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PHARMACOGNOSTICAL AND PHYTOCHEMICAL EVALUATION OF WHOLE PLANT OF *ASPLENIUM DALHOUSIAE* HOOK (FERN)

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ABSTRACT

The present communication attempts to evaluate the Pharmacognostical and preliminary phytochemical studies on the whole plant *Asplenium dalhousiae* Hook (Aspleniaceae). As there is no detailed work reported on this plant, therefore Pharmacognostical evaluation including physicochemical parameters, preliminary phytochemical standards were determined .The study revealed specific identities for the plant, which will be useful in identification, as a control to abet adulterants and for future standardization work. Physicochemical studies shows, total moisture content(Leaf 12% and roots 7%), total ash(Leaf1% and root 4%), total starch content (Leaf 0.13% and root 0.079%), total sugar content(Leaf 0.19% and root 0.02%), total tannins(Leaf 1.18% and root 0.38%),total phenolics(Leaf 9.27% and root 3.20),total flavonoids(Leaf 1.3% and root 0.09%), total proanthocynadines content (Leaf 0.01% and root0.002%).. Preliminary phytochemical analysis reaveled that various other phyto-components are also present.TLC analysis and method development shows the presence of various other components.

KEYWORDS: *Asplenium dalhousiae* Hook, Pharmacognostical, TLC.

INTRODUCTION

Asplenium dalhousiae Hook (Aspleniaceae) is a fern (pteriodphyte)found with a rosette of fronds from a rhizome, leaves are rachies green, scaly beneath, blade pinnatifid, 5-15 cm long, with 6 to 13 pairs of lobes. The lobes are 5-12 mm wide. They are found in shady, rocky and ravines in moist soil.[1] *Asplenium dalhousiae* Hook generally distiunguished by its allied species through its once pinnatified leaves from *asplenium exiguum* which has bipinnatified leaves. It is a genus (Asplenium) of about 700 species of ferns found

extensively worldwide, mostly native to northen Mexico and disjunction to the Himalaya mountains in Asia.[2] In India they generally habitat at IHR (Indian Himalayan Region) includes Kashmir, Himachal, Uttarakhand and many more. The plant found to possess many medicinal activities. Roots of the plant are used in snake bite[3] where as decoction of the roots used as Ghutti in infants.[4]

METHODOLOGY

The plant was collected from catchment of Bhimtal region in Uttarakhand located in North India, proclaimed as to have ethanopharmacological importance. It was preserved in 70% ethyl alcohol for various other studies. Pharmacognostical and physicochemical evaluation were carried out from shade dried plant powder. Physicochemical standardization methods of the plant were done including determination of moisture content (loss on drying), determination of total ash and acid insoluble ash, extractive values were carried out as per WHO recommendations and authentic procedures mention in Ayurvedic pharmacopoeia of India. Estimation of total Sugar and total starch in plant material was carried out with according to Mont Gomery, 1957 [Spectrophotometric method][15] taking dextrose and starch (soluble), respectively as a standard solution. Whereas total tannins were determined by using Tannic acid as standard and Gallic acid for the determination of total phenolics. In the determination of total flavonoids and total flavonols. Rutin was taken as a standard. Proanthocynidines were estimated by using Catechin as a standard. Fluorescence analysis of both leaves and roots were carried out using different dilutions shown below. In chromatographic processes pre-coated TLC plates were used. TLC method development process was also carried out using various successive extracts. Post-derivatization was done with Anisaldehyde Sulphuric acid reagent.

Table. 1 PHYTOCHEMICAL ANALYSIS OF LEAVES EXTRACTS

S.No.	Compounds	Tests	Hexane	Chloroform	Ethyl Acetate	Methanol	Water
1	Carbohydrate	Molish's	+	-	+	+	-
2.	Proteins and Amino Acids	Millon's	+	-	-	-	+
3.	Steroides and Triterpenoids	Choloform	+	+	-	-	-
4.	Alkaloids	Mayer's	-	-	-	-	-
5.	Resins	Test	-	+	+	-	-
6.	Tannis	Ferric Chloride	+	+	-	+	-
7.	Flavanoids	Alkaline Reagent	-	-	+	+	+
8.	Glycosides	Legal Test	-	-	-	+	+
9.	Phenols	Lead Acetate	-	-	-	+	-

Table. 2: PHYTOCHEMICAL ANALYSIS OF ROOTS EXTRACT

S.No.	Compounds	Tests	Hexane	Chloroform	Ethyl Acetate	Methanol	Water
1	Carbohydrates	Test	+	+	+	-	-
2.	Reducing Sugars	Benedict's	-	+	-	+	-
3.	Mono sacciharides	Borfoed's	-	-	-	-	-
4.	Non reducing Sugar	Fehling's	+	-	+	+	+
5.	Tannis	Ferric Chloride	-	+	-	+	-
6.	Proteins and Amino Acids	Millon's	-	-	+	+	-
7.	Oils and Fats	Filter Paper	+	+	+	-	-
8.	Steroides	Libermann Burchand	+(Green)	-	+	+(Deep Red)	-
9.	Glycocides	Legal's	-	-	+	+	+
10.	Flavanoids	Alkaline Reagent	-	-	-	+	-
11.	Resins	Test	+	+	+	-	-
12.	Phenols	Lead Acetate	+	+	+	-	-

Table. 3: FLOURSCENES ANALYSIS OF LEAVES EXTRACT

S.No.	Dilution	Visible Light	Long Wave Length	Short Wave Length
1.	Powder in Methanol	Green	Black	Green
2.	Powder in Ethanol	Dark Green	Black	Blackish Green
3.	Powder in 1N NaoH	Brownish Green	Black	Dark Green
4.	Powder in 1N HCL	Green	Black	Light Green
5.	Dil HCL	Light Green	Black	Light Green
6.	Methanol in NaoH	Yellowish Green	Black	Yellowish
7.	Ethanol in NaoH	Dark Green	Black	Blackish Green
8.	Conc. HCL	Green	Light Black	Light Green

Table. 4: FLOURSCENCES ANALYSIS OF ROOTS EXTRACTS

S.No.	Dilution	Visible Light	Long Wave Length	Short Wave Length
1	Powder in Methanol	Blackish Green	Blackish	Brownish
2	Powder in Ethanol	Blackish	Blackish	Blackish
3	Powder in1N NaoH	Brownish	Blackish	Dark Green
4	Powder in 1N HCL	Brownish	Blackish	Dark Green
5	Powderin Conc.HCL	Blackish	Blackish	Blackish
6.	Ethanol in NaoH	Blackish	Blackish	Blackish
7.	Methanol in NaoH	Blackish	Blackish	Blackish
8.	Dil HCL	Brownish	Blackish	Blackish

Table. 5: PHYSICOCHEMICAL ANALYSIS

S.No.	Parameter	Leaves	Roots
1.	Loss on drying	12%	7%
2.	Total Starch (%)	0.13 %	0.079%
3.	Total Tannins (%)	1.18%	0.38%
4.	Total Sugar (%)	0.19%	0.02%
5.	Total Phenolic (%)	9.27%	3.20%
6.	Total Flavanoid (%)	1.3%	0.09 %
7.	Total Proanthrocynidines (%)	0.01%	0.002%

Fig.1 Image of *Asplinium dalhousiae* Hook taken from the site of collection

Table-6: TLC Method Development for whole plant

S.No	Solvent system	H	C	Et	M	Wt
1.	Hex : Et (8:2)	4 spots	4 spots	1 spot	---	----
2.	Hex :Et (7:3)	5 spots	6 spots	1 spot	----	----
3.	Hex : Et (6:4)	4 spots	4 spots	---	---	---
4.	Hex : Et (5:5)	4 spots	5 spots	---	---	---
5.	Hex : Et (4:6)	3 spots	4 spots	1 spot	1 spot	1 spot
6.	Chlo: Met (8:2)	3 spots	2 spots	2 spots	2 spots	1 spots

NOTE: *H= Hexane C= Chloroform Et= Ethyl acetate M= Methanol W= Water

RESULTS AND DISCUSSIONS

The powdered drug showed yellowish green, bitter odor and taste. The % of moisture content of leaves and roots was 12 and 7 respectively , total ash 1% and 4 %, acid insoluble ash 0.65% and 0.42%, hexane soluble extractive 16.8%, alcohol soluble extractive 39% and

water soluble extractive 5.6%. A known quantity (10 gms) of dried powder was extracted in a Soxhlet with hexane, chloroform, ethyl acetate, methanol and water for 72 hrs successively and tested for phytochemical analysis. The % of successive extractive values were hexane 2.91, chloroform 3.56, ethyl acetate 0.24, methanol 0.26 and water 1.4. The results of phytochemical analysis are provided in Table _1 and Table_2. TLC analysis and method development of whole plant were also carried out and results found mentioned in Table_6. The result of total starch content, total sugar content, total tannins, total phenolics, total flavonoid, total flavonols content, total proanthocynadines content and total volatile content are presented in Table_5. Results of Fluorescence analysis of both leaves and roots extract were carried out using different dilutions shown in Table_3 and Table_4 respectively.

CONCLUSION

Pharmacognostical and Phytochemical evaluation on *Asplenium dalhousiae* Hook provides some specific pharmacognostical and chemical parameters that are useful in the future evaluation and standardization of the whole plant drug. The presence of considerable level of polyphenolic compounds including flavonoids, flavonols, proanthocyanidines, etc. suggested that plant part can also be considered to show antioxidant activity. TLC analysis predicts that hexane and chloroform extract contains maximum amount of spots. So in future the study can be consider to exploit the nature of Phytoconstituents and its pharmacological action.

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