SHORT REPORT

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Conflict of Interest: None Declared !

Phytochemical Analysis and Toxicity Study of *Actiniopteris dichotoma Bedd*

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ABSTRACT

The present study was aimed to find out phytochemical testing and acute toxicity effect of the methanolic extract of *Actiniopteris Dichotoma Bedd* in rats. For phytochemical testing, depending upon the type of natural drug under examination, the test solution may be a aqueous extract or specific menstrum like petroleum ether, chloroform, methanol and aquous etc. All the different extracts were then subjected to preliminary phytochemical analysis to assess the presence of various phytoconstituents. The acute oral toxicity of the crude ethanolic extracts of Actiniopteris dichotoma was evaluated in mice using the procedures described by Organization for Economic Co-operation and Development 423 guidelines. The acute toxicity study showed that animals fed by oral gavages tolerated the limit dose of 5000 mg/kg body weight of methanolic extract of *Actiniopteris dichotoma bedd.* There were no visible signs of acute toxicity during the 14 days of observation.

Keywords: Methanolic extract, *Actiniopteris Dichotoma Bedd,* acute toxicity, phytoconstituents.

Introduction

There are very few medicinal herbs of commercial importance which are not found in this country. India officially recognizes over 3000 plants for their medicinal value. It is generally estimated that over 6000 plants in India are in use in traditional, folk and herbal medicine, representing about 75% of the medicinal needs of the Third World countries.[1] Herbal medicine is still the mainstay of about 75 - 80% of the world population, mainly in the developing countries, for primary health care.[2] *Indian traditional medicine,* Ayurveda is a medical system primarily practised in India that has been known for nearly 5000 years. It includes diet and herbal remedies, while emphasizing the body, mind and spirit in disease prevention and treatment (Morgan, 2002).

Plant profile:

Actiniopteris dichotoma bedd

Figure-1: *Actiniopteris Dichotoma Bedd* plant

Figure-2: Actiniopteris Dichotoma Bedd plant

Classification	
Kingdom:	Plantae
División:	Pteridophyta
Subdivisión:	Pterophyta
Class:	Pteridopsida
Order:	Pteridales
Family:	Pteridaceae
Subfamily:	Pteridoideae
Génus:	Actiniopteris
Specific epithet:	dichotoma
Vernacular Name	
Hindi:-	Mayursikha
English:-	Peacock's Tail
Kannd:-	Mayursikha
Malayalum:- Nanmukappullu	
Sanskrit:-	Mayursikha
Tamil:-	Mayilatumsikha
Telgu:-	Mayursikha

Plant Description

Actiniopteris dichotoma, Bedd is Small fern about 4-inch high very much like a miniature palm; cuadex oblique stipes densely tufted, 2-4 inch high, scaly; fronds like leave of a small palm, fan-like; segments dichotomous, radiating narrowly-linear, rush-like in texture, Veins not many and parallel with indistinct midrib; segments of fertile frond longer than the segments of barren ones.

Distribution and Habitate:-

It is found throughout of India and very common in the lower hills of Atta paddy, upto 600m in Nilgiris and the rocky hillsides of Western Ghats. It is found practically in all the districts of the State on slopes of hills specially on the northern aspect.

Chemical Constituents:-

Several chemical constituents was separated and characterized like alkaloids, glycosides, carbohydrates, fixed oils and fats, phytosterol, triterpenoides, saponins, tannins and phenolic compound, proteins and free amino acids, flavonoids, lignin.

Medicinal Properties:-

Plant pacifies vitiated kapha, pitta, diarrhea, dysentery, worm infestations, skin discoloration, skin diseases, diabetes and fever. Useful part of this plant is whole plant.

METHODS:

Collection of drug material

The fresh whole plant was collected from Chitrakoot region, District Satana, Madhya Pradesh in the season of July and August.

Authentication

The plant material to be investigated can be selected on the basis of some specific traditional uses (ethnobotanical bioprospecting approach). The plant was authenticated by Dr. H.B. Singh, H.O.D. of National Institute of Science Communication and Information Resources (NISCAIR), near Pusa gate, New Delhi, India. Reference No. NISCAIR/RHMD/Consult/2010-11/1408/06.

Experimental animals

Male wistar albino rats weighing 150-180 gm were purchased from DRDE, Gwalior, and M.P. the animals were housed in polypropylene cages and maintained in control temperature (26+ 2^{0}C) and light cycle (12 h light and 12 h dark); they were fed with modern scientific animal feed, Jayendraganj, Gwalior. Water was supplied *ad libitum*. Initial body weight of each animal was recorded. Ethical clearance for the use of animal was obtained from CPCSEA and registration No. is 891/po/ac/05/CPCSEA.

Preparation of Extract:-

Soxhlet extraction is used widely in the extraction of plant metabolites because of its convenience. This method is adequate for both initial and bulk extraction. The plant powder is placed in a cellulose thimble in an extraction chamber, which is placed on top of a collecting flask beneath a reflux condenser. A suitable solvent is added to the flask, and the set up is heated under reflux. When a certain level of condensed solvent has accumulated in the thimble, it is siphoned into the flask beneath. The main advantage of Soxhlet extraction is that it is a continuous process. As the solvent (saturated in solubilized metabolites) empties into the flask, fresh solvent is recondensed and extracts the material in the thimble continuously. This makes Soxhlet extraction less time- and solvent-consuming than maceration or percolation. However, the main disadvantage of Soxhlet extraction is that the extract is constantly heated at the boiling point of the solvent used, and this can damage thermolabile compounds and/or initiate the formation of artifacts.[3]

The whole plant was dried and powdered. A fine coarse powder was obtained which was sieved through #40 to obtain uniformity. The powered obtained was successively extracted in petroleum ether, chloroform, acetone, methanol and distilled water. Continues soxhlet extraction method was used, the powder of crude drug was packed in a thimble made whatman filter paper and then inserted in to the extractor. Each batch extracted for about 35 cycles.

The extracts were then made to powder by using rotary evaporator under reduced pressure. Crude drug of *Actiniopteris dichotoma* yielded 0.9%, 1.5%, 2.4%, 3.4% and 4.6 % w/w powered extract with petroleum ether, chloroform, acetone, methanol and distilled water respectively. When the extraction was completed, the extract was concentrated under vacuum, for large volumes and by heating at low temperature. Aqueous extracts were generally freeze-dried and stored at 20^{o}C as this low temperature reduces the degradation of the bioactive natural product.

Phytochemical analysis

All the different extracts were then subjected to preliminary phytochemical analysis to assess the presence of various phytoconstituents. The plant extracts were subjected to qualitative tests for the identification of the phytoconstituents present in it viz., alkaloids, carbohydrates, glycosides, phytosterols, fixed oils & fats, phenolic compounds & tannins, proteins and free amino acids, gums & mucilage's, Flavonoids, lignin and saponin.

Qualitative phytochemical testing, depending upon the type of natural drug under examination, the test solution may be a aqueous extract or specific menstrum like petroleum ether, chloroform, methanol etc. All the different extracts were then subjected to preliminary phytochemical analysis to assess the presence of various phytoconstituents. The plant extracts were subjected to qualitative tests for the identification of the phytoconstituents present in it viz., alkaloids, carbohydrates, glycosides, phytosterols, fixed oils & fats, phenolic compounds & tannins, proteins and free amino acids, gums & mucilage's, Flavonoids, lignin and saponin.[4,5]

Test for Alkaloids

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

Test for carbohydrates

The minimum amount of extracts were dissolved in 5ml of distilled water and filtered. The filtrate was subjected to test for carbohydrates.

Molisch's test: The filtrate was treated with 2-3 drops of 1% alcoholic alpha naphthal and adds few drop of concentrated sulphuric acid through sides of the test tube purple to violet colour ring appear.

Benedict's test: To 0.5 ml of the filtrate, 0.5ml of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 minutes. A characteristic red colour precipitate indicates the presence of sugar.

Fehling's test: The filtrate was treated with 1ml of Fehling's solution and heated. Orange precipitate was obtained shows the presence of carbohydrates.

Test for glycosides

Borntrager's test: Boil the test material with 1ml of sulphuric acid in attest tube for five minutes. Filter while hot. Cool the filtrate and shake with equal volume of chloroform. Separate the lower layer of chloroform and shake it with half of its volume of dilute ammonia. A rose pink to red colour is produced in the ammonical layer.

Legal's test: Test solution was treated with pyridine and adds alkaline sodium nitroprusside solution then blood red colour appears.

Keller-killani test : Extract the drug with chloroform and evaporate it to dryness. Add 0.4 ml of glacial acetic acid with trace amount of ferric chloride. Add 0.5ml concentrated sulphuric acid by side of the test tube. Acetic layer shows blue colour.

Baljet's test: Treat the test solution with picric acid, orange colour is formed.

Test for Phytosterols and Triterpenoids

Libermann Burchard Test: 1 gram of the extract was dissolved in few drops of dry acetic acid; 3ml of acetic anhydride was added followed by few drops of conc, sulphuric acid. Appearance of bluish green colour shown the presence of phytosterols.

Salkowski test: Treat the extract with few drops of concentrated sulphuric acid red colour at lower layer indicate presence of steroid and formation of yellow coloured lower layer indicate presence of triterpenoids.

Test for fixed oils and fats

A small quantity of the various extracts 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 small quantity of various extracts along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 hours. Formation of soap or partial

neutralization of alkali indicates the presence of fixed oil and fats.

Test for Saponin

Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

Test for Tannins and Phenolic Compounds

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Test for Proteins and Free Amino Acids

Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

Ninhydrin Test: To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

Burette Test: Equal volume of 5% Solution of Sodium hydroxide and 1% solution of Copper sulphate were added. Appearance of pink colour shows the presence of proteins and free amino acids.

Test for Flavonoids

Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Shinoda's test: Extract were dissolved in alcohol, to that piece of magnesium followed by conc. Hydrochloric acid drop wise are added and heated. Appearance of magenta colour shows the presence of Flavonoids.

Test for lignin

With alcoholic solution phloroglucinol and hydrochloric acid appearance of red colour shows the presence of lignin.

Procedure of Acute Oral Toxicity Test

The acute oral toxicity of the crude ethanolic extracts of Actiniopteris dichotoma was evaluated in mice using the procedures described by Organization for Economic Co-operation and Development 423 guidelines.[6]

A total of 18 female animals were divided into three dosage groups with 6 animals per dose. The control group was given 10 ml/kg of normal saline. The second and third groups were given with a single dose of 2000 mg/kg and 5000 mg/kg of Actiniopteris dichotoma, respectively. Gavage dosing was performed using a curved, ball-tipped intubation needle affixed to a 5 ml syringe. All solutions were prepared just prior to dosing and were kept chilled and tightly capped. Body weight, food, and water consumption were monitored daily. Animals were fasted approximately 12 hours prior to dosing. Following administration of a single dose of herbal preparation, the animals were observed for behavioural changes and general toxicity signs. Results were recorded for the first 30 minutes and at hourly intervals for the next 24 hours.[7]

Results and Observation:

The results of the phytochemical and toxicity have been presented and discussed herewith.

Table-1: Preliminary phytochemical analysis of successive extract of *Actiniopteris dichotoma Bedd*

S. No.	Test	Extract				
		Petroleum ether	Chloroform	Acetone	Methanol	Aqueous
1	Alkaloid					
	Mayers Test	-	-	-	+	-
	Wagner's Test	-	-	-	+	+

	Dragendroff's Test	-	-	-	+	+
	Hager's Test	-	-	-	+	+
2	Glycoside					
	Borntrager's test	-	-	-	+	+
	Legal's test	-	-	-	+	-
	Killer-killani test	-	-	-	+	+
3	Phytosterols and Triterpenoids					
	Libermann Burchard Test	+	+	+	+	-
	Salkowski test	+	-	+	+	-
4	Fixed oils and fats	+	-	-	-	-
5	Saponin					
	Froth Test	-	-	+	+	+
	Foam Test	-	-	-	-	+
6	Tannins and Phenolic Compounds					
	Gelatin Test	-	-	-	+	-
	Ferric Chloride Test	-	-	-	+	-
7	Proteins and Free Amino Acids					
	Xanthoproteic Test	-	-	-	+	-
	Ninhydrin Test	-	-	-	+	-
	Burette Test	-	-	-	+	
8	Flavonoids					
	Alkaline Reagent Test	-	-	+	+	+
	Lead acetate Test	-	-	+	+	+
	Shinoda's test	-	-	+	+	+
9	Lignin	-	-	-	-	-
10	Carbohydrate					
	Molish test	-	-	-	+	+
	Benedict's test	-	-	-	+	+
	Fehling's test	-	-	-	+	+
11	Acidic compound	-	-	-	+	-
12	Aleurone grains	-	-	-	+	+
13	Free reducing sugar	-	-	+	+	+
14	Starch	-	-	-	-	-
15	Wax	-	-	+	-	-

Acute Oral Toxicity

The acute toxicity study showed that animals fed by oral gavages tolerated the limit dose of 5000 mg/kg body weight of methanolic extract of ***Actiniopteris dichotoma***. There were no visible signs of acute toxicity during the 14 days of observation. Absence of death at all doses up to 5000 mg mg/kg showed that the LD50 of the extract is greater than 5000 mg extract/kg body weight. There was a significant increase in weight gain of rats after 14 days of extract treatment at 5000 mg/kg as compared with control. The eating, drinking habit and behaviour of all the animals used were normal. The results obtained on the average water and food intake and weekly weight gain are presented.

A total of 18 female animals were divided into three dosage groups with 6 animals per dose. The control group was given 10 ml/kg of normal saline. The second and third groups were given with a single dose of 2000 mg/kg and 5000 mg/kg of *Actiniopteris dichotoma*, respectively. Gavage dosing was performed using a curved, ball-tipped intubation needle affixed to a 5 ml syringe. All solutions were prepared just prior to dosing and were kept chilled and tightly capped. Body weight, food, and water consumption were monitored daily. Animals were fasted approximately 12 hours prior to dosing. Following administration of a single dose of herbal preparation, the animals were observed for behavioural changes and general toxicity signs. Results were recorded for the first 30 minutes and at hourly intervals for the next 24 hours and thereafter for a total of 14 days. Body weight was recorded on Day 0 (before dosing), Day 7 and Day 14.

Table 2 : Feeding Pattern of Rats In Acute Toxicity Study of *Actiniopteris dichotoma*

Parameter	Control	2000 mg/kg	5000 mg/kg
Average water intake (ml/day)	22.34 ± 0.38[a]	26.30 ± 3.96[a]	28.70 ± 1.70[a]
Average food intake (g/day)	13.14 ± 0.80[a]	12.66 ± 0.34[a]	14.93 ± 0.25[a]
Average weekly weight gain (g)	9.08 ± 0.24[a]	11.05 ± 1.10[ab]	12.43 ± 0.80[b]

Each value represents the mean ± SEM (N = 6)

Superscripts a b Within row showed significant difference at (p<0.05)

DISCUSSION

Phytochemical Analysis

The plant extracts were subjected to qualitative tests for the identification of the phytoconstituents present in it viz., alkaloids, carbohydrates, glycosides, phytosterols, fixed oils & fats, phenolic compounds & tannins, proteins and free amino acids, gums & mucilage's, Flavonoids, lignin and saponin.

Acute Toxicity Study

In the acute toxicity study showed that animals were tolerated the limit dose of 5000mg/kg body weight of methanolic extract of Actiniopteris dichotoma bedd, there was no sign of acute toxicity during the 14 days observation. At the 2000mg/kg and 5000mg/kg slightly changes were recorded as compare to control maximum changes were obtained to average water intake, average food intake and average weight gain at the 5000mg/kg. the significant difference (P<0.05) as compare to control were obtained of different parameters.

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