

Phytochemical Screening and Comparative Anthelmintic Activity of Alcoholic Extracts of Some Herbal Plants

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Abstract

In Indian medicinal plant literature, leaves of *Annona squamosa* (Sitaphal), *Azadirachta Indica* (Neem) and barks of *Punica granatum* (Anar) have been traditionally reported to be used as anthelmintics. The scientific authentication about traditional claim of anthelmintic activity of *Azadirachta Indica* (Neem) and *Annona squamosa* (Sitaphal) is already established whereas the scientific data for *Punica granatum* (Anar) as anthelmintic is not yet available. Hence, it is essential to examine these plant species for anthelmintic potential to support traditional claim which can prove beneficial to the suffering people. The ethanolic extracts of the three plant parts were investigated for anthelmintic activity using earthworms. Experimental setup comprised of 5 groups viz; ethanolic extracts of three plants, saline water and reference drug (albendazole). The concentrations of extracts and albendazole were kept same for comparative activity (50mg/ml). Saline water was kept as control. Determination of anthelmintic activity was done by recording the paralysis time and death time of the worms. Phytochemical test on plant extracts were carried out. The result showed that the anthelmintic activity of plant extracts were comparable to that of the reference drug. Also the data obtained from study showed that extract of *Punica granatum* (Anar) was most potent in activity than the other two plant extracts.

Keywords : Anthelmintic activity, ethanolic extracts, helminthiasis, phytochemicals.

1. INTRODUCTION

Disease has been an integral part of man and the search for remedies to combat it is been ongoing. Plants are good sources for new, safe, biodegradable and renewable drugs. The medicinal plants are recognized for their ability to produce a wealth of secondary metabolites and mankind has used many species for centuries to treat a variety of diseases. Herbal medicines are prepared from a variety of plant materials-leaves, stems, roots, barks, fruits, seeds, flowers and so on. India has an ancient heritage of traditional medicines. Materia Medica of India provides lots of information on the folkore practices and traditional aspects of therapeutically important natural products.^[1] Anthelmintics are used to treat people who are infected by helminths, a condition called helminthiasis. Increasing problems of development of resistance in helminthes against anthelmintics have led to the proposal of screening medicinal plants for their anthelmintic activity. Anthelmintic plants offer a traditional alternative to manufactured anthelmintics that is both sustainable and environmentally acceptable.^[2]

From the literature survey of traditional medicine, three plants which are freely available and have been used traditionally as anthelmintics were selected viz. *Annona squamosa* (leaves), *Azadirachta Indica* (leaves) and *Punica granatum* (barks).^[5] The anthelmintic activity of *Annona squamosa* (Sitaphal) and *Azadirachta Indica* (Neem) have been proved scientifically^[2,3,4] but the traditional claim of *Punica granatum*(Anar) is not yet established scientifically. In addition no scientific data is available about comparative anthelmintic activity of these three common plants. Hence in present study, attempts

were made to establish scientific claim of these plants and to compare their biological activity.

2. MATERIALS

2.1 Plant collection

Required parts of all plant species were collected. Selected parts were washed, shade dried at room temperature for 15 days. The leaves and the barks were powdered with the help of electric grinder separately and stored in air tight bottles for further study.

2.2 Used organism

Indian adult earthworms (*Pheretima posthuma*; Annelida; Megascolecidae) collected from water logged area of soil and identified at the department of Zoology, S.K.Porwal, College, Kamptee.

3. EXPERIMENT

3.1 Preparation of extracts

Dried and coarsely powdered parts of all plants were macerated for 8 days using ethanol as a solvent. All the extracts were concentrated by evaporation in water bath and then dried in open air.^[2]

3.2 Anthelmintic activity

In vitro anthelmintic activity was carried out. All the dried extracts of plant species were dissolved in minimum amount of DMSO and then volume was adjusted to 10 mL with saline water. For carrying out comparative anthelmintic activity 5 groups each of 6 earthworms were released in to 10mL of desired formulations as follows:

- Ethanolic extract of *Annona squamosa*
- Ethanolic extract of *Azadirachta indica*
- Ethanolic extract of *Punica granatum*
- Control: Saline water
- Reference drug: Albendazole

All the solutions of various extracts were freshly prepared before starting the experiment. Observations were made for the time taken to paralyse and death of individual worms. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Death was concluded when the worms lost their motility followed with fading away of their body colours. [1,2]

3.3 Phytochemical screening:

Following phytochemical tests of plant extracts were performed: [8,9,10]

- 1] Test for Tannins (Ferric chloride Test): A little portion of extract was diluted with water in the ratio of 1:4 and few drop of 10% Ferric chloride solution was added, end colour was noted which indicated presence of tannins.
- 2] Test for Alkaloids: 0.5g of each extract was stirred with 5mL of 1% aqueous Hydrochloric acid on a steam bath. 1mL each of the filtrate was treated, and then it was treated separately with a few drops of Dragandroff's reagents, Meyer's reagent and Wagner's reagents. End colour was noted.
- 3] Test for Saponins: 10mL of distilled water was added to 0.5mL of each extract. Shake the content vigorously with the test tube for 2 minutes. The presence of frothing or bubbling indicates the presence of saponins.
- 4] Test for Steroids: 5 drops of Concentrated sulphuric acid was added to 1mL of the extract. A reddish brown colour indicates the presence of steroids.
- 5] Test for Flavonoids; 1mL of each extract was dissolved in 2mL of sodium hydroxide solution. The appearance of a yellow solution which disappeared on addition of Hydrochloric acid indicates the presence of flavonoids.
- 6] Test for Cardiac Glycosides (Keller-Killiani test): 0.5g of each extract was dissolved in 3mL of Ferric chloride in glacial acetic acid and leave for a minute. 15mL of concentrated sulphuric acid was added with the aid of pipette, so that it runs down the side of the test tube. A clear interphase with a blue layer indicates the positive test.

4. RESULT AND DISCUSSION

The results obtained by performing various phytochemical tests are summarized below:

Table 1. Observation Table for Phytochemical Test for Plant Extracts

Sr.No	Tests	<i>Azadirachta indica</i> (Neem)	<i>Annona squamosa</i> (Sitaphal)	<i>Punica granatum</i> (Anar)
1	Tannins	+	+	+
2	Alkaloids	+	+	+
3	Saponins	+	+	+
4	Steroids	+	+	+
5	Flavonoids	-	+	+
6	Cardiac Glycosides	+	-	+

The observations obtained for the anthelmintic activity are given below:

Table 2. Observation Table for Anthelmintic Activity

Sr. No	Groups	Concentration (mg/ml)	Mean time taken for paralysis (min)*	Mean time taken for death (min)*
1	Control	50	-	-
2	Neem	50	19.14	31.45
3	Sitaphal	50	5.80	8.93
4	Anar	50	4.99	8.31
5	Albendazole	50	9.58	15.39

*Average of Six Readings of Earthworms

From the above observation, the plant extracts used in the experimental setup showed the anthelmintic activity. The data revealed that ethanolic extracts of plant parts of all three plants showed significant anthelmintic activity when compared with standard drug albendazole at the same concentrations. Ethanolic extracts of all three plant species were found to be more effective in promoting paralysis as well as in causing death of the worms.

5. CONCLUSION

Based on the findings of the present study it is concluded that, the ethanolic extracts of leaves of *Annona squamosa* (Sitaphal) and *Azadirachta Indica* (Neem) and barks of *Punica granatum* (Anar) found to have confirm their anthelmintic activity. Besides, we can conclude that *Punica granatum* (Anar), exhibited most significant anthelmintic activity among the other extracts. This study can be further carried out in the direction that can lead to know the role of secondary metabolites of the plant extracts in the anthelmintic activity. Therefore, further study must be carried out so that the general people can get actual benefit from this important medicinal plant.

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