Research Article

GC-MS Analysis and Phytochemical Screening of A Rare Pteridophyte *Nephrolepis Cardifolia* (L.) Presl. from Tirumala Hills

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ABSTRACT

Plants have been an important source of medicine with qualities for thousands of years. Mainly on traditional remedies such as ferns for their history it has been used as a popular folk medicine. *Nephrolepis cardifolia*(L.) Presl. of family Nephrolepidaceae found in hilly area of Tirumala hills, Tirupathi, Andhra Pradesh, India. Ethanomedicinally, the genus is important and popularly known as Fishbone fern. The present paper reveals the phytochemical screening and GC-MS analysis of *Nephrolepis cardifolia.* Preliminary phytochemical studies showed the presence of alkaloids, flavonoids, phenols, tannins and saponins in higher to moderate concentrations. Acetone extract of the plant was analyzed 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 Neophytadiene and 2,6,10-trimethyl,14,ethylene,2 Hexadecen-1-ol and 3,7,11,15 tetramethyl R-R, hexadecanoic acid & palmitic acid, 9 octadecenoic acid(z) & oleic acid, octadecanoic acid & stearic acid,Stigmast-4-en-3-one & 4-Stigmasten-3-one. The six compounds were identified by comparing their retention time and peak area with that of literature and by interpretation of mass spectra. Hence, at any rate *Nephrolepis cardifolia* is an attractive material for further research leading to possible drug development.

Keywords: GC-MS analysis, *nephrolepis cardifolia,* phytoconstituents, tirumala hills

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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 [1]. Higher plants as sources of 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 [2- 4]. Plants are a rich source of secondary metabolites with interesting biological activities. In general these secondary metabolites are an important source with a variety of structural arrangements and properties [5]. *Nephrolepis cardifolia* (L.) Presl. is commonly known as fishbone fern, ladder fern, sword fern. It has bright green fronds that are from 16-32 inches (40-80 cm) long and about 4 inches (10 cm) wide at their widest point. *N.cardifolia* is commonly cultivated as ornamental fern in Shilong and often the tubers are eaten to quench thirst [6]. Juice of root tubers is taken to treat fever, indigestion, headache, cough, cold and hematuria. Whole plant is used to

cure renal, liver and skin disorder [7]. It is a vigorous grower and colony former, producing small scaly tubers on their roots. In nature, they will grow in a wide variety of situations, growing in the soil or among rocks as well as growing as an epiphyte.

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, there are more significant advantages for GC-MS [10]. Thus, GC-MS should be the most preferable tool for the analysis of the volatile chemical compounds in herbal medicines. The medicinal importance of the plant 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, leaves, tubers etc., considering all these facts, present investigation is designed to find out phytochemical screening and GC-MS of *N.cardifolia* which evokes various therapeutic effects. Now we are aimed to isolate the bioactive compounds of *N.cardifolia, a* rare plant of Tirumala hills.

MATERIALS AND METHODS

Collection and Identification of Plants

The whole plant was collected from the Japalitheertham of Tirumala hills, Tirupati [11]. 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.2679. 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 analysis of different extracts

The Extracts were analyzed for the presence of phytoconstituents such as Alkaloids, Flavonoids, Saponins, Tannins and Phenols. Following standard procedures were used [12 - 14].

Mayer's test for Alkaloids

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

Plant of *Nephrolepis cardifolia* (L.) Presl.

Ferric chloride test for Tannins

Small quantity of extract was mixed with water and heated on 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 magnesium foil was added and 3-4 drops of conc.HCL was added,change in colour from red to pink shows the presence of flavonoids.

Froth test for Saponins

About 0.2 g of the extract was shaken with 5 ml of distilled water and then heated to boil. Frothing (appearance of 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.

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 filtratre was then concentrated by bubbling nitrogen gas into the solution. The extract contained both polar and non-polar phyto-components in the plant material. 2 µl of this solution was employed for GC-MS analysis [15].

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 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 moss and calculated fragments.

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standardand Technology [NIST] having more than 62,000 patterns. 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.

RESULT AND DISCUSSION

In the present study, the phytochemical screening of *Nephrolepis cardifolia* qualitatively 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**). 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 [16]. Saponins have the property of precipitating and coagulating human RBC [17]. 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 [18,19]. Tannins have astringent property, hasten healing of wounds and inflamed mucous membrane.

Table 1: Results of Preliminary Phytochemical Evaluation of *Nephrolepis cardifolia*

Test for Secondary Metabolites	Alkaloids Mayers Test	Flavonoids Shinodas test	Tannins Fecl3 test	Phenols Ellagic test	Saponins Froth test
Acetone	++	+++	+	++	_
Benzene	+++	++	+++	+++	++
Ethylacetate,	+	+	_	++	+
Methanol	_	_	++	++	+++
Ethanol	_	+	++	_	+++

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

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 six major chemical constituents. They are identified as Neo phytadiene and 2, 6, 10-trimethyl, 14-Ethylene, 2 hexadecen-1-ol and 3,7,11,15- tetra methyl-R-R, hexadecanoic acid and palmitic acid, 9-octadecenoic acid(Z) and oleic acid, Octadecanoic acid and stearic acid, Stigmast-4-en-3-one **(Table 2) (Figure 1 & 2)**. The first compound identified with less retention (18.152 min) was Neophytadiene and 2,6,10 trimethyl 14-ethylene whereas Stigmast-4-en-3-one was the last compound which took longest retention time (26.400 min) to identify. N-Hexadecanoic acid – Palmitic acid (RT) can be an antioxidant, hypo-cholesterolemic, nematicide, pesticide, lubricant activities and hemolytic 5-alpha is a reductase inhibitors. 9,12-Octadecanoic acid and squalene were identified in the ethanol leaf extract of Aloe vera [20] and Vitex negundo [21]. Our results are in agreement with reports that many plant extracts have been as a source of medicinal plant to cure number of diseases [22].

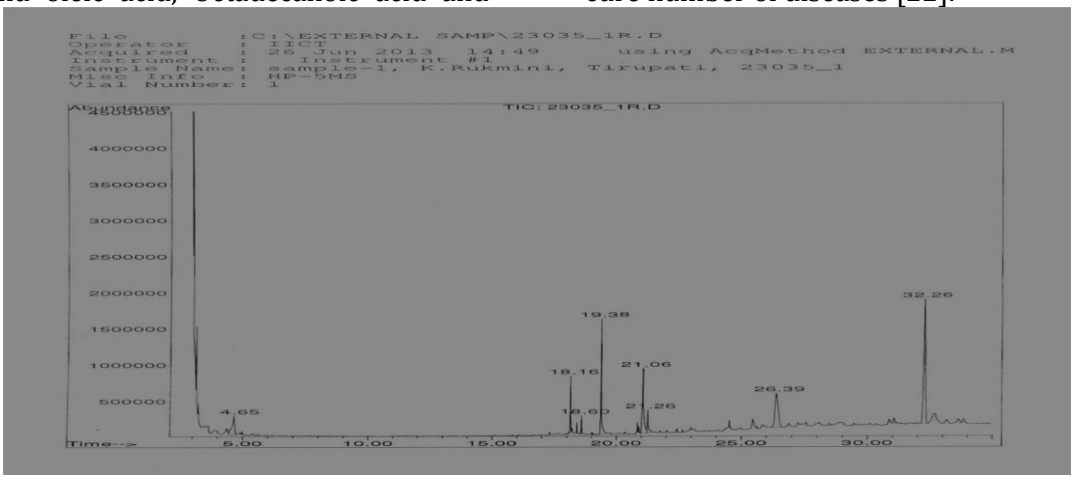
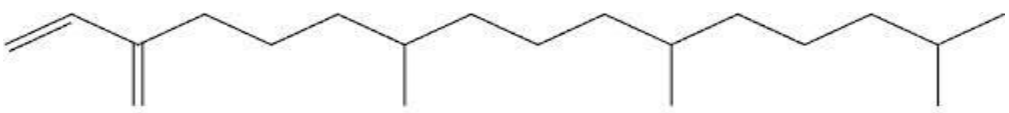


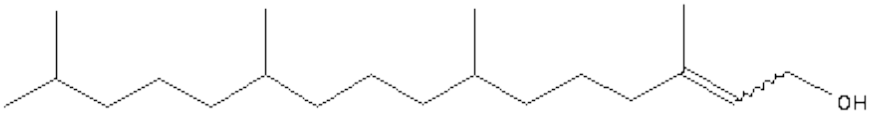
Figure 1: GC-MS Chromatogram of *Nephrolepis cardifolia*, acetone extract

Table 2: Name of the Chemical compounds identified by GC-MS Analysis

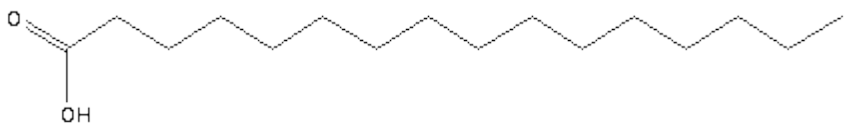
S.NO.	Retention time	Name of the compound	Base peak	Molecular Ion Peak
1	18.152	Neo phytadiene and 2,6,10-trimethyl,14-Ethylene (1)	68	278
2	18.594	2 hexadecen-1-ol and 3,7,11,15-tetramethyl-R-R (2)	68	278
3	19.376	hexadecanoic acid and palmitic acid (3)	73	256
4	21.065	9-octadecenoic acid(Z) and oleic acid (4)	55	264
5	21.255	Octadecanoic acid and stearic acid (5)	43	284
6	26.400	Stigmast-4-en-3-one and 4-Stigmasten -3-one (6)	124	412



neophytadiene

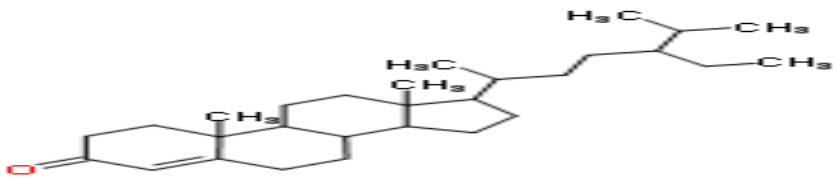


2Hexadecen-1-ol



Palmitic acid

Oleic acid



Stigmast-4-en-3-one

Figure 2: Acetone extract of *Nephrolepis cardifolia* were Identified by GC-MS analysis

CONCLUSION

It was concluded that acetone extract of the total plant of *Nephrolepis cardifolia* possesses various potent bioactive compounds and is recommended as a plant of phyto-pharmaceutical importance. Further subjects are required to explore the potential compounds responsible for the biological activity from N*ephrolepis cardifolia* for application in drug delivery, nutritional or pharmaceutical studies.

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