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Pharmacognostical and biological evaluation of *Mayurshikha (Actiniopteries dichotoma* Bedd*)*: An Ayurvedic medicinal plant

Research Article

Akshay K Patil[1*], Vishakha P Gaonkar[2], Supriya S Chimagave[2], Kirankumar Hullatti[3]

1. Assistant Professor, 2. Research Scholar, Department of Pharmacognosy, KLE College of Pharmacy, KLE Academy of Higher Education and Research, Belagavi. Karnataka. India.
3. Vice Principal, Cauvery College of Pharmacy, Mysuru. Karnataka India.

Abstract

Aim: To evaluate pharmacognostical parameters and biological activities for *Mayurshikha*. Methods: The freshly collected plant material was subjected to pharmacognostical study followed by extraction and fractionation. Extract and fractions subjected for phytochemical investigation.The hydro-alcoholic extract and their four fractions were evaluated for *in vitro* antioxidant, antimicrobial, anthelmintic and brine shrimp lethality assay. The extract was subjected for fluorescence analysis. Total phenol content was estimated by Folin-Ciocalteu's method. Results: The pharmacognostic study revealed the macroscopic characters, physicochemical constants and phytochemical nature of *Mayurshikha*. Phytochemical screening revealed the presence of lipids, phenols, terpenoids, sterols, alkaloids and flavonoids.Total phenol content was found to be 6.36 µg/mg of Gallic acid equivalents GAE/g. *In vitro* biological activities were performed to assess the biological efficacy. Fraction 4 showed promising antioxidant activity with IC_{50} of 1.49µg/ml. Amongst four fractions, F3 and F4 showed pronounced antimicrobial activity with minimum inhibitory concentration of 100µg /ml. Fraction 3 showed better anthelmintic activity as death time for same fraction was 4.78(±0.93) minutes. Further, with the LC_{50} value of 1.49µg/ml, Fraction 3 was found to exhibit better cytotoxicity amongst all fractions. Conclusion: From the above finding it clearly indicates that *Mayurshikha* consists of active phytoconstituents which can be utilized for therapeutic purpose.

Key Words: Extraction, Fractionation, Antioxidant, Antimicrobial, Anthelmintic, Brine Shrimp Lethality assay.

Introduction

Plants based products have indistinct role in human development, apart from three basic needs of human life, health is also one of the important need which is greatly depends on natural resources. Medicinal plants having therapeutic effects are remained base for the various Ayurvedic, Unani, Homeopathy, Herbal and Allopathic medicines. Around 25 % of medicines are plant based or their derivatives (1). Plant based medicines are utilized by early civilization to treat illness of human being as therapeutic agents and has gained more acceptance due to their better therapeutic efficiency (2, 3). Natural plant based products retained the human health due to fewer side effects, low price so there is an increasing demand for the plant based therapeutic products (4). The Indian medicinal systems utilized plants to cure different ailments. According to WHO, more than 40000 plant species have medicinal values and about 80 % of population utilizing herbal drugs for their therapeutic efficacies (5, 6, 7). India has great diversities consist of fern that shows therapeutic effects due to bioactive principles (8). The Indian system Sushruta, Charaka, Samhita has suggested use of pteridophytes for treatments of different ailments (9). *Actiniopteries dichotoma* is pretty fern belongs to family *Actiniopteridaceae*. The plant shows anti-inflammatory, antihistaminic, antimicrobial, anthelmintic, anticholinergic, analgesic, antitubercular activity (10). Herbal drugs quality can be affected by physical and environmental factors so purity and safety of herbal crude drugs can be concerned by assessing the pharmacognostical parameters (11). Herbal drug material are very susceptible for detoriation due to physiological, environmental, biological factors which may form toxic compounds hence there is need to each and every medicinal plant to standardize and develop a quality control guidelines for identification, collection, raw material processing and also to avoid any adulterants in herbal products (12).

Therefore, an attempt has been made for the development of pharmacognostical standardization parameters for *Mayurshikha*. *In vitro* biological activities such as antioxidant, antimicrobial, anthelmintic, and brine shrimp lethality assay were performed on the extract and its fractions.

*** Corresponding Author:**

Akshay K Patil

Assistant Professor, Department of Pharmacognosy, KLE College of Pharmacy, KLE Academy of Higher Education and Research, Belagavi.
Karnataka-590010 India.
Email Id: pakshay1577@gmail.com

Materials and Methods

Plant material and Chemicals

Mayurshikha (Actiniopteris dichotoma Bedd*)* was collected from local region of Belagavi, Karnataka and it was identified by Dr. Harsha Hegde (Scientist E, ICMR-NITM, Belagavi, Karnataka) with voucher specimen RMRC 1448. The whole plant was thoroughly cleaned and shade dried, converted into powder and used for further study. DPPH (2, 2-diphenyl-1-picrylhydrazyl) reagent was purchased from Himedia, India. All other chemical and reagents of analytical grade were used from KLE College of Pharmacy, Belagavi

Pharmacognostical study

The freshly collected plant material was subjected to the macroscopic evaluation. Macroscopic characters of *Mayurshikha* such as shape, size, color, odor, taste, surface characteristics and texture. Physicochemical evaluation including moisture content, ash value and extractive value carried out on powdered plant material (13, 14).

Extraction and fractionation

The extraction was carried by maceration followed by soxhlet apparatus. Initially, accurately weighed quantity of dried plant material was subjected for maceration for 24 hours using ethanol: water (70:30) as a solvent. The mark from maceration was subjected to soxhlet extraction. Both the filtrate were combined and concentrated on rotary evaporator and evaporated to get the crude extract. The resultant extract was subjected for fractionation by *Cos et al* method to separate Lipids (Fraction 1); Phenols, Terpenoids, Sterols (Fraction 2); Alkaloids (Fraction 3); and Polyphenols (Fraction 4) (15). The extract and fractions were subjected for qualitative phytochemical analysis (16).

Fluorescence Analysis

The plant extract was placed on clean glass slide and freshly prepared reagents were added to slide with gentle tilting. After standing for few minutes the slides were examined under UV chamber (17).

Total phenolic content

Test sample with reagents (0.2 mL of test sample +0.6mL of distilled water +0.2mL of Folin-Ciocalteu's reagent) was added and kept for 5 minutes. To this, 1 ml of saturated sodium carbonate solution (8% w/v in water) added, volume was made up to 3 ml with distilled water and test tubes were kept in dark for 30 minutes, the absorbance was measured at 765nm. The phenol content was calculated as gallic acid equivalents GAE/g of a standard curve of gallic acid (18, 19).

Biological activities

In vitro antioxidant activity

In-vitro antioxidant potential of *Mayurshikha* extract and fractions were evaluated by DPPH radical scavenging assay (20). 1 ml of test fraction and 3 ml of standard DPPH reagent (0.1 mmol/L prepared in methanol) was added in test tube. Similarly, control was prepared by adding methanol to DPPH solution. The test tubes containing test solutions were properly closed and kept in dark at room temperature for 30 minutes. After 30 minutes, Absorbance was measured at 517 nm. The IC_{50} value was calculated based on the concentration at which DPPH radicals scavenged by 50% and value obtained by interpolation from linear regression analysis (21, 22). The scavenging activity was calculated by using equation:

$$Radical\ scavenging\ activity(\%) = \frac{Abs\ Control - Abs\ sample}{Abs\ control} * 100$$

In vitro antimicrobial activity

In vitro antimicrobial activity were evaluated by agar well diffusion method. Microbial strains such as *Staphylococcus aureus, Streptococcus pneumonia*, *Escherichia coli* and *Salmonella typhi* were used. Nutrient broth was prepared by pouring 20 ml of molten agar in clean and sterilized petri plates. The plates were cooled and examined for any contaminations. Agar wells of 5 mm diameter in each petri plate were made by sterilized stainless steel bore and labeled as C, E, F1, F2, F3 and F4 on the back side of the petri plates representing control, Extract, Fraction 1, Fraction 2, Fraction 3 and Fraction 4 respectively. The agar well were loaded with control as gentamicin, whole extract of *Mayurshikha*, fraction 1, fraction 2, fraction 3 and fraction 4 in C, E, F1, F2, F3 and F4 respectively. The petri plates containing microorganism and samples were incubated at 37°C. Zone of inhibition were examined which appear as a clear area around the wells and it was measured using a meter ruler and the mean value for each organism was recorded and expressed in millimeter (23).

In vitro anthelmintic activity

In vitro anthelmintic activity of extract and fractions were performed on *Lumbricus terrestris* (Indian earthworms) due to morphological resemblance with the worms that causes helminthiasis. Paralysis time and death time were compared with standard Albendazole at concentrations of 25 mg/mL, 50 mg/mL and 100 mg/mL (24).

Brine shrimp lethality assay

Few milligrams of brine shrimp eggs were sprinkled in glass compartment containing saline water with small hole between the compartments and light source was kept 'ON' for 24 hours. After 24 hours, hatched nauplii move towards the light source as they are phototropic in nature. Different test tubes containing concentration of 10,100 and 1000 µg / ml of test sample, exactly 10 shrimps transferred and volume was made up to 5 ml using sea water. Little quantity of yeast suspension (3 mg in 5 ml sea water) used as food for shrimps. Potassium dichromate were used as standard. All test samples were kept on illumination for 24 hours. After 24 hours, shrimps were counted, number of survivals and number of deaths recorded. The lethal concentration (LC_{50}) values were calculated by using SPSS-20 (Statistical Package for the Social Sciences) software (25, 26).

Results

Pharmacognostical study

Macroscopic study

Mayurshikha (Actiniopteris dichotoma) is a pretty, green to dark brown fern, about 4 inch height, faint odor, tasteless rooted usually in the cervices of rocks in moist and shade places. Macroscopic characters are depicted in table1.

Figure 1: *Mayurshikha* whole plant

Table1: Macroscopic characters of *Mayurshikha*

Macroscopic Parameters	Conclusion
Rhizomes	Horizontal
Segments	Dichotomous
Leaves	Circinate vernation
Sporangia	Sub marginal
Spores	Trilete

Physicochemical study

The physicochemical parameters such as moisture content, ash values and extractive values are represented in table 2. Physicochemical parameters highly affects the stability, quality of any herbal crude drugs. The moisture content of whole plant of *Mayurshikha* is less, so there will not be microbial attack. Ash values are within limit. The result concludes alcohol will be better solvent for extraction.

Table 2: Physicochemical analysis

Sr.no	Physico-chemical Parameters	Test	Results %(w/w)
1	Moisture content	Loss on drying	2.66 (±0.34)
2	Ash values	Total ash	8.94 (±0.59)
		Acid insoluble ash	0.83 (±0.29)
		Water soluble ash	1.50 (±0.50)
3	Extractive values	Aqueous	8.33 (±0.58)
		Alcohol	9.00 (±1.0)
		Petroleum ether	4.67(±0.58)

Phytochemical analysis

The qualitative phytochemical analysis revealed the presence of Lipids, Phenols, Terpenoids, Sterols, Alkaloids and Flavonoids.

Table 3: Phytochemical analysis

Phyto-chemicals	Extract	Fraction 1	Fraction 2	Fraction 3	Fraction 4
Lipids	+	+	-	-	-
Phenols	+	-	+	-	-
Terpenoids	+	-	+	-	-
Sterols	+	-	+	-	-
Alkaloids	+	-	-	+	-
Flavonoids	+	-	-	-	+

('+'indicates positive results and '-' indicates Negative results)

Fluorescence analysis

The plant extract showed different fluorescence reveals the presence of different chemical components.

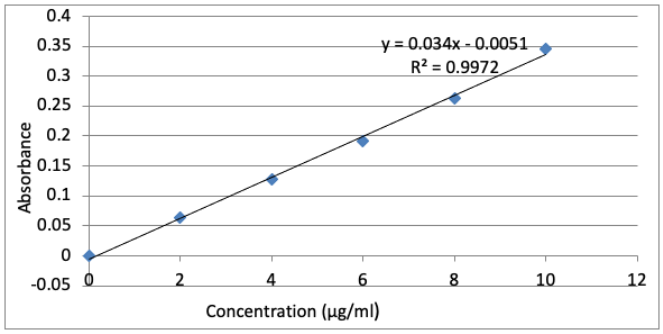
Table 4: Fluorescence analysis

Sr. No	Test	Visible light	UV light (Wavelength)	
			Short (254nm)	Long (366nm)
1	Extract + 1 mol/L NaOH (aq)	Orange	Brown	Orange
2	Extract + 1 mol/L NaOH (alc)	Green	Yellow	Green
3	Extract + Ethyl alcohol	Yellow	Green	Green
4	Extract + Methanol	Green	Green	Yellow
5	Extract + Ethyl acetate	Light green	Red	Red
6	Extract + 50% H2SO4	Pale yellow	Yellow	Yellow
7	Extract + 50% HCl	Pale yellow	Green	Blue
8	Extract + Petroleum ether	Pale yellow	Orange red	Pale yellow
9	Extract + Picric acid	Yellow	Green	Green
10	Extract + Ammonia	Orange	Green brown	Reddish brown

Total Phenol Content

The total phenol content was estimated by Folin-Ciocalteu's reagent using UV Spectrophotometer. The phenol content was found to be 6.36 µg/mg of Gallic acid equivalents GAE/g of a standard curve of Gallic acid.

Figure 2: Total Phenol Content



Biological activities

***In vitro* antioxidant activity**

In vitro antioxidant activity on *Mayurshikha* extract and fractions were carried by DPPH free radical scavenging assay, the percent inhibitions are depicted in Table 5. The IC_{50} values are compared with standard ascorbic acid.

***In vitro* antimicrobial activity**

In vitro antimicrobial efficacy of *Mayurshikha* extract and fractions were carried by agar well diffusion method using gentamicin as standard with gram negative and gram positive microbial samples. The zone of inhibition and minimum inhibitory concentration depicted in table 6 and 7 respectively.

***In vitro* anthelmintic activity**

In vitro anthelmintic activity on *Mayurshikha* extract and fractions were carried using earthworms (*Lumbricus terrestris*). Paralysis time and death time were compared with standard albendazole. Mean time taken for paralysis and death were depicted in table 8.

Brine shrimp lethality assay

The brine shrimp lethality assay on *Mayurshikha* extract and fractions were carried for cytotoxicity using potassium dichromate as standard. Table 9 represents the number of survivals and number of deaths of shrimps. LC_{50} values were calculated with the help of SPSS-20 software based on the number of survivals and number of deaths.

Figure 3: Absorbance

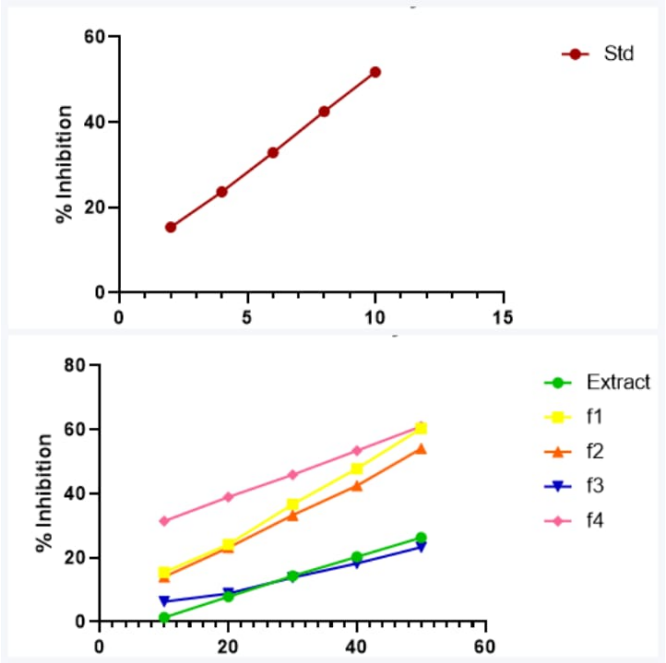


Table 5: Percentage Inhibition

Sr. No.	% Inhibition							
	Conc (µg/ml)	Standard (Ascorbic acid)	Conc (µg/ml)	Extract	F1	F2	F3	F4
1	2	15.35	10	1.38	15.46	14.01	6.29	31.45
2	4	23.68	20	7.83	24.15	23.19	8.81	38.99
3	6	32.89	30	14.29	36.71	33.33	13.84	45.91
4	8	42.54	40	20.28	47.83	42.51	18.24	53.46
5	10	51.75	50	26.27	60.39	54.11	23.27	61.01
IC_{50}		0.757	**IC_{50}**	1.44	1.47	1.46	1.49	1.45

Table 6: *In vitro* antimicrobial activity (Zone of Inhibition)

	Microorganism	Zone of Inhibition (mm)					
-	-	Extract	F1	F2	F3	F 4	Standard
Gram positive	***S. aureus***	15	7	9	14	11	24
	S.pneumonia	14	-	5	12	10	21
Gram negative	***E.Coli***	13	5	7	14	11	18
	S. typhi	14	-	5	11	9	20

Table 7: Minimum Inhibitory Concentration (µg /ml)

	Samples	Extract	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Control
Gram positive	*S. aureus*	100	>300	>300	100	100	10
	S.pneumonia	100	>300	>300	100	100	10
Gram negative	*E. Coli*	100	>200	>200	100	100	10
	S. typhi	100	>300	>300	100	100	10

Table 8: *In vitro* anthelmintic activity

Sr. No.	Sample	Concentration	Earthworm	
			Time taken for paralysis (Mins)	Time taken for death (Mins)
1	Control	NA	NA	NA
2	Standard	25	4.05(±0.49)	4.67(±0.53)
		50	3(±0.42)	3.92(±0.55)
		100	2.2(±0.41)	3.07(±0.69)
3	Extract	25	5.35(±0.79)	6.37(±0.84)
		50	3.98(±0.39)	5.03(±0.73)
		100	2.82(±0.51)	4.45(±1.02)
4	Fraction 1	25	8.6(±0.53)	9.97(±0.94)
		50	7.2(±0.54)	8.55(±0.73)
		100	6.13(±0.67)	7.58(±0.70)
5	Fraction 2	25	10.5(±0.98)	12(±0.85)
		50	9.35(±0.90)	10.85(±1.39)
		100	7.35(±0.79)	8.95(±1.06)
6	Fraction 3	25	4.63(±0.42)	5.98(±0.70)
		50	3.92(±0.50)	5.08(±0.97)
		100	3.05(±0.45)	4.78(±0.93)
7	Fraction 4	25	5.98(±0.50)	7.23(±0.85)
		50	4.35(±0.40)	5.92(±0.81)
		100	3.3(±0.52)	4.48(±0.99)

Table 9: Brine shrimp lethality assay

Plant /standard	Extract /fraction	Mean Percent Death after 24 hours			LC_{50}
-	-	10 µg/ml	100 µg/ml	1000 µg/ml	-
Control	-	-	-	-	0
Standard	Potassium dichromate	80	100	100	8.70
Mayurshikha	Extract	80	100	100	8.70
	Fraction 1	80	90	100	3.63
	Fraction 2	70	80	90	2.39
	Fraction 3	60	70	100	8.43
	Fraction 4	60	90	100	4.10

Discussion

Mayurshikha is an important fern which can be used for treatment of several diseases. In the present work, attempt has been made on pharmacognostical and biological evaluation of *Mayurshikha* plant. The macroscopic study resembles the correct identification of plant. The stability of the crude drug depends on moisture which affects the shelf life of drug. Lower moisture content lead to high stability and higher moisture contents results to low stability (12). The ash values are important quantitative tools for evaluating purity of powdered crude drug (27). An extractive value confirms the amount of the active constituents present based on the solvent (28). Based on the results, all the physicochemical parameters are found to be within limit. The phytochemical analysis revealed presence of lipids, phenols, terpenoids, sterols, alkaloids and flavonoids. The chemical nature of plant constituents can be identified by fluorescence analysis (12). Due to presence of chemical constituents, *Mayurshikha* exhibit different fluorescence. It has reported that polyphenols present in plants shows the various biological activities which are due to free radical scavenging property of polyphenols (29, 30, 31).The phenolic content in *Mayurshikha* was found to be 6.36 µg/mg of Gallic acid equivalents GAE/g of a standard curve of Gallic acid.

Furthermore, *Mayurshikha* extract and its fractions were subjected for *in vitro* biological assays. *In vitro* antioxidant activity were evaluated by DPPH free radical assay. It is revealed that fraction 4 showed promising effects due to presence of active chemical constituents responsible for antioxidant properties. *In vitro* antimicrobial activity on extract as well as on fractions were performed by agar well plate method. Based on the results it can be concluded that the fraction 3 and fraction 4 has promising antimicrobial efficiency as compared with other fractions. The anthelmintic activity showed the dose dependent activity. *In vitro* anthelmintic activity was performed on earthworms. Based on the results, it can be concluded that fraction 3 showed the better anthelmintic activity. Due to higher risk of adverse reactions associated with synthetic drugs, it will be promising plant based anthelmintic drug candidate. Furthermore, brine shrimp lethality assay on *Mayurshikha* extract and fractions were carried for cytotoxicity using potassium dichromate as standard. Based on results, Fraction 3 showed better LC_{50} value as compared with other fraction.

Conclusion

Mayurshikha is an enormous valuable medicinal plant used since ancient times due to their medicinal properties. In the present study, attempt has been made for pharmacognostical evaluation followed by *in vitro* biological activity evaluation on extract and fractions. The pharmacognostical study will be bench marker for the identification and standardization of the plant material. *In vitro* biological activity assessment on extract and their fractions will helps to identify which fraction shows better activity and thereby focusing on same fractions for future lead molecule identification. Concerning the present work, it was found that fraction F3 containing alkaloids showed promising antimicrobial, anthelmintic and cytotoxic effect. Based on the results, it can be concluded that present work can be used as scientific tool for the pharmacognostical evalution as well as for future plant research.

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Abbreviations

- GAE- Gallic Acid Equivalent
- WHO- World Health Organization
- ICMR-Indian Council of Medical Research
- NITM- National Institute of Traditional Medicine
- RMRC- Regional Medical Research Centre
- DPPH -2, 2-diphenyl-1-picrylhydrazyl.
- IC_{50} -Half maximal inhibitory concentration
- LC_{50} - Lethal concentration 50
- SPSS-20- Statistical Package for the Social Sciences software

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