Physico-Chemical Analysis and Phytochemical Screening of Dryopteris Nigropaleacea Leaves

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ABSTRACT

Dryopteris nigropaleacea is a species of perennial leptosporangiate fern endemic to parts of Afghanistan, Pakistan, western Nepal, and Himachal Pradesh and Uttar Pradesh in India. Plants are mainly multicellular, predominantly photosynthetic eukaryotes of the kingdom Plantae. The term is generally limited to the green plants, which form an unranked clade Viridiplantae. There are about 300–315 thousand species of plants, of which the great majority, green plants provide most of the worlds molecular oxygen and are the basis of most of Earths ecologies, especially on land. These plants are also having great importance to the health of individuals and communities. Hence the Author proposes the screening of the above mentioned plant. The present paper deals with the investigation or screening of various phytochemicals present in various extract i.e. Methanol, with different phytochemical tests.

KEYWORDS: *physico-chemical analysis, phytochemical screening, dryopteris nigropaleacea.*

INTRODUCTION

For thousand years herbal medicines are the major remedy in traditional medicinal system and are they used in medicinal practice. The practice continues even today because of the biomedical benefits in many parts of the world. There is phenomenal increase in the demand for the herbal medicines especially for those, which have been scientifically validated. Review of literature suggests that there is tremendous focus on anti-oxidant molecules with respect to their diverse pharmacological properties such as analgesis, anti diabetic, anticancer and cancer prevention, protective roles on cardiac myopathy, neuropathy and nephropathy, anti-inflammatory and wound healing promotion. Disorders of inflammation have been implicated in various complications of diabetes as well as cardiovascular disorders. Antioxidants molecules have been used to alleviate inflammation and because of this reason they are being increasingly researched in the prevention or treatment of metabolic syndrome, aging and cancer. As the life expectancy in India is increasing the metabolic diseases and cancer are likely to be major burden on healthcare, which would need utilizing all the arsenals available in the nature[1]

Many species of *Dryopteris* has been used for diverse ethno medical applications since time immemorial in India. Its leaves has antifungal property and has been used as an antidote to snake bite, in diverse diseases like epilepsy, leprosy, cuts, ulcers, swelling, blood purification

and to alleviate muscular and rheumatic pain. The leaf extract has been reported to have antibacterial activity, antiepileptic activity, analgesic activity and mental disorders. It has reported significant antioxidant property in the leaves but the leavess have not been studies for the presence of antioxidant molecules. The efficacy of the plant in the metabolic disorders, wound healing, and hepatoprotective properties have not been investigated. Moreover, Leaves of *Dryopteris nigropaleacea* has not been investigated for its pharmacological properties to the best of my knowledge. Hence the aim and objectives of the present study is to investigate phytochemical and unexplored pharmacological properties related to analgesic, anti inflammatory, would healing and anti diabetic.[2]

From ancient time the tribal communities throughout the world are utilizing different parts of pteridophytes like leaves, stem, fronds, pinnae and spore in various ways for the treatment of various ailments as folk medicine. The numbers of contributors about the taxonomy, ecology and distribution of Pteridophytes have been published from time to time but enough attention has not been paid towards their useful aspects, specially the phytochemical part of Pteridophytes.[3]

MATERIAL & METHODS

1. Collection of plant material

The whole plants *Dryopteris nigropaleacea* was chosen for the present investigation, from the selected plants leaves has been collected in the months of Nov. 2019- Dec. 2019, from Himachal Pradesh Region and were identified and authenticated by botanist through sample number DN/19/001.

2. Preparation of plant powder

The leaves of plant were dried under shade and then powdered with a mechanical grinder. The powder was passed through Sieve No. 40 and stored in an airtight container for further use.

3. Physico-chemical evaluation[4-6]

The dried parts of *Dryopteris nigropaleacea* Leaves were subjected to standard procedure for the determination of various physicochemical parameters.

(i) Determination of ash values

The determination of ash values is meant for detecting low-grade products, exhausted drugs and sandy or earthy matter. It can also be utilized as a mean of detecting the chemical constituents by making use of water-soluble ash and acid insoluble ash.

a) Total ash value

Accurately about 3 gms of air dried powder was weighed in a tared silica crucible and incinerated at a temperature not exceeding 4500C until free from carbon, cooled and weighed and then the percentage of total ash with reference to the air dried powdered drug was calculated. The percentage of total ash with reference to the air-dried drug was calculated.

b) Acid insoluble ash

The ash obtained in the above method was boiled for 5 minutes with 25ml of dilute HCl. The residue was collected on ash less filter paper and washed with hot water, ignited and weighed. The percentage of acid insoluble ash was calculated with reference to the air dried drug.

c) Water soluble ash

The ash obtained in total ash was boiled for 5 minutes with 25 ml of water. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited to constant weight at a low temperature. The weight of insoluble matter was subtracted from the weight of the ash. The difference in weights represents the water soluble ash. The percentage of water soluble ash with reference to the air dried drug was calculated.

(ii) Determination of moisture content (Loss on drying)

Place about 10 g of drug (without preliminary drying) after accurately weighing in a tared evaporating dish and kept in oven at 1050 C for 5 hours and weigh. The percentage loss on drying with reference to the air dried drug was calculated.

(iii) Determination of foreign organic matter

Accurately weighed 100 g of the drug sample and spread it out in a thin layer. The foreign matter should be detected by inspection with the unaided eye or by the use of a lens (6X). Separate and weigh it and the percentage present was calculate.

(iv) Determination of swelling index

Swelling index is determined for the presence of mucilage. Accurately weigh 1 g of the powdered plant part and placed in 150 ml measuring cylinder, add 50 ml of distilled water and kept aside for 24 hours with occasional shaking. The volume occupied by the seeds after 24 hours of wetting was measured.

4. Determination of extractive values[7]

(i) Alcohol Soluble Extractive

5 gm of coarsely powdered air dried drug was macerated with 100 ml of alcohol in a closed flask for 24 hour, shaking frequently for six hours and allowed to stand for eighteen hours. It was then filtered rapidly taking precaution against loss of alcohol. 25 ml of the filtrate was evaporated to dryness in tared flat bottomed shallow dish, dried at 1050c and weighed. The percentage of alcohol soluble extractive was calculated with reference to the air dried drug.

(ii) Water Soluble Extractive

5 gm of coarsely powdered air dried drug was macerated with 100 ml of chloroform water in a closed flask for 24 hours, shaking frequently for six hours and allowed to stand for eighteen hours. It was then filtered rapidly taking precautions against loss of chloroform water. 25 ml of the filtrate was
evaporated to dryness in tared flat bottomed dish dried at 1050c
and weighed. The percentage of water soluble extractive was calculated with reference to air dried drug.

(iii) Ether soluble extractive

100 gm of coarsely powdered air dried drug was extracted in soxhlet apparatus with solvent ether for six hours. The extract is filtered into a tared evaporating dish and

evaporates off the solvent on a water bath. The residue is dried at 1050C to constant weight. The percentage of ether extractive was calculated with reference to air dried drug Extraction and phytochemical screening

5. Preparation of extract[8-10]

The already prepared coarse powder drugs of selected plants were used for the preparation of different extracts.

Chemicals: Methanol (85%), Distilled water with chloroform (2.5%)

The dried powder was extracted with methanol in a soxhlet apparatus. Aqueous extract was prepared by cold maceration process by using separate quantity of powder. The solvents were removed by distillation under reduced pressure and the resulting semisolid mass was vacuum dried using rotary flash evaporator. The percentage yields are presented in table.

6. Preliminary Phytochemical Screening[11-14]

a) Tests for carbohydrates and glycosides

Molisch's test

Sample was treated with 2-3 drops of 1% alcoholic - napthol solution and 2 ml of conc. sulphuric acid was added along the sides of the test tube. Appearance of brown ring at the junction of two liquids shows the presence of Carbohydrates.

Legal's test

To the sample add 1 ml of pyridine and few drops of sodium nitropruside solutions and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red colour shows the presence of glycosides.

Borntr ager's test

Sample was treated with chloroform and then the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. Ammonia layer acquires pink color, showing the presence of glycosides.

b) Test for alkaloids

A small portion of the sample was stirred separately with few drops of dilute hydrochloric acid and was tested with various reagents for the presence of alkaloids. The reagents are

Dragendroff's reagent: Reddish brown ppt o Wagner;s reagent - Reddish brown ppt

Mayer's reagent: Cream color ppt o Hager's reagent - Yellow color ppt

c) Test for proteins and free amino acids

Small quantities of the sample was dissolved in few ml of water and treated with following reagents.

Million's reagent: Appearance of red color shows the Presence of protein and free amino acid.

Ninhydrin reagent: Appearance of purple color shows the Presence of Proteins and free amino acids

Biuret's test: Equal volumes of 5% sodium hydroxide solution & 1% copper sulphate solution was added.

Appearance of pink or purple color shows the presence of proteins and amino acids.

d) Test for tannins

A small quantity of the sample was taken separately in water and test for the presence of phenol compounds and tannins was carried out with the following reagents.

Dilute Ferric chloride solution (5%): Violet color.

10% lead acetate solution: White ppt

e) Test for flavonoids

Alkaline reagent test

To the test solution add few drops of magnesium hydroxide solution, intense yellow color is formed which turns to colorless on addition of few drops of dilute acid indicates presence of flavonoids.

Shinoda's test

Small quantities of the sample was dissolved in alcohol, to them piece of magnesium followed by conc. hydrochloric acid drop wise added and heated. Appearance of magenta color shows the presence of flavonoids **.**

f) Tests for fixed oils and fats Spot test

: A small quantity of sample was separately pressed between two filter papers. Appearance of oil stain on the paper indicates the presence of fixed oil.

: Few drops of 0.5 N alcoholic potassium hydroxide were added to a small quantity of sample along with a drop of phenolphthalein, the mixture was heated on a water bath for 1-2 hours, format ion of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

g) Tests for steroids and triterpenoids

Libermann-burchard test

Treat the sample with few drops of acetic an hydride, boil and cool. Then add con. sulphuric acid from the side of test tube, brown ring is formed at the junction two layers and upper layer turns green which shows presence of steroids and format ion of deep red color indicates presence of triterpenoid.

Salkowski test

Treat the sample with few drop of conc. sulphuric acid, red color at lower layer indicates presence of steroids and formation of yellow colored lower layer indicates presence of triterpenoids.

h) Test for mucilage's and gums

Small quantities of sample was added separately to 25 ml. of absolute alcohol with constant stirring and filtered. The precipitates was dried in oil and examined for its swelling property for the presence of gum and mucilage.

i) Test for waxes

To the test solution add alcoholic alkali solution, waxes get saponified.

RESULTS AND DISCUSSION

The various extracts of the plant of *Dryopteris nigropaleacea* Leaves was subjected to phytochemical screening which reveals the presence of various pharmacological active components later. *Dryopteris nigropaleacea* Leaves was screen up and give a report on the possible preliminary phytochemical screening and exhaustive extraction of the plant material

with extraction of aqueous & methanolic further the extracts were screened for the presence of various medicinally active phytoconstituents.

Methanolic extract shows presence of alkaloids, carbohydrates, glycosides, tannins, protein, amino acids, and steroids. Chloroform extract shows presence of carbohydrates, tannins Aqueous extract shows presence of alkaloids, carbohydrates, glycosides, tannins, protein, amino acids, and steroids and Petroleum ether extract shows presence of alkaloids, carbohydrates, glycosides, protein and amino acids, steroids. Since, the major active constituents are present in Methanolic and aqueous extract, therefore, the Methanolic and Aqoeous extract were taken for further investigation. The results are shown for Screening in Table 1, 2, 3 and 4

Table No. 1. Physico-chemical analysis of *Dryopteris nigropaleacea.*

S.No.	Parameters (% w/w)	Values obtained (DNL)
1	Total ash (TA)	5.6
2	Water soluble ash (WSA)	3.2
3	Acid insoluble ash (AIA)	0.75
4	Moisture content (MC)	6.0
5	Swelling index (SI)	-
6	Foreign organic matters (FOM)	3.0

All reading are average of three values, n=3.

Abbr.: DNL=*Dryopteris nigropaleacea*. (Leaves)

Table No. 2. Extractive values of *Dryopteris nigropaleacea.*

S.No.	Solvents	Values obtained (DNL)
1	Alcohol soluble extractive value	16.4
2	Water soluble extractive value	18.5
3	Ether soluble extractive value	09.2

All reading are average of three values, n=3.

Abbr.: DNL=*Dryopteris nigropaleacea*. (Leaves)

Table No. 3. Percentage yield value of various extracts of *Dryopteris nigropaleacea.*

S.No.	Extract	Estimated percentage (%w/w)	Color of extract
1	Aqueous	14.3	Light Brown
2	Methanol	11.2	Brown

All reading are average of three values, n=3.

Table No. 4. Preliminary phytochemical screening of *Crotalaria burhia* Hamilt

S.No.	Constituents	Test	DNL	
			AQ	ME
1	**Alkaloids**	**Wagner's test**	+	-
		Mayer's test	+	-
		Hager's test	+	-
		Dragendroff' test	-	-
2	**Glycosides**	**Brontrager's test**	-	+
		Legal's test	-	+
3	**Carbohydrates**	**Fehling's test**	-	-
		Molisch's test	-	-
4	**Fixed oil and fats**	**Soap formation test**	-	-
		Spot test	-	-
5	**Tannins**	**Fecl3**	+	+
		Alkaline reagent Alkaline reagent	+	-
		Vanillin	-	-

		hydrochloride		
6	**Protein and amino acid**	**Biuret test**	+	+
		Ninhydrin test	+	-
		Spot test	-	-
		Million's test	-	-
7	**Flavanoids**	**With H_2SO_4**	-	-
		With NaOH	-	+
8	**Steroids and triterpenoids**	**Libermann's Burchard test**	+	+
		Salkowski's test	+	+
9	**Mucilage and gum**	**With 90% alcohol**	+	+
10	**Waxes**	**With alc. KOH**	+	-

Abbr.: +=Present, - = Absent, Aq= Aqueous extract, Me=Methanolic extract, DNL=*Dryopteris nigropaleacea*. (Leaves)

REFERENCES

1. *Bahadori MB et al. Antibacterial evaluation and preliminary phytochemical screening of selected ferns from Iran RJP 2015; 2(2): 53-59*
2. *Shakoor a mir. Preliminary phytochemical screening of some pteridophytes from district shopian (J & K) Int J Pharm Pharm Sci, Vol 5, Suppl 4, 632-637*
3. *Emily B. Sessa Thomas J. Givnish. Leaf form and photosynthetic physiology of Dryopteris species distributed along light gradients in eastern North America Functional Ecology 2014: 28, 108–123*
4. *Gautam GK, Vidyasagar G. Physicochemical and Preliminary Phytochemical Screening of Salvadora oleoides Dene. (root bark) and Salvadora persica Linn. (root bark) IJDDHR 2011; 1(2): 91-94*
5. *Gautam GK, Vidyasagar G, Dwivedi SC. Phytochemical Investigation of Crotalaria burhia Hamilt. IJRPBS 2011; 2(4): 721-72*
6. *Gautam GK, Vidyasagar G. Studies on Physicochemical Evaluation of leaves of Abutilon muticum dc. Farmavita.net Journal- Life science files (Pharmaceutical technology transfer, Licensing and regulatory affairs network) 2011; http://www.farmavita.net/content/view/1759/84/*

7. *Harbone J.B., Methods of Plant Analysis Chapter II In: Phytochemical methods: A guide to modern techniques of plant analysis Toppan Company Ltd, Japan: (1), 4 – 5, (1973).*

8. *Gautam GK, et al. Phytochemical Evaluation of The Methanolic Extracts of Zizyphus Xylopyrus (Willd.). Int. Jou. of Drug Discovery and Herbal Research 2011; 1(4): 232-233*

9. *Gautam GK, et al. Phytochemical Evaluation of The Ethanolic Extracts of Bauhinia tomentosa Linn. (Root) International Journal of Pharmacy and Life sciences 2012; 3(5): 675-676*

10. *Gautam GK, et al. A review on Indian medicinal plant "Salvadora persica" International Journal of Universal Pharmacy And Bio Sciences 2013; 2(1): 308-316*

11. *Gautam GK, Vidyasagar G. Phytochemical Screening of Abutilon muticum (del.ex dc.) And Celosia argentea linn Int. Jou. of Pharm. and Bio Sciences 2011; 2(3): 463-467*

12. *P. M. Werchan, W. R. Summer, A. M. Gerdes and K. H. McDonough. Right ventricular performance after monocrotaline-induced pulmonary hypertension. Am. J. Physiol. Heart. Circ. Physiol. 256: H1328-H1336, 1989*

13. *Soetan K.O. and Aiyelaagb, "The need for bioactive-safely evaluation and conservation of medicinal plant", Journals of medicinal plant and Research: 3(5):324-328, May, 2009*

14. *C.S.I.R. (Council of Scientific and Industrial Research). 1948-1976. The wealth of India. 11 vols. New Delhi.*

15. *Kokate, C. K., "Practica l Pharmacognosy", Nirali Prakashan, Pune: (2007).*