

Pharmacognostical Standardization, Phytochemical Investigation, and Anthelmintic Activity of *Arisaema propinquum* Schott Rhizomes

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ABSTRACT

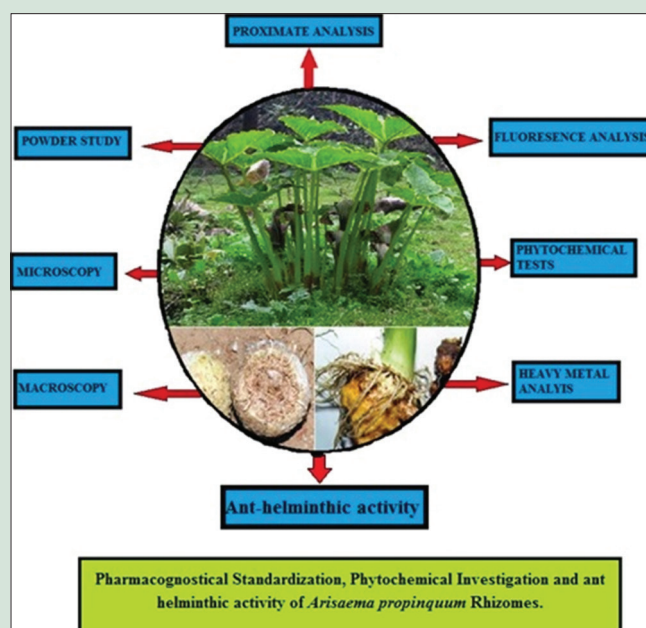
Ethnopharmacology: *Arisaema propinquum* Schott (*Araceae*) is commonly known as cobra lily. Traditionally, the rhizomes were used as vermifuge, in rheumatism, as stomachache, and in snake bites. **Objectives:** The present study was designed to evaluate the pharmacognostical parameters and anthelmintic activity of *A. propinquum* rhizomes. **Materials and Methods:** The rhizomes of *A. propinquum* Schott was collected, shade dried and then powdered, and then evaluated for pharmacognostic parameters such as macro- and microscopical characters, physico-chemical parameters, and phytochemical analysis using standard procedures. Anthelmintic activity of the extracts was evaluated against *Pheretima posthuma*. **Results:** The rhizomes are light brown in color with pungent odor and astringent taste. Transverse section of rhizomes showed intercellular schizogenous cavities, xylem vessels, phloem vessels, and parenchymatous cells. Phytochemical screening of the extracts reveals the presence of alkaloids, carbohydrates, cardiac glycosides, coumarins, proteins, amino acids, phenols, tannins, flavonoids, saponins, steroids, and terpenoids. Physicochemical parameters including ash values showed 6.32% total ash, 1.77% acid insoluble ash, 5.15% water-soluble ash, and 8.55% sulfated ash. Other parameters such as extractive value, foreign matter, moisture content, swelling index, foaming index, pH of different solvents, and fluorescence analysis were also determined. Both the methanolic and aqueous extracts of *A. propinquum* showed dose-dependent anthelmintic activity against *P. posthuma* compared to standard albendazole. **Conclusion:** This is the first report on the pharmacognostic studies and anthelmintic activity of *A. propinquum* Schott. Data composed from such studies can be used as a standard in the quality control of this plant used as an herbal medicine for the treatment of various diseases.

Key words: Anthelmintic, *Arisaema propinquum*, pharmacognostical standardization, *Pheretima posthuma*, phytochemical investigation

SUMMARY

- In the present era, the use of herbal products and herbal formulations are predominantly increased due to their least side effect and tremendous level of active ingredients. Till date, no detailed standardized work has been reported for this plant. The pharmacognostical parameters such as acid values, extractive values, foaming index, swelling index, fluorescence analysis, and preliminary phytochemical screening provide significant information by which one can easily authenticate the crude drug and also check the adulteration for the quality of raw material. The pharmacognostic parameters studied during the present research work are being reported for the first time and will be quite beneficial for the identification of herbal plant and also for the preparation of herbal monographs. The present study will also be helpful in differentiating the present species from its closely related species of the same genus and family.
- Indian adult earthworms (*Pheretima posthuma*) were used for studying

Anthelmintic activity of the herbal drugs in the present study as they share similar anatomical and physiological resemblance with the helminthic parasites of humans. In the present work, both methanolic and aqueous extracts of *A. propinquum* Schott showed good anthelmintic activity in a dosage-dependent manner as compared to aqueous and standard drug albendazole.



Abbreviations Used: TS: Transverse section; SE Tibet: Southeast Tibet; HIV: Human immunodeficiency virus; CBT: Centre for Biodiversity and Taxonomy; SEM: Standard error of mean.

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INTRODUCTION

The genus *Arisaema*, commonly known as cobra lilies, comprised more than 399 herbaceous species belonging to family *Araceae*. *Arisaema propinquum* Schott is commonly known as Wallich's cobra lily or cobra lily, and in Kashmir, the plant is known as Hapat-makei or Hapat-mundh. *A. propinquum* Schott is a tuberous plant that grows at an altitude of 2400–3600 m. The plant is widely distributed throughout temperate to tropical areas, mainly distributed in the Himalayas, from Kashmir to

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Southeast Tibet.^[1] The plant grows up to 5–6 feet in height with three large yellowish-green glossy leaves. The spathe or hood is thick at the base and ends in a pointy beak, which comprised 8–20 cm wide such as long tongue emerging out from inside. At the curvature of the hood, there is a white netting effect which presents the side edges such as a stained window. Leaf stalk and stem are often brown-spotted. The plant flowers in the month of May–June.^[2] Traditionally, the dried leaves, fruits, and roots are used to treat diabetic neuropathy, rheumatoid arthritis,^[3] chronic boils, and skin eruptions.^[4] Pastes prepared from the roots were applied externally for the treatment of erysipelas and scabies.^[5]

Helminthiasis or worm infection is among the most prevalent infections, effecting billions of people throughout the world.^[6] The disease spreads by contamination or inadequate sanitation.^[7] The helminthic disease decreases the immune responses to various pathogens and leads to serious clinical complications such as undernourishment, anemia, eosinophilia, pneumonia,^[8] malaria, tuberculosis, and human immunodeficiency virus.^[9] Due to the resistance of gastrointestinal helminthes to various available anthelmintic drugs, the demand for natural anthelmintics is increasing extremely day by day.^[10,11] Indian adult earthworms (*Pheretima posthuma*) were used for studying anthelmintic activity of the herbal drugs in the present study as they share similar anatomical and physiological resemblance with the helminthic parasites of humans.

MATERIALS AND METHODS

Collection of plant material and authentication

The rhizomes of *A. propinquum* Schott were collected from Doodhpathri area of Budgam district at 2730 m in the month of July 2013. The plant was identified and authenticated by Dr. Akhtar H. Malik, Taxonomist, Centre for Biodiversity and Taxonomy, Department of Botany, University of Kashmir, with reference number 1896-KASH.

Chemicals and reagents

All the chemicals used were of analytical grade and were procured from registered dealers such as HiMedia Laboratories Pvt. Ltd., Mumbai, and Central Drug House Ltd., New Delhi, India.

Macroscopical and Microscopical Evaluation

Macroscopical and microscopical evaluations of *A. propinquum* Schott were studied according to the methods described in Trease and Evans Pharmacognosy.^[12,13] The study was carried out to prevent adulteration which mainly occurs during the selection procedures of raw medicinal material from genus *Arisaema*.

Preparation of extracts

The fresh air-dried rhizomes of *A. propinquum* Schott was powdered and then subjected to hot extraction using methanol as a solvent in a Soxhlet apparatus. Aqueous extract was prepared by the decoction method. The extracts obtained were filtered through Whatman filter paper and concentrated under reduced pressure using a rotary vacuum evaporator. The extracts were then dried and transferred in a closed airtight container for further use.

Physicochemical analysis

The physicochemical analysis of *A. propinquum* Schott rhizomes was carried out by determining ash values, extractive values, foreign organic matter, moisture content, and pH of different solvents.^[14] Other parameters such as swelling index, foaming index,^[15] total tannin content, and total fat content^[16] were also determined.

Fluorescence analysis

Many herbs show fluorescence when cut surface or powder is exposed

to ultraviolet (UV) light, and this can be useful in their identification. The fluorescence character of the powdered plant parts (40 mesh) was studied both in daylight and UV light (254 and 366 nm) and after treatment with different reagents such as sodium hydroxide, picric acid, acetic acid, hydrochloric acid, nitric acid, iodine, and ferric chloride.^[17,18]

Phytochemical investigation

Weighed quantity of plant material was extracted separately with petroleum ether, chloroform, ethyl acetate, methanol, and water by hot extraction method. The extracts obtained were subjected to preliminary phytochemical investigation for the detection of following compounds: carbohydrates, protein, amino acids, fats and oils, sterols and steroids, glycoside, coumarins, flavonoids, alkaloids, tannins and phenolic compounds, acidic compounds, saponins, terpenes and terpenoids, mucilage resins, and lipids/fats.^[19,20]

Heavy metal analysis

Wet digestion procedure

1.0 g of plant material was digested with 5 mL mixture of nitric and perchloric acids in a digestion flask. The mixture was heated at 110°C until the solution became clear, indicating the complete digestion of the organic matter. The mixture was allowed to cool at room temperature. The digested solution was then diluted with 100 mL of deionized water and was analyzed using an atomic absorption spectrometer (AAS) for the determination of lead, copper, cadmium, and chromium.^[21]

Anthelmintic activity study

Earthworms were collected locally from Hazratbal district, Srinagar. Earthworms of 6–8 cm in length and 0.2–0.4 cm in width were collected and washed thoroughly in saline water to remove the external debris to be used for anti-helminthic activity. The earthworms were acclimatized to the laboratory condition before experimentation. The earthworms were divided into 6 groups of 5 earthworms in each and placed in Petri dishes containing 15 mL of sample/drug solutions, as mentioned below:

Group 1: Received 2% gum acacia which served as the control

Group 2: Received albendazole suspension at a dose of 10 mg/mL which served as the standard

Group 3: Received methanolic extract at a dose of 50 mg/mL

Group 4: Received methanolic extract at a dose of 100 mg/mL

Group 5: Received aqueous extract at a dose of 50 mg/mL

Group 6: Received aqueous extract at a dose of 100 mg/mL.

Anthelmintic potential of methanolic and aqueous extracts of rhizomes of *A. propinquum* was carried out using the method previously described by Ajaiyeoba *et al.* 2001, with necessary modifications.^[22] The Indian earthworms (*P. posthuma*) of nearly equal size were taken in Petri plates containing 15 mL of different concentrations (50 and 100 mg/mL) of methanolic and aqueous extracts suspended in normal saline. Albendazole suspension of the same concentration prepared in normal saline was taken as standard. All Petri dishes were kept under room temperature and under close observation. Observation was made for time taken to complete paralysis (PT) and death (DT) for individual worms. Time for paralysis was noted either when any movement could not be observed except when the worms were shaken vigorously or when dipped in warm water (50°C). Death was included when the worms lost their motility followed by white secretions and fading away of their body color.^[23]

RESULTS AND DISCUSSION

Macroscopic characters

The rhizomes of *A. propinquum* Schott are spherical in shape with light brown color outside and cream color inside with pungent odor and

astringent taste. The outer covering of the rhizomes is smooth and is of varying size ranging from 2 to 6 cm in diameter [Figure 1].

Microscopic characters

The transverse section (TS) of the rhizomes was circular in outline and showed the outer multilayered cortex cells, parenchymatous cells, schizogenous intercellular cavities, and well-developed xylem and phloem vessels [Figure 2]. The powdered microscopy of the rhizomes revealed the presence of cork cells, reticulate vessels, and prismatic calcium oxalate crystals [Figure 3].



Figure 1: Rhizomes of *Arisaema propinquum* Schott

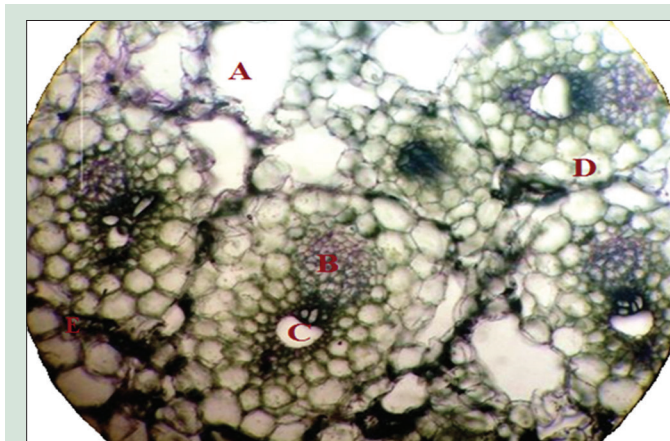


Figure 2: TS of *Arisaema propinquum* rhizome shows (A) schizogenous intercellular sacs, (B) xylem vessels, (C) phloem vessels, (D) parenchymatous cells, (E) cork cells

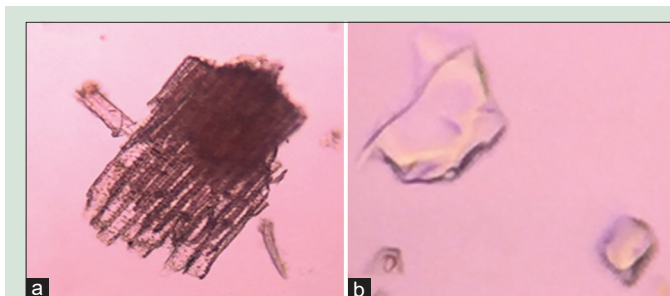


Figure 3: Powdered microscopy of *Arisaema propinquum* showed (a) reticulate vessels, (b) prismatic calcium oxalate crystals

Physicochemical analysis

For the evaluation of pharmacognostic characters of *A. propinquum* Schott rhizomes, various physicochemical parameters were studied. *A. propinquum* rhizomes showed the presence of total ash – 6.32%, acid insoluble ash – 1.77%, water-soluble ash – 5.15%, sulfated ash – 8.55%, moisture content – 18.50%, swelling index – 25.66%, and foreign organic matter – 1.1% [Table 1]. For determination of the extractive value, the powdered drug was extracted by three methods, namely cold extraction, hot extraction, and successive extraction [Table 2]. The fluorescence analysis, an important parameter for the determination of quality and purity of powdered drug material, was also evaluated [Table 3]. The powdered drug is also tested for heavy metal analysis using AAS [Table 4].

Table 1: Physicochemical analysis of rhizomes of *Arisaema propinquum*

Parameters	Content obtained
Total ash value (%w/w)	6.325
Acid insoluble ash value (%w/w)	1.77
Water-soluble ash value (%w/w)	5.15
Sulfated ash value (%w/w)	8.55
Foreign organic matter (%w/w)	1.1
Loss on drying (%w/w)	18.50
Swelling index (%w/w)	25.66
Foaming index	<100
Total tannin content (%w/w)	7.15
Total fat content (%w/w)	1.68
pH of 1% solution	6.50
pH of 10% solution	6.75

Table 2: Extractive values of rhizome part of *Arisaema propinquum*

Extractive values	Methanolic (%)	Aqueous (%)
Cold extractive value	12.8	10.3
Hot extractive value	15.9	15.2
Successive extractive value	16.7	14.8

Table 3: Fluorescence analysis of *Arisaema propinquum* rhizomes

Experiment	Visible/daylight	UV light (254 nm)	UV light (365 nm)
Powder as such	Greyish brown	Greyish brown	Black
Drug + water	Light brown	Greenish brown	Black
Drug + acetone	Greyish brown	Greenish brown	Black
Drug + pet ether	Greyish brown	black brown	Black
Drug + CHCl ₃	Dark brown	Greenish brown	Black
Drug + ethyl acetate	Dark brown	Greyish brown	Black
Drug + methanol	Greyish green	Greenish brown	Black
Drug + glacial acetic acid	Brown	Greyish brown	Dark brown
Drug + NH ₃	Light brown	Greenish brown	Black
Drug + HNO ₃ (Concentrated)	Reddish brown	Greenish brown	Black
Drug + HNO ₃ (Diluted)	Reddish brown	Greenish brown	Black
Drug + HCL (Concentrated)	Dark brown	Black brown	Black
Drug + HCL (Diluted)	Brown	Greenish brown	Black
Drug + H ₂ SO ₄	Brown	Dark greenish brown	Black
Drug + H ₂ SO ₄ (Diluted)	Brown	Dark brown	Black
Drug + Picric acid	Yellowish brown	Greenish brown	Black
Drug + 5% NaOH	Dark brown	Greenish brown	Black
Drug + 5% FeCl ₃	Greyish green	Greenish black	Black

UV: Ultraviolet

Phytochemical analysis

The results of qualitative phytochemical analysis of *A. propinquum* Schott rhizomes are shown in Table 5. The rhizomes showed the presence of alkaloids, carbohydrates, cardiac glycosides, coumarins, proteins, amino acids, phenols, tannins, flavonoids, saponins, steroids, and terpenoids.

The anthelmintic activity of methanolic and aqueous extracts of *A. propinquum* Schott was evaluated against Indian adult earthworms (*P. posthuma*) due to its similar anatomical and physiological resemblance with the intestinal worms. Methanolic and aqueous extracts of *A. propinquum* Schott showed dose-dependent anthelmintic activity as

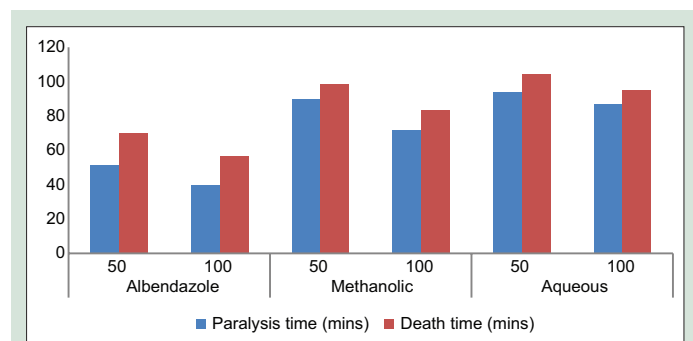


Figure 4: Death time and paralysis time of methanolic and aqueous extracts of *Arisaema propinquum*

Table 4: Heavy metal content in the rhizome of *Arisaema propinquum*

Heavy metal	Heavy metal in drug (ppm)	Permissible limit of heavy metals in crude drugs (ppm)
Arsenic	Not detected	NMT 10.0 ppm
Cadmium	Not detected	NMT 0.3 ppm
Lead	Not detected	NMT 1.0 ppm
Mercury	Not detected	NMT 10.0 ppm

NMT: Not more than

Table 5: Phytochemical screening of rhizome of *Arisaema propinquum*

Plant constituent	Methanol extract	Aqueous extract
Alkaloids	+	+
Carbohydrates	+	+
Antraquinone glycosides	-	-
Cardiac glycosides	+	+
Coumarin	+	+
Proteins and amino acids	+	+
Flavonoids	+	+
Steroids and terpenoids	+	+
Saponins	+	+
Resin	-	-
Tannins and phenolics	+	+

+: indicates Present, -: indicates Absent

Table 6: Anthelmintic activity of methanolic and aqueous extracts of *Arisaema propinquum*

Treatment	Concentration mg/mL	Paralysis time (min)	Death time (min)
Control	-	-	-
Albendazole	50	51.40 ± 0.81	70.2 ± 1.15
	100	39.60 ± 0.92	56.6 ± 1.80
Methanolic	50	89.53 ± 3.07	98.4 ± 1.05
	100	72.02 ± 0.56	83.41 ± 1.45
Aqueous	50	94.20 ± 1.02	104.2 ± 1.24
	100	86.76 ± 3.01	95.33 ± 2.11

compared to standard albendazole. However, methanolic extract showed potent activity as compared to aqueous extract [Table 6 and Figure 4].

Statistical analysis

Results are expressed as mean ± standard error mean ($n = 6$), and the comparisons are made by ANOVA followed by Tukey's test. GraphPad Prism 6 (GraphPad Software, San Diego, California) was used for the analyses.

The present endeavor of the study is to establish, as possible, the diagnostic characteristics of *A. propinquum* Schott rhizomes. In this regard, the macro- and microscopic study was carried out, showed the rhizomes are spherical in shape with light brown color outside and cream color inside with pungent odor and astringent taste. The TS of the rhizome showed the multilayered cortex cells, parenchymatous cells, schizogenous intercellular cavities, and well-developed xylem and phloem vessels. Physicochemical analysis showed 6.32% total ash, 1.77% acid insoluble ash, 5.15% water-soluble ash, and 8.55% sulfated ash. Extractive values, which are important parameters for the evaluation of crude drugs, determine the amount and nature of chemical constituents present within the crude drug. The pH value provides information about the acidic or basic nature of constituents present in the crude drug. For quality assurance, it is necessary to perform heavy metal analysis of the crude drug which was carried out according to the wet digestion method, and the results showed the absence of heavy metals in the crude drug indicating their safety for human consumption. The phytochemical analysis of the extracts showed the presence of alkaloids, carbohydrates, cardiac glycosides, coumarins, proteins and amino acids, phenols, tannins, flavonoids, saponins, steroids, and terpenoids.

Anthelmintic activity of methanolic and aqueous extracts was evaluated against Indian adult worm (*P. posthuma*). Both methanolic and aqueous extracts of *A. propinquum* Schott rhizomes showed good anthelmintic activity in a dosage-dependent manner. Methanol extract showed the highest anthelmintic activity as compared to aqueous and standard drug albendazole.

CONCLUSION

This study revealed that methanolic and aqueous extracts of *A. propinquum* Schott rhizomes possess good anthelmintic activity. Therefore, the extracts mainly methanolic which shows the highest activity has the potential for development as an anthelmintic agent against helminthic diseases which however needs further study to understand the underlying mechanism of extracts as an anthelmintic agent. Also, the data generated from the pharmacognostic and phytochemical analysis can be used as a standard in the quality control of this plant.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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