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Preliminary Phytochemical screening and HPTLC Studies of Extracts of Dried Rhizomes of *Aspidium cicutarium*

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ABSTRACT

Objective: Many herbal formulations are widely used in Ayurveda since ancient times. However it is not possible to attribute their pharmacological activity to a particular phytoconstituent present in the extract unless thorough phytochemical investigation is carried out. The present study aims at phytochemical screening of methanolic and aqueous extracts of rhizomes of *Aspidium cicutarium* Family Dryopteridaceae. The powdered dried rhizome was subjected to initial studies to determine the physical constants. **Results:** Extracts were made and subjected to various chemical tests and showed presence of steroids, flavonoids, saponins. Further investigation of the extracts by HPTLC is an attempt to deduce the varied composition of methanolic and aqueous extract of *Aspidium cicutarium.* The chromatogram shows presence of multiple peaks which indicate diverse composition of extract. **Conclusion:** Further detailed investigations of the extracts can be helpful in making formulations of these extracts so that they can successfully and safely be used to treat some diseases.

Keywords: *Aspidium cicutarium,* Rhizome, Physical Constants, Phytoconstituents, HPTLC.

1. Introduction

Natural products are a source of many traditional medicines and even some synthetic herbal medicines .In some parts of the world the herbal medicines are commonly used to treat a number of diseases. The alternate systems of medicine such as Unani, Siddha, Ayurveda comprise of the natural products.[1] The modern Herbal medicines have been widely used all over the world. The pharmaceutical industry itself still relies largely on the diversity of secondary metabolites in plants. The synthesized aromatic substances (metabolites) are used by plants as defensive weapon against predation by microorganisms, insects and herbivores. The search for the alternative systems of medicines having potential anti-inflammatory, antibiotic and other activities has gained an importance considering the harmful side effects which the modern synthetic medicine has. Medicinal plant is defined as any plant with one or undesirable side effect of some antibiotics. Herbal medicines have been widely used and form an integral part of primary health care of many countries and may constitute a reservoir of new antimicrobial substances to be discovered. [2] Similarly many disease conditions are a result of excessive stress and require an individual to be on anti-oxidant therapy. Antioxidants are abundantly found in a number of trees and in their various parts. In the present study the plant material selected was rhizomes of *Aspidium cicutarium* commonly known as a fern species belonging to family Dryopteridaceae. These rhizomes are used mainly as anthelmintic. Methanolic extract is reported to contain phenolic compounds and tannins. The rhizomes are known as kombank in Marathi and find a wide use in Ayurveda as an anti-arthritic drug. Free radical scavenging activity of the methanolic extracts is also reported. Further investigation in this regard needs to be done to explore the potential of this extract as anti-inflammatory and anti-oxidant, with this objective this drug was selected in the present study [3-6]. Standardization of plant materials is the need of the day. Several pharmacopoeia containing monographs of the plant materials describe only the physicochemical parameters.

Hence the modern methods describing the identification and quantification of active constituents in the plant material may be useful for proper standardization of herbals and its formulations. Also, the WHO has emphasized the need to ensure the quality of medicinal plant products using modern controlled techniques and applying suitable standards. HPTLC offers better resolution and estimation of active constituents can be done with reasonable accuracy in a shorter time [7-9].

2. Materials and Methods

2.1 Plant material

The plant specimens for the proposed study were purchased from Ayurvedic Bhandar that sells crude drugs used in Ayurveda located in Bibwewadi, Pune, Maharashtra, India. The dried rhizomes of *Aspidium cicutarium* were authenticated by Dr. Upadhyay Senior Scientist at Botany Department at Agharkar Research Institute, Pune, India.

2.2 Preparation and Extraction of Plant Material

The dried Rhizomes of *Aspidium cicutarium* were first powdered coarsely in a grinder and defatted with petroleum ether and 100 g was packed in a Soxhlet apparatus and extracted with methanol. Similarly for aqueous extract 100 g of dried powdered drug was defatted using Petroleum ether (60-80 °C) and then extracted with distilled water. The extraction was carried out until the extractive becomes colorless. The methanolic extract was filtered and evaporated under reduced pressure using Rotary vacuum Evaporator.

2.3 Phytochemical Screening

The phytochemical investigation of the methanolic and aqueous extracts of rhizomes of *Aspidium cicutarium* was carried out using standard protocol [10]. The extracts were finally weighed. The phytochemical tests were performed on the liquid and dried extracts using standard methods and the physical constants were evaluated. The results are stated in table 1.

2.4 HPTLC Profile (High Performance Thin Layer Chromatography)

HPTLC studies were carried out following the method of Harborne [11] and Wagner [12] *et al.*

2.4.1 Sample Preparation

Each extract residue was re-dissolved in 1ml of chromatographic grade methanol and water which was used for sample application on pre-coated silica gel 60 F 254 aluminium sheets.

2.5 Developing Solvent System

A number of solvent systems were tried, for extracts, but the satisfactory resolution was obtained in the solvent n Hexane: Ethyl Acetate in the ratio of 7:3 for methanolic extract of Aspidium cicutarium. The solvent system selected for the aqueous extract was Ethyl Acetate: n Butanol in the ratio of 6:4.

2.5.1 Sample Application

Application of bands of each extract was carried out (4mm in length and 1µl in concentration) using spray technique. Samples were applied in duplicate on pre-coated silica gel 60 F254 aluminium sheets (5 x 10 cm) with the help of Linomat 5 applicator attached to CAMAG HPTLC system, which was programmed through WIN CATS software.

2.6 Development of Chromatogram

After the application of sample, the chromatogram was developed in Twin trough glass chamber 10 x 10 cm saturated with and n hexane: Ethyl Acetate in the ratio of 7:3 for methanolic extract and Ethyl Acetate: n Butanol in the ratio of 6:4 for aqueous extract.

2.6.1 Detection of Spots

The air-dried plates were viewed in ultraviolet radiation to mid-day light. The chromatograms were scanned by densitometer at 200 nm for methanolic extract and 220 nm after spraying with anisaldehyde with sulphuric acid for aqueous extract The R_f values and finger print data were recorded by WIN CATS software.

3. Results and Discussion

3.1 Physical constants.

The proximate analysis showed satisfactory result with respect to foreign matter, moisture content, Ash value and Extractive values [13, 14]. The water soluble extractive value is 5.69% and methanolic extractive value is 6.47%. The physical constants are given in table 1.

Table 1 Evaluation of Physical constants of powdered rhizomes of *Aspidium cicutarium*

Sr. no.	Evaluation Parameter	Value (%)			Mean±Std Deviation (n=3)
1	Foreign Matter	1	1.09	1.1	1.06 ± 0.551
2	Moisture Content	10.6	10.55	10.5	10.55 ± 0.0550
3	Total Ash Value	13.5	13.9	13.8	13.73 ± 0.2082
4	Water Soluble Ash Value	3.5	3.6	3.87	3.66 ± 0.1914
5	Acid Insoluble Ash Value	12	12.39	12.1	12.16 ± 0.2026
6	Water Soluble Extractive Value	5.6	5.68	5.8	5.69 ± 0.1007
7	Chloroform Soluble Extractive Value	0.1	0.12	0.1	0.11 ± 0.0115
8	Methanol Soluble Extractive Value	6.6	6.3	6.5	6.47 ± 0.1528
9	Ethanol Soluble Extractive Value	5.3	5.2	5.2	5.23 ± 0.0577

3.2 Phytochemical Screening

The methanolic and aqueous extracts of rhizomes of *Aspidium cicutarium* showed presence of sugars, carbohydrates, tannins, steroids, flavonoids and saponins. The results are reported in Table 2. The presence of steroids and flavonoids is mostly responsible for the anti-inflammatory activity proven in Ayurveda. Alkaloids are present in methanolic extract but not present in aqueous extract [15-16].

Table 2: Phytochemical screening of Methanolic and Aqueous extracts of *Aspidium cicutarium*.

Phytochemical constituents		Methanolic Extract	Aqueous Extract
Alkaloids	Mayer's reagent	+	-
	Dragendorff's reagent	+	-
	Hager's reagent	+	-
	Wagner's reagent	+	-
Phenolic compounds	Ferric chloride test	+	+
	Lead acetate test	+	+
Carbohydrates	Molisch's reagent	+	+
	Barfoed's test	+	+
	Fehling's test	+	+
	Benedict's test	+	+
Flavonoids	Lead acetate test	+	+
	Ferric chloride test	+	+
	Sodium Hydroxide test	+	+
	Shinoda test	+	+
Steroids	Liebermann-Burchard test	+	-
	Salkowski reaction	+	-
	Liebermann's test	+	-
Saponins	Foam test	+	+
Tannins	Ferric chloride test	+	+
	Lead acetate test	+	+
	Potassium Dichromate	+	+
	Dilute Potassium Permanganate	+	+
Cardiac Glycosides	Keller-Kiliani test	+	-
	Legal's test	+	-
Anthraquinone Glycosides.	Borntrager's test	-	-
Saponin Glycosides	Foam Test	-	+
	Hemolytic test	-	-
Carbohydrates	Molisch's test	+	+
	Fehlings test	+	+
	Benedict's test	+	+
Proteins	Biuret's test	-	-
	Millon's test	-	+

3.3 HPTLC studies

3.3.1 HPTLC studies of methanolic extract of *Aspidium cicutarium*

The mobile phase selected for the HPTLC studies of methanolic extract was n hexane: Ethyl Acetate in the ratio of (7:3). The resolution was obtained at 200nm. The result is depicted in figure 1. The following HPTLC figure for the methanolic extract of *Aspidium cicutarium* sample whose 10 µl was injected shows 5 peaks at R_f values 0.41, 0.61, 0.81, 1.21, 1.41. The maximum concentration of 350 is obtained at R_f value 0.61 and 0.81 indicating presence of phytoconstituents eluting out at 200 nm with the mobile phase of n Hexane: Ethyl acetate (7:3).

3.3.2 HPTLC studies of aqueous extract of *Aspidium cicutarium*

The mobile phase for the aqueous extract of *Aspidium cicutarium* was ethyl acetate: n Butanol at the ratio (6:4). The resolution was obtained at 220 nm. The results are depicted in Figure 2. The Figure shows 06 peaks. The peaks at R_f value 1.01 shows the maximum concentration with an area under the curve of 200. The other peaks are obtained at 0.61, 0.81, 1.21 and 1.41. However the intensities are low.

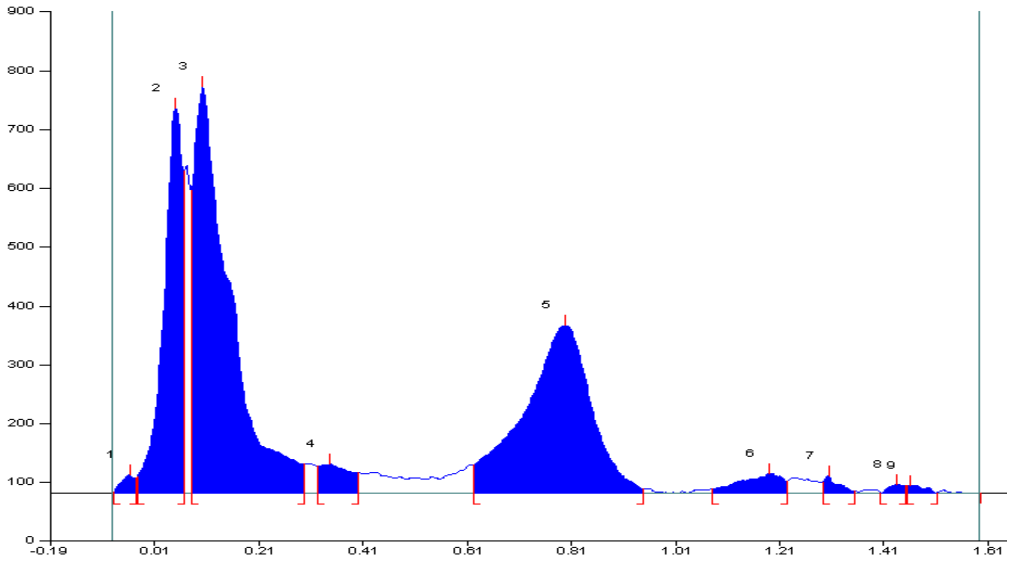

Fig 1: HPTLC of methanolic extract of *Aspidium cicutarium.*

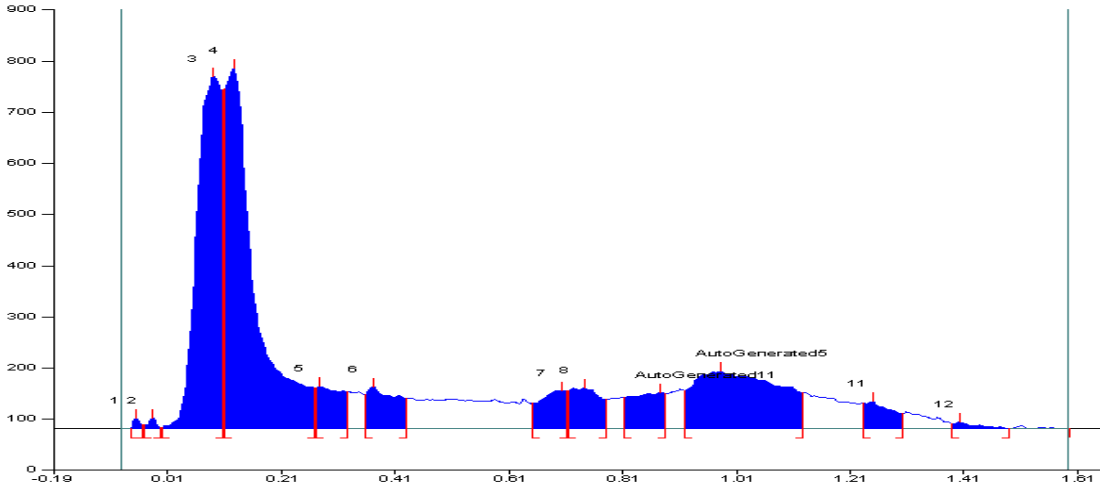

Fig 2: HPTLC of aqueous extract of *Aspidium cicutarium.*

4. Conclusion:

Herbal medicines are composed of many constituents and are therefore very capable of variation. Hence it is very important to obtain reliable chromatographic fingerprints that represent pharmacologically active components of the herbal medicine. HPTLC fingerprinting profile is very important parameter of herbal drug standardization for the proper identification of medicinal plants. *Aspidium cicutarium* is widely used in Ayurveda in treatment of rheumatoid arthritis and has anti-inflammatory activity which has been proven in the traditional systems since ancient times. Different preparations made from different parts of the plant have been used in different ailments but their exact chemical components were unknown for a long time. To understand the actual chemical constituents responsible for the pharmacological activities of the plant detailed chromatographic studies are needed to be carried. Present study is the first report pertaining to deduce the varied composition of methanolic and aqueous extract of *Aspidium cicutarium* using the HPTLC technique. In this study multiple peaks correspond to various pigments and the other lipid soluble compounds like alkaloids, flavonoids, steroids, anthraquinones. Several peaks observed in this experiment indicate the diverse composition of the extracts. The data and HPTLC fingerprint profile could be used as a valuable analytical tool in the routine quality control and standardization. Further characterization of these fractions by applying more sophisticated separation and purification techniques are necessary to find out the exact chemical compounds and their relation to the pharmacological activity.

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