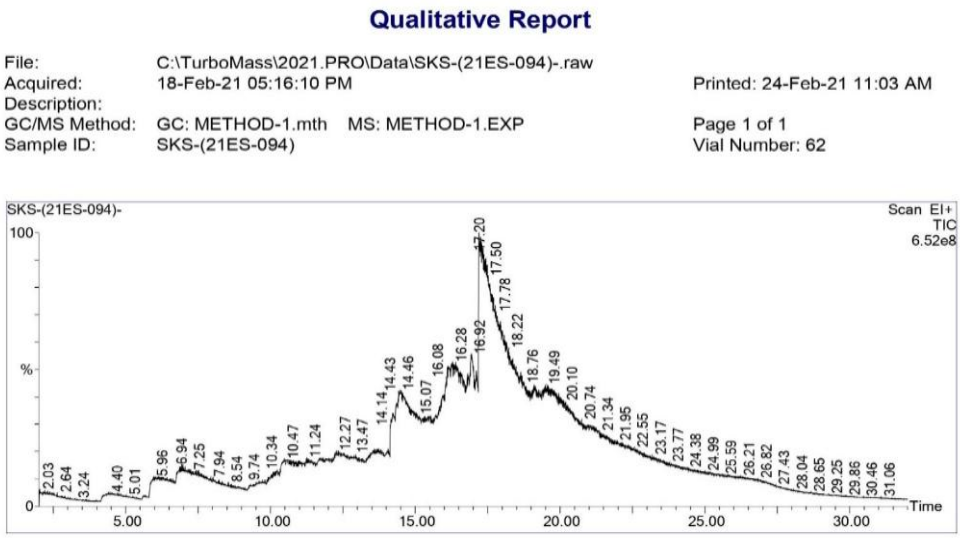

Fig. 3: Total ion chromatogram of Methanolic extract of *Hemionitis opposita.*

Table 3: Compounds identified in GC-MS analysis of methanol extract of Hemionitis *opposita*.

RT	Compound Name	Molecular Weight	Molecular Formula	Area %	CAS No.	Biological Role
6.135	2-Pentanone	86	$C_5H_{10}O$	3.963	107-87-9	Plant Metabolite
6.135	Methanecarbothiolic acid	76	$C_2H_4O_5$	3.963	507-09-5	Antimicrobial agent
10.587	2-Nonanol	144	$C_9H_{20}O$	2.538	628-99-9	Plant Metabolite, a pheromone and volatile oil component
11.237	2,3-Anhydro-D-Galactosan	144	$C_6H_8O_4$	2.879	900129-98-9	Preservative
12.338	Tetraacetyl-D-Xylonic Nitrile	343	$C_{14}H_{17}O_9N$	4.724	900130-04-4	Antioxidant, Anti-tumour property
12.338	D-Glycero-D-Ido-Heptose	210	$C_7H_{14}O_7$	4.724	900130-14-3	Anti- inflammatory, Anti-septic property
12.338	Ethanimidic acid, Ethyl ester	87	C_4H_9ON	4.724	1000-84-6	Antioxidant, Cancer preventive
14.518	Carbamic acid, Phenyl-,1-Methylethyl ester	179	$C_{10}H_{13}O_2N$	18.308	122-42-9	-
14.518	5-Dodecen-7-yne	164	$C_{12}H_{20}$	18.308	16336-82-6	-
16.394	9-Octadecenoic acid(Z)-, Phenylmethyl ester	372	$C_{25}H_{40}O_2$	10.365	55130-16-0	Cancer preventive, Anti-inflammatory
16.394	Eicosanoic acid	312	$C_{20}H_{40}O_2$	10.365	506-30-9	Anti cancer, antioxidant and cardioprotective agent
16.394	Methoxyacetic acid, Tridecyl ester	272	$C_{16}H_{32}O_3$	10.365	900281-82-0	Antimicrobial
16.944	Nitric acid, Nonyl	189	$C_9H_{19}O_3N$	2.599	20633-13-0	-

	ester					
16.944	2-Dodecen-1-yl(-)Succinic anhydride	266	$C_{16}H_{26}O_3$	2.599	19780-11-1	Antioxidant, Antimicrobial and antineoplastic agent
17.199	1-(P-Toluidino)-1-deoxy-.beta.-D-Idopyranose	269	$C_{13}H_{19}O_5N$	49.472	900226-06-4	-
17.199	n-Decanoic acid	172	$C_{10}H_{20}O_2$	49.472	334-48-5	Insecticide, aromatic compound
17.199	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	49.472	57-10-3	Antioxidant, Antimicrobial and antimalarial
19.105	Propanoic acid,2-(aminooxy)-	105	$C_3H_7O_3N$	5.153	2786-22-3	Antioxidant, Antiviral, Antimicrobial, Antinflammatory
19.105	Pentanoic acid,2-(aminooxy)-	133	$C_5H_{11}O_3N$	5.153	5699-55-8	Aromatic phenolic group of salicylic acid
19.105	Stevioside	804	$C_{38}H_{60}O_{18}$	5.153	77-05-4	Sweetening agent, Antineoplastic, Antioxidant, Antiinflammatory
19.105	5-O-Methyl-D-Gluconic acid dimethyl amide	237	$C_9H_{19}O_6N$	5.153	13096-67-8	Aromatic acid

The compounds identified in methanol extract of *H.opposita* have important biological activities which were illustrated in Table-3. The major peak was observed with 49.472 area% at 17.199 retention time (RT) which mainly possess the fatty acid compounds (n-Decanoic acid, n-Hexadecanoic acid) and sugar compound (1-(P-Toluidino)-1-deoxy-.beta.-D-Idopyranose). The next major peak was observed with 18.308 area% at 14.518 retention time which has Carbamic acid, Phenyl-,1-Methylethyl ester compound and an alkyne, 5-Dodecen-7-yne. The area% 10.365 at 16.394 RT has fatty acid (eicosanoic acid) and ester compounds (9-Octadecenoic acid(Z)-, Phenylmethyl ester and Methoxyacetic acid, Tridecyl ester). The prevailing compounds observed in 5.153 area% at 19.105 RT were carboxylic acid derivatives (Pentanoic acid,2-(aminooxy)- and Pentanoic acid,2-(aminooxy)-), diterpene glycoside (Stevioside) and an aromatic amide (5-O-Methyl-D-Gluconic acid dimethyl amide). The prevailing compounds noted in 4.724 area% at 12.338 RT were sugar compound (D-Glycero-D-Ido-Heptose), organic acid ester (Ethanimidic acid,ethyl ester) and Tetraacetyl-D-Xylonic Nitrile. A Ketone (2-Pentanone) was noted with 3.963 area% at 6.135 RT. A carbohydrate derivative (2,3-Anhydro-D-Galactosan), an alcohol compound (2-Nonanol) and carboxylic acid anhydride (2-Dodecen-1-yl(-)Succinic anhydride) were also identified.

CONCLUSION

The present study concludes that the phytochemical analysis of *Hemionitis. opposita* revealed the presence of alkaloids, tannins, terpenoids, glycosides and cardiac glycosides in the methanol extract and flavonoids in the chloroform extract. Inorganic nutrients of methanolic extract showed the presence of calcium, chloride, phosphate and nitrate. The quantitative estimation of total alkaloid, total tannin and total terpenoid revealed that the *H.opposita* possess more terpenoid content and traces of alkaloid. GC-MS analysis revealed the presence of biologically important compounds in methanol extract of *H.opposita.*

CONFLICT OF INTEREST

Authors have no conflict of interest.

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