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Phytochemical Screening and FTIR Analysis in *Dryopteris hirtipes* (Blumze) *Kuntze* Linn

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A B S T R A C T

In this present study, to identify the active phytocomponents present in methanolic extracts of *D. hirtipes* by using column fractionation and identification of homogenous and heterogenous spots using TLC analysis of *D. hirtipes*. In FTIR, to identify the presence of functional group depends upon the active components present in the methanolic extract of *D. hirtipes*. Leaves of *D. hirtipes* was extracted with various organic solvents (Hexane, Ethyl acetate and Methanol) using Soxhlet apparatus. Phytochemical analysis was carried out with standard Protocols. Different Fractions were separated from methanolic extract of *D. hirtipes* by column chromatography and followed by thin layer chromatographic studies indicated that the leaf of *D. hirtipes* contains secondary metabolites and identify the spots with different R_f values and FTIR Analysis to find out the presence of functional groups with their characteristic peaks. The methanolic leaf extracts of *D. hirtipes* was found to be rich in phytochemicals like glycosides, steroids, alkaloids, phenols, terpenes, flavanoids and tannins. TLC was carried out with different solvent systems and identified the presence of Phenolic compounds. In FTIR analysis, showed the presence of groups namely alcohols, phenols, alkanes, alkenes, esters, and amine groups. The results obtained in this present study indicated in *D. hirtipes* contain more phytoconstituents and this provides more therapeutic information in future.

***Key words*:** *Dryopteris hirtipes,* Phytocomponents, Column fractionation, Thin layer chromatography, Fourier Transform Infra-Red Spectroscopy

Medicinal plants hold the potent bio-resource of drugs, as food additives, as pharma products and chemical compounds as drugs to meet their human health care needs [1-2]. According to WHO, Scientific reports concludes 80% of the world population needs natural sources for satisfying their health demands with less cost and minimal side effects [3]. Medicinal therapy provides great attention towards researchers to discover the new biological compounds for various illness [4-5]. Medicinal plants produce various secondary metabolites namely flavanoids, steroids, alkaloids, phenols and triterpenes [6] and found to be treated for illness. Due to more exploitation of medicinal plants leads a scarcity in getting bioactive compounds. Therefore, the researchers found an alternative to rectify the problem to use Pteridophytes for future use by humans.

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Pteridophytes constitutes the primitive vascular plants, contain major resource with diverse biochemical compounds [7]. Pteriophytes constitutes in larger extent in the world and consists of about 225-230 genera containing more than 1200 species in all over the parts of the world [8]. Ferns and fern allied group of plants are considered as reptile group of plants. Pteridophytes grow luxuriantly in moist tropical and temperate regions and found eco-geographically threatened regions from the sea level to high attitudes. Some of the Pteridophytes are in threatened list and some are in endangered in the Red Data Book of IUCN [9]. Bioactive compounds of fern mainly consist of phenols, flavanoids, alkaloids and terpenes [10]. Some of the researchers reported that the phenols, alkaloids, flavanoids, tannins distributed in plants have more biological effects includes antibacterial, antioxidant and anti-inflammatory effects [11-13] Presence of tannins has the ability to act as antiparasitic, antiviral and antibacterial activities. Flavanoids are related to polyphenols having antioxidant, anti-inflammatory properties. Alkaloids used to treat antimicrobial, anticancer and analgesic activities. Steroid Components act as a signaling molecule and have cardio-tonic activity. Phenol possesses an antioxidant and antiseptic property. Terpenes, a lipid derivative show a cytotoxic action. This phytochemical information reveals the identification of compounds in ferns which creates the new discovery in Pharma products [14].

Ferns, constitutes the major class of Pteridophytes, are reported for their medicinal uses to treat infections and contain many bioactivities such as anti-oxidant, antimicrobial, antiviral, anti-inflammatory, anti-tumor properties [15]. Recently, ferns and fern allied species is reported to have a great economic potential due to some interesting pharmacological properties and they have adapted with varied climatic conditions than the other primitive vascular plants [16]. Researchers also concluded that the Pteridophytes are not infected by pathogenic microorganisms and important factors for the evolutionary success of Pteridophytes to survive more than 350 million years [17]. *Dryopteris* species (Dryopteridaceae) genus contain more than 225 species found in tropical and sub-tropical regions [18]. In *Dryopteris* spp., rhizome parts are used for rheumatism, treat epilepsy and to cure leprosy [19], paste of the same plant applied on the snake bite, to cure the infection, pain, antifungal and insecticidal properties [20-22]. Young fronds *Dryopteris* species are edible which are used to cure anti-helminthic [23]. *Dryopteris hirtipes* leaf juice given in epilepsy and also used as antibiotics [18].

The genera of Dryopteris. *filix D. crassirhizoma*, *D. cochleata*, *D. chrysocoma*, and *D. syrmatica* possess good antimicrobial property [24-25]. Among *Dryopteris* species, *D. cochleata* and *D. affinis* shows the strong activity on the phytoconstituents like phenols, tannins and quinines possess antimicrobial and antifungal properties [26-27]. *D. cochleata* leaves possess antioxidant activity with good radical scavenging properties [28]. Some plant species belonging to the genus *Dryopteris* species shows bioactive components like alkaloids, flavanoids, quinones used for the treatment of disease and also reported to fight against bacterial, fungal infections [29]. Some Dryopteris sps., also possess tannins [30]. Now days, to treat various diseases in humans, modern techniques were used to isolate the secondary metabolites from Rhizome, fronds and stem. In the present study, investigates to identify the qualitative phytochemical screening present in *D. hirtipes* and Column chromatography separations were achieved with different solvent systems and TLC Profiling methods were carried out to separate organic compounds in fractions [31]. In FTIR analysis, showed the presence of C-OH alcohol and phenol, C=C-H-Alkyne groups, C=O Carbonyl groups, NH_2-C=O aromatic compounds. The detailed investigation was done with these processes helps in screening of bioactive compounds from plants which lead to further discovery of new drugs.

MATERIALS AND METHODS

Collection of the plant

The leaves of *D. hirtipes* were collected from Yercaud hills, Salem District, Tamil Nadu. The plant identification was confirmed with the help of Herbarium specimens in Botanical Survey of India, Coimbatore. The leaves were carefully separated, washed twice in tap water and then air dried under shade for more than two weeks. The shade dried fronds with spores of *D. hirtipes* were powdered by Electric blender. The powdered plant materials were stored in a closed glass container till their use in extract preparation. About 100 g of powdered leaves was extracted with various organic solvents like Hexane, Ethyl acetate and Methanol using Soxhlet apparatus and then concentrated by using rotary evaporator and noted down the weight of each crude extracts in (Table 1).

Phytochemical screening

Various extracts of polar and non-polar solvents (Hexane, Ethyl acetate, Methanol) were subjected to phytochemical analysis for the presence of bioactive constituents [32] in (Table 2).

Separation of bioactive compounds using column chromatography

From Various extracts, Methanolic extract was subjected to column chromatography for the separation of phytocompounds. Borosilicate glass column was used for the separation and to fractionate the samples. The glass column was properly rinsed with acetone and allowed to dry before packing the sample. A piece of cotton was placed at the end of the column and 20gm of slurry of about (60-120 mesh) size was used as a packing material and sample was poured on the top of the column. To prevent drying, the solvent level was maintained up to 6cm. By using a gradient elution technique, different fractions were collected for further analysis of bioactive compounds. The flow rate was adjusted to 5ml/minutes and 40 ml solvent was collected for each fraction (Fig 2).

Thin layer chromatographic studies

TLC Profile of sample was performed as per the standard methods. TLC was carried out on the 20x20cm TLC plates precoated with silica gel $60F_{254}$ (Merck). About 1µl of each extracts (Hexane, Ethyl acetate, Methanol) solutions were used as standards. The sample were loaded on the plates and it was kept in TLC glass chamber containing mobile phase (solvent saturated) was allowed to move through adsorbent phase up to $3/4^{th}$ of the plate. Different solvent systems were used for the identification of unknown bioactive compounds. The R_f values of the separated fractions were calculated by using the formula:

R_f = Distance travelled by the solute / Distance travelled by the solvent

FT-IR analysis

D. hirtipes leaf dried powder was subjected to IR Spectroscopy which showed the presence of functional groups. The chemical groups were spotted by the transverse frequencies present infrared regions represented in wave number in cm^{-1}. The frequencies shows the different chemical groups from each other.

Table 1 The result of preliminary phytochemical screening of *D. hirtipes*

Phytochemical test	Hexane extract	Ethyl Acetate extract	Methanol extract
Phenols	-	+	+
Flavonoids	+	+	+
Alkaloids	-	-	+
Saponins	-	-	-
Tannins	-	-	+
Glycosides	+	-	+
Quinones	-	-	+
Carbohydrates	+	+	+
Proteins	+	+	+
Terpenes	-	-	+

RESULTS AND DISCUSSION

Percent yield of concentrated crude fraction is shown in (Table 1). Crude Hexane (0.800mg), Crude Ethyl acetate (1.2gm), Crude Methanol (2.34gm). The results of the qualitative Phytochemical screening in various extracts are shown in (Table 1) and it shows the presence of several metabolites which have been involved in various medicinal properties. Some research investigated the presence of Phytochemical compounds in the extracts of various solvents

and explored the presence of Alkaloids, Phenol compounds, Flavanoids were influenced in Methanolic fern extracts and more specifically effective in the identification of Phenolic compounds for the analysis of antioxidant property [33-35]. Phenolic compounds are commonly reported to have the antioxidant activities [36].

Table 2 Column chromatography fractionation of the methanol extracts of the leaves of *D. hirtipes*

Eluent	Ratio	Fractions
Chloroform / Methanol	5%	F (1-10)
Chloroform / Methanol	10%	F (11-21)
Chloroform / Methanol	15%	F (22-32)
Chloroform / Methanol	20%	F (33-40)

Fig 1 Separations of compounds from F_3 and F_4 fractions by TLC

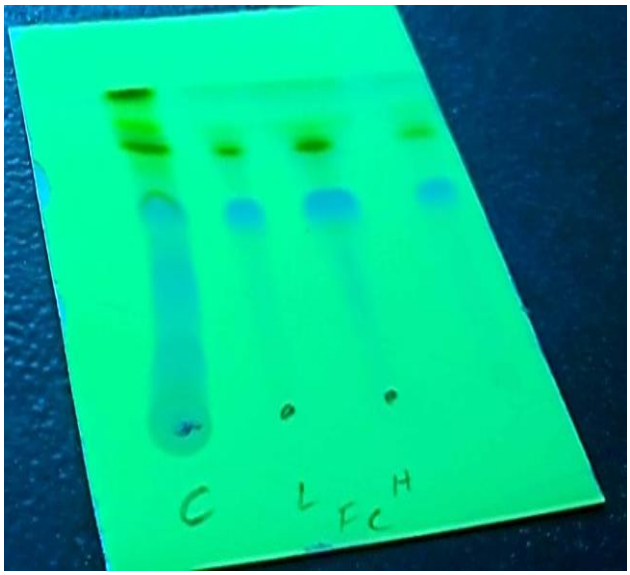

Fig 2 TLC profile of F_3 fraction (Low and high concentration)

The profile of each extract was subsequently analyzed by TLC. The Column chromatographic technique was carried out in Methanolic extract (2.34gm) in different solvent systems like Methanol with Ethyl acetate in the ratio (9:1,7:3,5:5) and chloroform and methanol in the ratio (9.5:0.5,9:1,8:2) and Hexane and ethyl acetate in the ratio (7:3,5:5). Fractions of 50 ml were collected in each tube (F_1 - F_4) using the eluents in combination of Methanol and Chloroform in the percentage ranges from 5%-20% represented in (Table 2) and a single spot identified in chloroform: methanol (8:2) in F_3 - F_4 in (Fig 1-2). Samples showing similar spots on their TLC Profiles were mixed. Fractions 22-40 which showed one homogenous spot-on TLC were combined and dried to afford the component. No specific spots were identified in F_1, F_2 fractions. These fractions (F_3 and F_4) were recrystallized and dried to get 0.13mg of compound for further spectroscopic analysis. This technique characterizes both organic and inorganic materials suggesting in potential chemical analysis of complex extract material [37]. In our TLC study, the most suitable TLC system for analysis was shown to be chloroform: methanol (8:2) and the R_f values were identified and it ranges about 0.18 to 0.20. In methanolic extract, Violet color spots were observed under UV light (254nm). This R_f indicates the presence of the bioactive compounds when sprayed with the strong KMNO4, it oxidized the functional groups (phenols and alcohols) and turns into brown color in (Fig 3). The suggested research finding concludes the spots may possess phenol and alkaloid derivatives compounds in methanol extract [38-39].

Fig 3 TLC plate (F_3) stained with strong KMNO $_4$ for visualization of compounds

FTIR Analysis is used to find out the presence of specific functional groups like alkenes, alkanes, carboxylic acids, aromatics, phenols, alcohols, alkyl halides, esters, sulphonates and also proved to be the reliable, sensitive method to identify the symmetrical and asymmetrical stretching frequency of different groups and some of the researchers reported the existence of functional groups in the Indian Medicinal Plants using different solvents [40]. The result of FTIR analysis confirmed the presence of major groups like alcoholic and phenol groups, alkanes, alkenes, aromatic amines and carboxylic acid functional groups. 619. 4, 721.67, 825.25, 1034.2, 1101.13, 1165.7, 1384.8, 1458, 1739.72395.9, 2854.0, 2925.1, 3009.6, 3423.74 (Fig 4, Table 3). Similarly, Vijay *et al*. [41] reported that the peak value 3423.7 shows hydrogen bonded OH Stretching indicates the presence of phenol, alcohols as functional groups, H-C-H stretching (2925.14, 2854.06) indicates alkanes, C=O (1739.71, 1034.2) indicates carboxylic acids, esters and ethers as functional groups. The result of this study offers a platform of using *D. hirtipes* as a herbal alternative for various diseases including anti-oxidant, anti-inflammatory, antimicrobial activities.

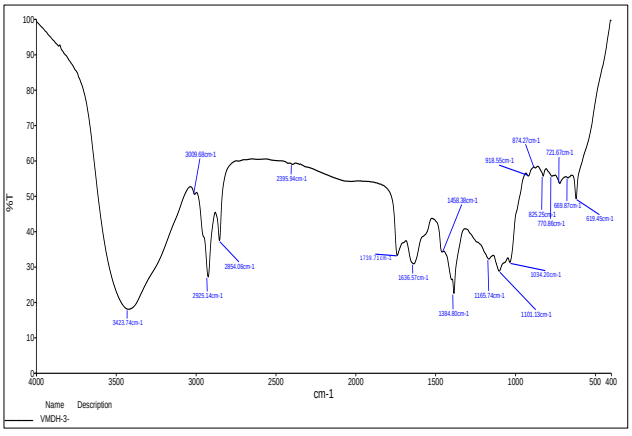

Fig 4 FTIR analysis of *D. hirtipes* leaf powder

CONCLUSION

The phytochemical tests performed on the various extracts of *Dryopteris hirtipes* and show the presence of phenols, flavanoids, terpenes were reported in methanolic extracts. By comparing with the various extracts, methanol was found to be the best solvent for extraction purpose. FTIR is used to identify the functional groups. Further study is needed to analyze the structure of compounds by using different analytical methods such as GC-MS and NMR studies. Further investigation is required for the pharmacological activity of specified compounds in *Dryopteris hirtipes* which may lead to explore the development of new drug by using it as a herbal medicine on scientific ground.

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