e-ISSN: 2249 – 7781
Print ISSN: 2249 – 779X

International Journal of Pharmaceutical Research & Analysis
www.ijpra.com

Research Article

SCRUTINY OF ANTI – RHEUMATOID ACTIVITY OF ETHANOLIC EXTRACT OF LEAVES OF NEPHROLEPIS CORDIFOLIA

Johnsy Mary F*, Leelavathi A

Assistant professor, Annai Veilankanni's College of Pharmacy, Saidapet, Chennai - 600015, Tamil Nadu, India.

ABSTRACT

Rheumatic arthiritis is an autoimmune and inflammatory disease, which means that immune system attacks healthy cells by causing inflammation in the affected parts of the body. The plant *Nephrolepis cordifolia* belonging to the family Nephrolepidaceae has traditional claim for Anti - rheumatic activity. Evaluation of crude drug can be attempted by different method which includes morphological and microscopical studies of the crude by analyzing the chemical, physical and biological behaviour. Shade dried powdered plant material of leaves of *Nephrolepis cordifolia* was used for the determination of physio chemical constants in accordance with WHO guidelines. Phytochemical evaluation is used to determine the nature of phyto-constituents present in the plant by using suitable chemical tests.The anatomical examination of Nephrolepis cordifolia leaf exhibited important microscopical characters such as xylem, phloem, palisade parenchyma, spongy parenchyma. Powder microscopy revealed the presence of sclereids, Epidermal cells. In phytochemical Screening, successive solvent extraction was carried out with ethanol, chloroform and hexane as solvent. Ethanol extract leaves showed maximum yield of 5.25% W/W. Efficacy of the extract was screened by evaluating anti rheumatic activity. FT-IR spectra had amply evidenced the occurrence of OH group. The FT-IR spectrum showed the presence of an alkyl amine, phenol ring, cyclo-alkane and carbonyl compounds. The results confirm the fact that this plant possess important bioactive constituent, hence to ensure the anti rheumatoid activity further scientific investigation will be proposed.

Keywords Nephrolepis cordifolia, FT-IR, Powder Microscopy, Phyto constituents.

INTRODUCTION

It is an autoimmune and inflammatory disease, which means that immune system attacks healthy cells by causing inflammation (painful swelling) in the affected parts of the body. A chronic inflammatory disorder affecting many joints, including those in the hands and feet .It is associated with increased morbidity and mortality. To standardize the leves of Nephrolepis Cordifolia by carrying out the pharmacognostical and phytochemical parameter. In India, young leaves are cooked as vegetables. Whole plant used for kidney, liver, and skin disorders.

Corresponding Author

F. Johnsy Mary

Email:

Phytochemical evaluation is used to determine the nature of phyto – constituents present in the plant by using suitable chemical tests. FTIR spectra had amply evidenced the occurrence of OH group. The FT- IR spectrum showed the presence of an alkyl amine, phenol ring, cyclo – alkane and carbonyl compounds.

MATERIAL AND METHODS

Preparation of Extracts:

The fern was chosen for the present study. *Nephrolepis cordifolia* was collected from a nursery. The ferns were washed thoroughly thrice with tap water, shade dried at room temperature and powdered.

Preparation of Plant Extracrt For FTIR Analyis

Dried powder (ethanolic extract) of test plant was used for FTIR analysis.1mg of the dried powder was encapsulated in 10mg of KBr pellet , in groups were

represented in table 2. FTIR gave broad peak at 1731.17cm $^{-1}$ which indicated the presence of c = o stretching. It gave a peak at 2833.52cm $^{-1}$ presence of OH bond. The peaks at 1080. 17, 1139.97,1181.44cm $^{-1}$ presence of alkyl amine.The peak obtained at 1487.19cm^{-1} indicated the presence of c=c ring stretching and the peaks at 592.17cm^{-1} indicated the presence of halogen compounds.There was no absorbance in between the region 3000-3500cm $^{-1}$ there was no alcohol group present in drug extract.The FTIR spectrum confirmed the presence of alkyl amine , carbonyl compound, halogen compounds, and cyclo alkane in powder pellet.

ETERMINATION OF PHYSIO – CHEMICAL CONSTANTS

Shade dried powdered plant material of leaves of *Nephrolepis cordifolia* was used for the determination of physio chemical constants in accordance with WHO guidelines.

DETERMINATION OF ASH VALUES

Ash values are helpful in determining the quality and purity of a crude drug in the powdered form. The residue remaining after incineration is the ash content of the drug, which simplifies represents inorganic salts, naturally occurring drug or adhering to it or deliberately added to it, as a form of adulteration.

TOTAL ASH

Total ash is designed to measure the total amount of material remaining after ignition. This includes both physiological ash which is derived from plant tissue itself and non – physiological ash which is the residue of the extraneous matter adhering to the plant surface.

PROCEDURE

Silica crucible was heated to red hot for 30 minutes and it was allowed to cool in desiccator. About 2gm of powdered sample was weighed accurately and evenly distributed in the crucible. Dried at 100-105 ^{o}c for 1 hour and ignited to constant weight in a muffle furnace at 600 +25^{o} c. The crucible was allowed to cool in a desiccator .The percentage of ash with reference to the air dried substance was then calculated.

WATER SOLUBLE ASH

The ash was boiled for 5min with 25ml of water. The insoluble matter was then collected in an ash less filter paper. It was washed with hot water and ignited for 15min at a temperature not exceeding 450 ^{o}c . The weight of the insoluble matter was subtracted from the weight of the ash and the difference in weight represented the water soluble ash, the percentage of water soluble ash with reference to the air dried substance was collected.

$$\text{Water soluble ash value} = \frac{\text{Weight of residue obtained}}{\text{weight of sample obtained}} \times 100$$

Acid Insoluble Ash

Acid insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid, igniting the remaining insoluble matter. This measures the amount of silica present, especially as sand and siliceous earth.

PROCEDURE

To the total ash obtained, 25ml of hydrochloric acid was added, covered with a watch glass and boiled gently for 5min on a burner. The watch glass was rinsed with5ml of hot water and these washings were added to the crucible. The insoluble matter was collected on an ash less filter paper by filtration and the filter paper was rinsed repeatedly with hot water until the filtrate is neutral and free from acid. Filter paper containing the insoluble matter was transferred to the crucible, dried on a hot plate and ignited to a constant weight in the muffle furnace at 450 – 500 ^{o}c. The silica crucible was removed from the muffle furnace and allowed to cool in a desiccator for 30 min, and then weighed without delay. The content of acid insoluble ash was calculated.

$$\textbf{Acid insoluble ash} = \frac{\textbf{weight of the residue obtained}}{\textbf{weight of the sample taken}} \textbf{ X 100}$$

Determination of Extractive Values

Extractive values are useful for the evaluation of Phyto constituents especially when the constituents of drug cannot be readily estimated by any other means. Further these values indicate the nature of the active constituents present in a crude drug.

Determination of Water-Soluble Extract

5gm of the powder was weighed and macerated with 100 ml of chloroform water (95 ml distilled water and 5ml chloroform) in a closed flask for 24 hours. It was shaken frequently for six hours and allowed to stand for eighteen hours. It was then filtered rapidly, taking precautions against loss of solvent and 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish. 2ml of alcohol was added to the residue and it was dried at 105^{o}c for 1 hour in the hot air oven and cooled in desiccators for 30min and weighed. The process was repeated till a constant weight was obtained; the percentage of water-soluble extractive value with reference to the air dried drug was calculated.

$$\textbf{Water soluble extractive value} = \frac{\textbf{weight of the dried extract}}{\textbf{weight of the sample taken}} \textbf{ X 100}$$

Determination of Alcohol Soluble Extractive

5gm of the powder was weighed and macerated with 100ml 90% ethanol in a closed flask for 24 hours. It was shaken frequently for six hours and allowed to stand for eighteen hours. It was then filtered rapidly, taking precautions against loss of solvent and 25ml of the filtrate was evaporated to dryness in a tarred flat-bottomed shallow dish. It was dried at 105 ^{o}c for 1hour in a hot air oven. The dish was cooled in desiccator and weighed. The process was repeated till the constant weight was obtained. The percentage of alcohol soluble extractive value with reference to the air-dried drug was calculate

$$\textbf{Alcohol soluble extractive} = \frac{\textbf{weight of the dried extract}}{\textbf{weight of the sample taken}} \textbf{ X 100}$$

Loss on Drying

Specified quantity of the substances was taken in a previously ignited and cooled silica crucible and the substance was evenly distributed by gentle side wise shaking. The crucible with the contents was weighed accurately. The loaded crucible and the lid were placed in the drying chamber (105 ^{0}c). The substance was heated for a specified period of time to a constant weight. The crucible was covered with the lid and allowed to cool in a desiccator at room temperature before weighing. Finally, the crucible was weighed to calculate the loss on drying with reference to the air-dried substance.

$$\textbf{Loss on drying} = \frac{\textbf{wt.of the sample before drying} - \textbf{wt.of the sample after drying}}{\textbf{weight of the sample taken}} \textbf{ X 100}$$

Determination of Foaming Index

1gm of the coarsely powdered drug was weighed and transferred to 500ml conical flask containing 100ml boiling water. The flask was maintained at temperature 80 – 90 ^{0}c for about 30min. It was then cooled and filtered into a volumetric flask and sufficient water was added through the filtrate to make up the volume to 100ml. The decoction was poured into 10 stopper test tube (height 16cm, diameter 16mm) in successive portions of 1ml, 2ml, 3ml, 4ml up to 10ml and the volume of the liquid in each tube was adjusted with water to 10ml. The test tubes were stoppered and shaked in a length wise motion for 15 seconds, two shakes per second. Allowed to stand for 15mins and the height of the foam was measured. The results are assessed as follows.

If the height of the foam in every tube is less than 1cm, the foaming index is less than 100. If a height of 1cm is measured in any tube, the volume of the plant material decoction in the tube (a) is used to determine the index. If this tube is the first or second tube in a series, prepare an intermediate dilution in a similar manner to obtain a more precise result.

If the height of the foam is more than 1cm in every tube, the foaming index is over 1000. In this case repeat the determination using a new series of dilution of the decoction in order to obtain a result. Calculate the foaming index using the following formula

Foaming index = 1000 /a

Where, a= the volume in ml of the decoction used for preparing the dilution in the tube where foaming to a height of 1cm is observed.

Determination of Swelling Index

The swelling index is the volume in ml occupied by the swelling of 1gm of plant material under specified conditions. A specified quantity of the plant material were previously reduced to the required fineness was accurately weighed and transferred into a 25ml glass stoppered measuring cylinder. The internal diameter of the cylinder should be about 16mm, the length of the graduated portion about 125mm, marked in 0.2ml divisions from o to 25ml in an upward direction. Unless otherwise indicated in the test procedure, add 25ml of water and shake the mixture thoroughly every 10min for 1hour, allowed to stand for 3 hours at room temperature. The volume in ml occupied by the plant material was measured including any sticky mucilage. Calculate the mean value of the individual determination, related to 1gm of plant material.

QUALITATIVE ANALYSIS

1. Detection of alkaloids

Dragendorff s reagent:

The substance was dissolved in 5ml of distilled water, to this 2M HCL was added until an acid reactions occurs , then 1 ml of dragendroff reagent was added and examined for an immediate formation of an orange red precipitate.

Mayer s reagent :

The substance was mixed with little amount of dilute hydrochloric Acid and Mayer s reagent examined for the formation of white precipitated.

Hager s reagent :

The substance was mixed with little amount of dilute hydrochloric acid and Hager s reagent examined for the formation of yellow coloured precipitate.

2. Detection of Tannin

Ferric chloride test :

To the extract solution few drops of 5% of Fecl3 solution were added the presence of phenolic compounds was determined by dark green colouration.

Lead acetate test :

To the extract solutions add few drops of 10 % of lead acetate solution formation of white precipitate, indicates the presence of tanni

Gelatin test :

To the extract solution few drops of 10% gelatin were added which results the formation of white precipitate, indication for presence of tannin.

3. Detection of Saponin:

Libermannburchard s test:

To the test solution few drops of glacial acetic acid and two drops of con. Sulfuric acid were added, suddenly colour changes indication for presence of saponin.

4. Detection of flavonoid:

Lead acetate test:

Extracts were treated with few drops of lead acetate solution. Indicated the presence of flavonoids by the formations of yellow colour precipitate.

Shinoda test :

To the extract 95% of ethanol was added with few drops of conc. HCL and 0.5g of magnesium turnings were also added. The presence of flavonoids was confirmed by observation of pink colour.

Detection of proteins

Biuret test:

To 3ml of the extract solution add few drops of 4% NaOH and few drops of 1% cuso4 solution. The appearance of pink or violet colour indicated the presence of protein

Detection of carbohydrates

Fehling test:

1ml of fehling's solution A and B was mixed and boil for 1min. equal volume of extract was added then it was heated in boiling water bath 5-10 minutes. First a yellow, brick red precipitate was observed.

Table 1: Preliminary phytochemical screening on leaf of Nephrolepis cordifolia.

SI.NO	PHYTO CONSTITUENTS	POWDER	HEXANE	CHLOROFORM	ETHANOL
1.	Alkaloid	+	+	-	+
2.	Saponin	-	-	+	-
3.	Glycosides	+	+	-	-
4.	Flavonoid	-	-	+	-
5.	Tannin	+	+	+	-
6.	Protein	-	+	+	-
7.	Carbohydrate	-	+	-	-

(+) - PRESENT (POSITIVE) (-) - ABSENT (NEGATIVE)

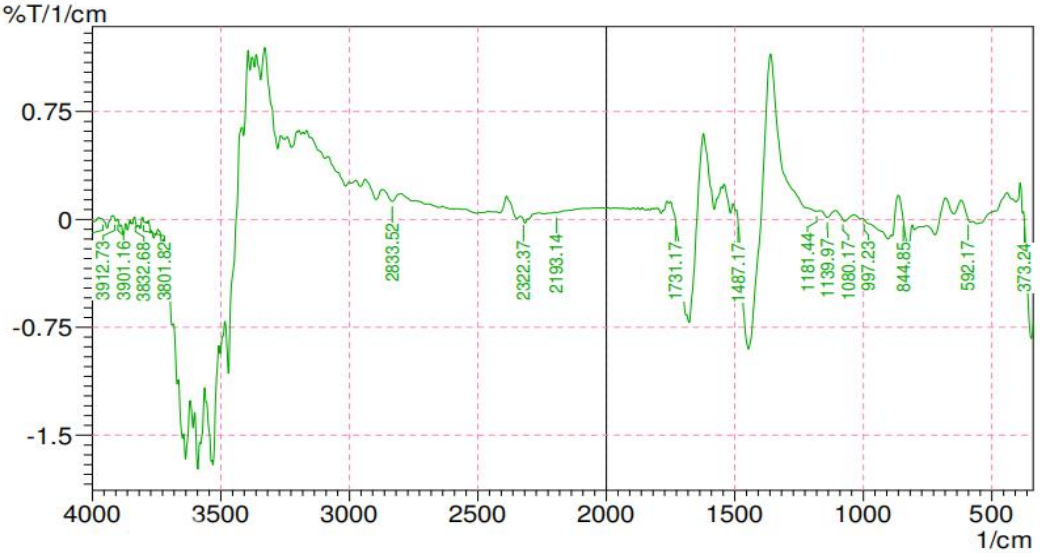
Table 2: The physio-chemical constant analysis leaf of Nephrolepis cordifolia

Si.no	Physiochemical constant	Percentage (%w/w)
1.	Ash values	
	Total ash	14.28 ± 0.72
	Water soluble ash	9.52 ± 0.48
	Acid insoluble ash	4.76 ± 0.24
2.	Extractive value	
	Alcohol soluble extractive value	32 + 1.0
	Water soluble extractive value	16 ± 0.95
3.	Loss on drying	4.05 + 0.50
4.	Foaming index	Less than 100
5.	Swelling index	Less than 100

	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	373.24	0	-0.01	374.2	372.27	385.78	94.53
2	592.17	0	0	593.13	591.2	385.78	94.19
3	844.85	0	-0.01	845.81	843.88	385.78	94.44
4	997.23	0	0	998.2	996.27	385.78	94.04
5	1080.17	0	0	1081.14	1079.21	385.78	94.28
6	1139.97	0.01	0.05	1165.04	1108.14	196.85	13.94

7	1181.44	0.06	0.09	1360.82	1166.01	534.24	31.08
8	1487.17	0	-0.02	1488.13	1486.2	385.78	94.44
9	1731.17	0	0	1732.13	1730.21	385.78	94.38
10	2193.14	0.05	0	2201.82	2136.23	212.91	0.48
11	2322.37	0	0	2323.34	2321.41	385.78	94.04
12	2833.52	0.13	0.07	2872.1	2798.8	204.5	5.42
13	3801.82	0	-0.01	3802.79	3800.86	385.78	94.32
14	3832.68	0	-0.01	3833.65	3831.72	385.78	94.17
15	3901.16	0	0	3902.12	3900.2	385.78	94.23
16	3912.73	0	0	3913.7	3911.77	385.78	94.35
17	3959.99	0	0	3960.96	3959.03	385.78	94.17
18	4179.88	0	0	4180.85	4178.92	385.78	93.42
19	4285.97	0	0	4286.94	4285.01	385.78	94.19
20	4344.8	0	0	4345.77	4343.84	385.78	94.17
21	4389.17	0	0	4390.13	4388.2	385.78	94.3
22	4491.4	0	0	4492.36	4490.43	385.78	94.29
23	4564.69	0	0	4565.66	4563.73	385.78	94.12
24	4629.31	0	0	4630.28	4628.35	385.78	94.53
25	4663.07	0	0	4664.03	4662.1	385.78	94.43

FT-IR spectrum of pellets of Nephrolepis cordifolia



CONCLUSION

In phytochemical screening, successive solvent extraction was carried out with ethanol, chloroform, hexane solvent. Ethanol extract of leaf showed maximum yield of 5.25 % w/w. The ethanolic extract was used in the pharmacological studies showed anti rheumatic activity. In the present study FTIR analysis of whole plant of *Nephrolepis cordifolia* showed the presence of phenolic compounds which are responsible for the various medicinal properties of test plant. Further research studies will be needed to find out the structural analysis of compound by use of different analytical methods such as NMR and Mass spectrometer.

REFERENCE

1. The Ayurvedic Pharmacopoeia of India , New Delhi : The controller of Publications : 2005.
2. Indian Pharmacopoeia. New Delhi The controller of publications, 1996.
3. Mukarjee PK , Quality of herbal drugs , New Delhi ; Business Horizons : 2002.
4. British pharmacopoeia General Medical council, London: Pharmaceutical press 1968.
5. Tyler VE , Brady LR , Robbers JE, *et al*. pharmacognosy , 9th edition , Philadelphia USA ; LEA and febrigoree. 1988.

6. Khandelwal KR , Practical pharmacognosy , Pune ; Niraliprakashan , 1998.
7. Wallis TE. Text book of pharmacognosy, CBS published and distributors: New Delhi, 1985.
8. Test methods for evaluating solid waste , physical / chemical methods , SW _ 846 , 3RD EDITION .Revision 1 , 1992.

Cite this article:
Johnsy Mary F, Leelavathi A**.** Scrutiny of Anti – Rheumatoid activity of ethanolic extract of leaves of *Nephrolepis cordifolia*. *International Journal of Pharmaceutical Research & Analysis, 13(1), 2023, 26-31*.