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Anti-inflammatory and Anti-helminthic potential of Methanolic and Aqueous extract of *Polygonum alpinum* rhizomes

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ABSTRACT

Aim: The present research work was carried out to evaluate the anti-inflammatory and anthelminthic activity of the extracts of *Polygonum alpinum* rhizomes. **Methods:** Fresh rhizomes of the *P. alpinum* were collected, washed and shade dried. The methanolic and aqueous extracts were first tested for phytochemical screening. *In-vitro* anti-inflammatory activities of crude extracts were evaluated by HRBC (Human Red Blood Cell) membrane stabilization method and percentage inhibition of protein denaturation method. Similarly anti-helminthic activity was evaluated using earthworms of 6-8 cm in length and 0.3-0.4 cm in width and the results were compared with standard drug Albendazole. **Results:** Phytochemical analysis of the extract reveals the of presence of Carbohydrates, Cardiac glycosides, Coumarins, proteins, amino acids, flavonoids, saponins, steroids, terpenoids, tannins and phenolics. The methanolic extract showed potent membrane stabilizing and significantly inhibit protein denaturation as compared to standard indomethacin. Methanolic extract at the concentration of 125µg/mL showed 81.29% membrane stabilizing activity as compared to aqueous extract 64.72%. Standard indomethacin showed 95.56 % activity at the same concentration. Similarly, methanolic extract at 500µg/mL showed 72.70% inhibition of protein denaturation as compared to aqueous extract 64.72%. Standard indomethacin showed 88.26% inhibition of protein denaturation at the same concentration. Both the methanolic and aqueous extracts showed dose dependent anthelminthic activity as compared to standard Albendazole. **Conclusion:** These results suggest that both the extracts from *P. alpinum* have promising anti-inflammatory and anthelmintic activity and that more broadly; plant extracts are a potential rich source of anti-inflammatory and anthelmintics to combat these diseases.

Keywords: *Polygonum alpinum*, anti-inflammatory, anti-helminthic, rhizomes, phytochemical analysis

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INTRODUCTION

Inflammation is a natural healing process, initiated by activating immune cells, molecular mediators and blood vessels against microbial infection or tissue/organ damage[1]. Inflammation that only takes place in vascularized tissue is a series of complicated biological reactions. The main aim of these biological reactions are to initiate tissue repair by removing or destroying injured or necrotic cells and induce healing and repairing tissue damage[2]. The various available drugs like NSAIDS and opioids used for treatment and are not useful in all cases, due to their side effects and potency[3]. Hence, search for other alternatives seems necessary and beneficial. The study of natural herbs that are used traditionally to cure inflammation is quiet fruitful and logical research strategy in the source of new anti-inflammatory drugs[4]. On the other hand Helminthiasis is an infection in which a body of host is infested by worms like tapeworm, pinworm or roundworm. The main cause of the disease is contamination and inadequate sanitation, in which the worm eggs or larvae are transmitted to the host through contaminated soil. These worms reside in gastrointestinal tract and may also burrow into other organs like liver[5]. The disease is among the most prevalent infections in humans, effects billions of people throughout the world and lead to serious clinical complications like undernourishment, anemia, eosinophilia, and pneumonia[6, 7]. The helminthic infection may also decreases the immune responses to various pathogenic diseases includes malaria, tuberculosis and HIV[8]. Due to resistance of gastro-intestinal helminthes to various available drugs, the demand for natural anthelmintics is increasing extremely day by day[9, 10]. Indian adult earthworms (*Pheretima posthuma*) sharing similar anatomical and physiological resemblance with the helminthic parasites of humans and hence used for studying anti-helminthic activity of the herbal drugs.

Polygonum alpinum All is a perennial herb growing up to 1.5 to 3m in height, belonging to the family Polygonaceae. The plant is widely distributed on alpine slopes in temperate Himalaya: Kullu to Kashmir. The plant is also distributed westward to S. Spain, Siberia and N. America. Traditionally the water extract of the roots are used to prepare rice and fed to arthritic patients. Poultices prepared from the rhizomes along with the seeds of *Medicago falcate* are applied locally on aching joints for relief in these patients[11, 12].

2. MATERIALS AND METHODS

2.1. Collection of Plant Material and Preparation of Extracts

The rhizomes of *Polygonum alpinum* were collected in the month of August 2013 from Aharbal area of J&K. The plant was authenticated in the center of Plant Taxonomy, Department of Botany, University of Kashmir, Hazratbal, Srinagar and specimen preserved there under voucher number 1896-KASH. The rhizomes were washed, shade dried and powdered. The powdered drug material (500 gm) was taken in a Soxhlet apparatus for (hot extraction) using methanol as solvent for 12 hours. Aqueous extract was prepared by decoction method. The crude extract was filtered through Whatt man No-2 filter paper and concentrated under vacuum by evaporating to dryness. The preliminary phytochemical analysis of the various fractions was carried out as per the standard methods[13, 14, 15].

2.2. Chemicals and Reagents

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

3. Determination of Anti-inflammatory Activity of *P. alpinum* rhizomes

3.1. The human red blood cell (HRBC) membrane stabilization method

Fresh Human blood (10 mL) was collected from the volunteers who had not taken any anti-inflammatory drugs for two weeks prior to the experimental plan and the blood is transferred to the heparinized centrifuged tubes, centrifuged at 3000 rpm for 10 min. The supernatant was carefully removed with a sterile pipette. The packed cells were resuspended in an equal volume of normal saline and centrifuged again. The washing was continued for 3 to 4 times until the supernatant was clear. A 10% Red Blood Cell (RBC) suspension was then prepared with normal saline and kept at 40° C undisturbed before use. The test sample consists of 0.5 mL of HRBC suspension, 1 mL of Phosphate buffer, pH 7.4, different concentrations of the drug (500, 1000, 1500, 2000, 2500 µg/mL and 2 mL hypotonic or hypo saline (0.25%, *w/v* NaOH). Indomethacin at varying concentrations is used as standard. All the assay mixtures were incubated at 37° C for 30 min and then centrifuged at 3000 rpm. The supernatant liquid was decanted and the hemoglobin content was estimated by a spectrophotometer at 560 nm. The percentage membrane-stabilizing activity was determined using following equation[16, 17].

$$\% \text{ Stabilizing activity} = \frac{\text{Optical density of drug}}{\text{Optical density of control}} \times 100$$

3.2. Inhibition of protein denaturation

Protein denaturation was carried according to method described by sakat *et al*., with minor modifications. The reaction mixture (5 mL) consists of 0.2 mL of egg albumin from fresh hen's egg. 2.8 mL of phosphate buffered saline pH 6.4 and various concentration of drug. Final volume was made with double distilled water. The reaction mixture is then incubated at 37 ± 2° C in a BOD incubator for 15 min and then heated to 51º C for 20 min, after cooling the samples the turbidity was measured at 660 nm using vehicle as blank. The percentage inhibition of protein denaturation was calculated by following equation[18, 19].

$$\% \text{ Inhibition} = \frac{\text{Abs. control} - \text{Abs. sample}}{\text{Abs. control}} \times 100$$

3.3. Anti-helminthic Activity

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, 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, 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 10mg/mL which served as the standard

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

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

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

Group - 6: Received Aqueous extract at a dose of 100mg/mL

Anthelmintic potential of methanolic and aqueous extracts of rhizomes of *P. alpinum* was carried out using the method previously described by Ajaiyeoba et al., with necessary modifications. The Indian earthworms (*Pheretima posthuma*) of nearly equal size were taken in petriplates containing 15 mL of different concentrations (50, 100 mg/mL) of methanolic and aqueous extracts suspended in normal saline. Albendazole suspension of 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[20, 21].

4. RESULTS AND DISCUSSION

4.1. Phytochemical screening

In the preliminary phytochemical screening of methanolic and aqueous extracts of *Polygonum alpinum* secondary metabolites like carbohydrates, cardiac glycosides, Coumarins, tannins, flavonoids, phenols, phytosterols, proteins, saponins, diterpenes, anthraquinones and alkaloids were tested. Phytochemical screening of methanolic and aqueous extracts of rhizomes of *Polygonum alpinum* demonstrated the presence of Carbohydrates, cardiac glycosides, coumarins, flavonoids, tannins, phenols, saponins, diterpenes, proteins and aminoacids. However resins, alkaloids and anthraquinone glycosides were found absent in all the extracts (Table-1).

Table 1: Phytochemical Screening of *Polygonum alpinum*

Plant constituent	Methanolic extract	Aqueous extract
Alkaloids	–	–
Carbohydrates	+	+
Anthraquinone glycosides	–	–
Cardiac glycosides	+	+
Coumarins	+	+
Proteins and amino acids	+	+
Flavonoids	+	+
Steroids and terpenoids	+	+
Saponins	+	+
Resin	–	–
Tannins and Phenolics	+	+

"+" positive & "-" Negative

4.2. *In-Vitro* Anti-Inflammatory Activity

4.2.1. Membrane stabilization

This study is based on the principle of stabilization of HRBC membrane by using a methanolic and aqueous extracts of *P. alpinum*, and the same was compared with that of standard indomethacin. The percentage membrane stabilizing activity shown by methanolic and aqueous extracts of *P. alpinum* was found to be 81.29% and 62.27% respectively at concentration of 125µg/mL, while indomethacin showed 95.56% of membrane stabilizing activity at the same concentration (Table 2 and Figure 1).

Table 2*: In vitro* membrane stabilization activity of methanolic and aqueous extracts of *Polygonum alpinum*

	% membrane stabilization		
Conc. µg/mL	Standard	Alcoholic	Aqueous
25	67.82±0.63	32.01±1.70	23.89±0.34
50	79.34±0.43	41.84±1.30	29.06±0.70
75	85.59±0.17	51.39±1.00	40.68±0.19
100	91.81±0.10	68.70±0.12	55.83±0.33
125	95.56±0.10	81.29±0.07	62.27±0.34

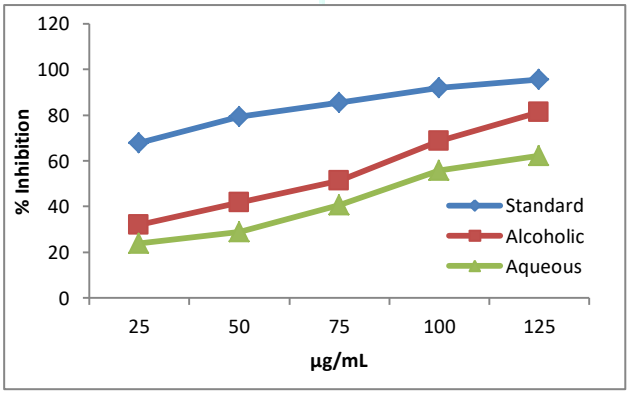


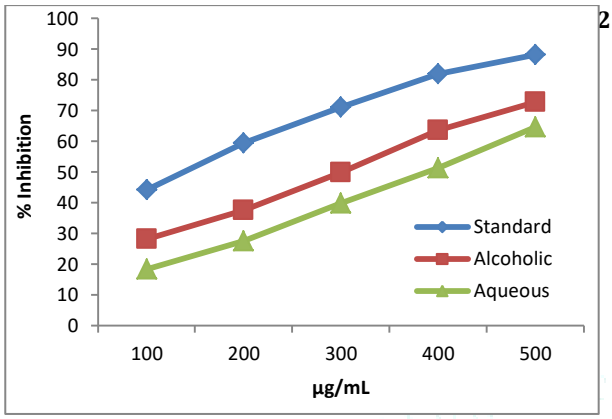
Figure 1: *In vitro* membrane stabilization activity of methanolic and aqueous extracts of *Polygonum alpinum.*

4.2.2 Protein denaturation

The percentage membrane stabilizing activity shown by methanolic and aqueous extracts of *P. alpinum* was found to be 72.70% and 64.72% respectively at concentration of 500µg/mL, whereas indomethacin showed 88.26% of membrane stabilizing activity at the same concentration (Tables 3 and Figure 2).

Table 3*: In vitro* protein denaturation activity of methanolic and aqueous extracts of *Polygonum alpinum.*

	% inhibition of protein denaturation		
Conc. µg/mL	Standard	Alcoholic	Aqueous
100	44.28±0.90	28.21±0.38	18.44±0.49
200	59.43±0.90	37.64±0.31	27.56±0.08
300	71.20±0.32	49.93±0.63	39.86±0.12
400	82.01±0.70	63.55±0.32	51.38±0.11
500	88.26±0.38	72.70±0.38	64.72±0.07



2: *In vitro* protein denaturation activity of methanolic and aqueous extracts of *Polygonum alpinum.*

4.3. Anti-helminthic activity

Similarly, in current study earthworms collected were subjected to anti-helminthic activity of *Polygonum alpinum* plant extract (Figure-3 & Table-4). Earthworms serve as preferred replacement as it shares similar anatomical and physiological resemblance with the intestinal parasites of humans which serves the objective of production of the influential anti-helminthic drug. Based on the current observations, methanolic extract showed anthelmintic activity in a dose-dependent manner giving the shortest time of paralysis and death with 100 mg/ ml concentration, for all worms.

Table 4: Ant-helminthic activity of methanolic and aqueous extracts of *Polygonum alpinum.*

Treatment	Conc. mg/mL	Paralysis time (mins)	Death time (mins)
Control	---	---	---
Albendazole	50	51.40 ± 0.81	70.2 ± 1.15
	100	39.60 ± 0.92	56.6 ± 1.80
Methanolic	50	71.60 ± 3.07	84.8 ±1.71
	100	59.80 ± 0.86	71.4 ±1.69
Aqueous	50	84.40 ± 2.42	90.4 ±2.27
	100	76.80 ± 5.05	83.4 ±3.14

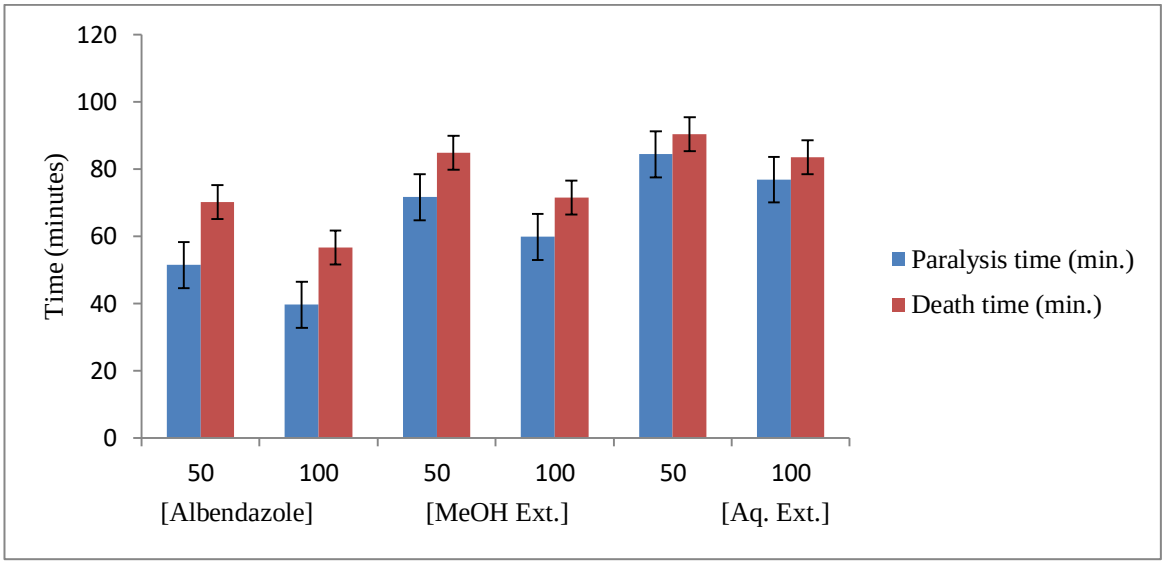


Figure 3: Death time and paralysis time of methanolic and aqueous extracts of *Polygonum alpinum.*

Statistical analysis

Results are expressed as Mean ± SEM (n=6), the comparisons are made by ANOVA followed by tukey test. Graph pad Prism 6 was used for the analyses.

4.4. CONCLUSION

As shown in results phytochemical analysis of both the extracts reveal the presence of Carbohydrates, Cardiac glycosides, Coumarins, proteins, amino acids, flavonoids, sapo-

nins, steroids, terpenoids, tannins and phenolics. The anti-inflammatory activity of the extracts was evaluated using HRBCs membrane stabilization and protein denaturation and the results were found to be directly proportional to its concentration. Methanolic extract showed potent membrane stabilizing as compared to aqueous extract and standard indomethacin. Methanolic extract at the concentration of 125µg/mL showed 81.29% membrane stabilizing activity as compared to aqueous extract 64.72%. Standard indomethacin showed 95.56% activity at the same concentration. Similarly methanolic extract at 500µg/mL showed 72.70% inhibition of protein denaturation as compared to aqueous extract 64.72%. Standard indomethacin showed 88.26% inhibition of protein denaturation at the same concentration. It is concluded from the above results that both alcoholic and aqueous extracts showed potent anti-inflammatory activity when compared with standard indomethacin. Both the methanolic and aqueous extracts showed dose dependent anthelminthic activity as compared to standard Albendazole. However further investigation is required for chemical and pharmacological properties.

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Conflicts of interest

There are no conflicts of interest

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