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Anthelmintic Activity of *Elaeagnus latifolia* Juss. on Earthworms



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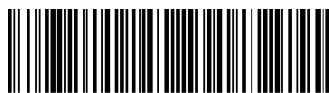
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ABSTRACT

Helminth infections occur in poverty-stricken areas and developing countries with warm and humid environments and where sanitary conditions are poor. Helminthiasis is the most common infection caused by worms that contaminate parts. Normally, the worms live in the gastrointestinal tract, liver and other organs. Only a few drugs are regularly used for the treatment of these parasite infections. The present study aimed to evaluate the anthelmintic activity of Hydroalcoholic extract of fruits of *Elaeagnus latifolia* Juss. using *Pheretima posthuma* as a test worms. The time of paralysis and time of death were studied and the activity was compared with Albendazole as a reference standard drug. The results thus support the use of *Elaeagnus latifolia* Juss. as an anthelmintic agent.

INTRODUCTION

Helminths are parasitic worms. The most common helminthiasis are those caused by infection with intestinal helminths, ascariasis, trichuriasis, and hookworm, followed by schistosomiasis and lymphatic filariasis (LF).^[1] Helminthiasis is an infection with either flukes (trematodes), tapeworms (cestodes), or roundworms (nematodes).^[2]

Helminthiasis is a disease that affects both man and animals leading to stunted growth. In developing countries, they pose a large threat to public health and contribute to the prevalence of malnutrition, anemia, consinophilia and pneumonia.^[3] Helminth infections are commonly found in villages of developing countries and are being recognized as the cause of many acute as well as chronic illnesses among humans.^[4]

Anthelmintics are drugs that either kill or expel infesting helminths and the gastrointestinal tract is the abode of many helminths, although some also live in tissues, or their larvae migrate into tissues. Helminthiasis is rarely fatal, but is a major cause of morbidity.^[5]

Currently, available anthelmintic drugs, including albendazole, mebendazole, thiabendazole, niridazole, diethylcarbamazine, ivermectin, praziquantel, are widely used to control helminthiasis.^[6]

Most of the existing anthelmintic drugs produce side effects such as abdominal pain, loss of appetite, nausea, vomiting, headache and diarrhea. To eliminate the harmful side effects of these synthetic anthelmintic drugs, it is important for us to promote the studies of traditionally used anthelmintic plants which will lead to the development of new anthelmintic substances with ease of availability and lesser side-effects.^[7]

Elaeagnus latifolia Juss. belongs to the family Elaeagnaceae locally known as Soh-shang in Khasi hills of Meghalaya. The flowers are hermaphrodite and are pollinated by Bees. The fruit is oblong in shape with dark pink color at the time of ripening.^[8]

The plant *Elaeagnus latifolia* Juss. is a thorny scandent shrub. The leaves are green colored and characteristic silvery-white at the beneath while the flowers are silver coloured. The fruits are green at early stage and dark pink/orange color at ripening.^[9]

Alkaloids, flavonoids, saponins and tannins have been demonstrated to possess anthelmintic activities. Chemically, tannins are polyphenolic compounds. They also suggested that the presence of steroidal alkaloids Anthelmintic activity of *Elaeagnus latifolia* Juss. Fruits. ^[10]

Most diseases caused by helminthes are of a chronic, debilitating nature; they probably cause more morbidity and greater economic and social deprivation among humans and animals than any single group of parasites. ^[11]

Today plants play an important role in the health care of about 80% of the world population and is estimated that more than half of the drugs under clinical use at present owe their origin to plants. For the acceptance of medicinal plants, it is necessary that their effectiveness and safety be evaluated and confirmed through *in vitro* and *in vivo* testing's.

However, the anthelmintic activity of *Elaeagnus latifolia* Juss. Fruits has not been scientifically investigated. Hence, the present study is undertaken for pharmacological evaluation of *Elaeagnus latifolia* Juss. fruits to evaluate its traditionally claimed anthelmintic activity. ^[12]

The fruits of *Elaeagnus latifolia* Juss. has traditionally been used for centuries as one of the most potential underutilized fruit crops among the tribal habitat of North Eastern Himalayan region, India. ^[13]

The fruit is considered to be a very rich source of vitamins, fatty acids and minerals and other bioactive compounds. The fruits are eaten raw with salt and used for making chutney and fruit pulp is used for making jam, jelly and refreshing drink. ^[8]

The flowers are used as astringent. The plant is investigated as a good source of food plant inhibiting growth of cancer cells. ^[9]

MATERIALS AND METHODS

1. Plant material

The fresh fruits of the plant of *Elaeagnus latifolia* Juss. Were collected from Shahuwadi region of Kolhapur district of Maharashtra state, India, in March 2023. The specimens were identified and authenticated by Dr. S.Y. Jadhav, M. Sc, M.Phil./SET, Ph.D., Head of Botany Department of Yashwantrao Chavan Warana Mahavidyalaya, Warananagar.

2. Preparation of extract

The collected plant materials were washed in running tap water and dried. It was then grinded properly, and then the powder was labeled and stored in airtight bottles for further use. The dried powder (120 gm) was extracted in a Soxhlet extractor with Hydroalcohol and a total of 45 cycles were run to obtain a thick slurry. This slurry was then vacuum evaporated to yield solid extract. The percentage yield of Hydroalcoholic extract was found to be 40.93%w/w. Extracts were prepared using an exact weighed sample powder (120 g) in the measured volume of solvent (500ml). Extraction was carried out using Soxhlet extraction method and solvent is poured into the round-bottom extraction flask, weighed and placed on the heating mantle. After this, the thimble containing the sample powder (120 g) was placed into the extraction chamber. Lastly, the condenser was placed on top of the extraction flask and all these parts were fixed vertically.

3. Phytochemical screening-

Test for alkaloids, glycosides, phytosterols, tannins, flavonoids, amino acid and fats are performed. ^[14]

4. Animals used

Indian adult earthworms *Pheretima posthuma* (Annelida) were used to study anthelmintic activity. The earthworms were collected from Shyam Agro, At Post Charan, Tal. Shahuwadi, Dist. Kolhapur and washed with normal saline to remove the faecal matter. The specimens were identified and authenticated by Dr. U. B. Chikurdikar, M.Sc, Ph.D, I/C Head, Department of Zoology, Yashwantrao Chavan Warana Mahavidyalaya, Warananagar. The earthworms of 8 cm in length were used for all experimental protocols. The earthworm resembles both anatomically and physiologically to the intestinal roundworm parasites of human beings and, hence can be used to study anthelmintic activity.

5. Drugs and Chemicals

Albendazole (Cipla Ltd.) and Ethanol were used during the experimental protocol.

6. Evaluation of Anthelmintic Activity

The anthelmintic activity was performed on the adult Indian earthworm *Pheretima posthuma*.

Albendazole, the standard drug, was diluted with water to obtain 25, 50 and 100 mg/ml concentrations and was poured into Petri dishes. Hydroalcoholic extracts of fruits of the plant were diluted with water to obtain 25, 50, and 100 mg/ml concentrations.

All these dilutions were poured into the Petri dishes accordingly. Six groups of earthworms ($n = 6$) were taken for the study. Earthworms, nearly equal sizes (about 8 cm), were placed in each Petri dish at room temperature. Time for paralysis was noted down when no movement of any sort could be observed, except when the worms were shaken vigorously. The time of death for worms was recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water (50°C). The paralysis time and lethal time were recorded in terms of minutes. ^[15]

RESULT

The percentage yield of hydroalcoholic extracts was found to be 40.93%w/w. Preliminary phytochemical screening of the extracts showed the presence of glycosides, flavonoids, alkaloids, steroids, and tannins. From the observations made, the extracts of fruits of *Elaeagnus latifolia* Juss. were found to show a potent anthelmintic activity when compared to the standard drug. It causes paralysis followed by death of the worms at all tested concentrations. As the concentration of the extracts was increased there was increase in anthelmintic activity. The potency of the extracts was inversely proportional to the time taken for paralysis and death of the worms.

DISCUSSION

Helminths are parasitic worms. They are the most common infectious agents of humans in developing countries and produce a global burden of disease. Helminth infections are commonly found in villages of developing countries and are being recognized as the cause of many acute as well as chronic illnesses among humans. ^[4]

Helminths infection occurs due to the warm, humid equatorial regions and inadequate hygiene sanitation facilities. The medicinal plants contain various secondary metabolites that exhibit anthelmintic activity. ^[16]

Tannins (phytoconstituents) are responsible for producing anthelmintic activities. Tannins can bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite (earthworms) and may cause death. [17]

CONCLUSION

The results of the present study indicated that the hydroalcoholic extract of *Elaeagnus latifolia* Juss. fruits did produce anthelmintic activity. Further studies using *in vivo* models are required to carry out and establish the effectiveness and pharmacological rationale for the use of *Elaeagnus latifolia* Juss. fruits as an anthelmintic drug. Further studies to isolate and reveal the active compound (s) contained in the crude extracts of *Elaeagnus latifolia* Juss. fruits and to establish the mechanism (s) of action are required.

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Tables –

Table 1. Qualitative chemical tests of hydro-alcoholic extract of *Elaeagnus latifolia* Juss. fruits.

Sr. No.	Plant Constituents	Test	Result	Observation
1	Test for alkaloids	Dragendroff's test	+	Reddish brown precipitate
		Mayer's test	+	White or creamy precipitate
2	Test for carbohydrates	Molisch test	+	Violet ring at the junction of two liquids
		Fehling's test	-	Formation of red precipitate
		Barfoeds test	+	Formation of red precipitate
		Benedict's test	+	Characteristic colored precipitate
3	Detection of glycoside	Bontrager's test	-	Formation of pink color
4	Detection of saponins	Foam or froth test	+	A two-centimeter layer of foam which is stable for 10 min.
5	Detection of phytosterols	Salkowski test	+	Red color in the lower layer
6	Detection of triterpenoids	Salkowski test	-	Golden yellow color
7	Detection of tannins	Ferric chloride test	+	Formation of violet colour
		Lead acetate test	+	Bulky white precipitate
8	Detection of flavonoids	Alkaline reagent test	+	Formation of intense yellow colour
		NH ₄ OH test	+	Development of yellow fluorescence
9	Detection of amino acids	Millions test	+	Formation of red color
		Ninhydrin test	+	Indicates purple color
10	Detection of fats	Solubility test	-	Soluble in water and insoluble in chloroform

Table 2. Anthelmintic activity of hydroalcoholic extracts of *Elaeagnus latifolia* Juss. fruits.

Sr. No.	Groups	Concentration (mg/ml)	Time (min)	
			Paralysis	Death
1.	Water	-	-	-
2.	Standard (Albendazole)	25	17.33	22.05
		50	16.66	21.16
		100	13.33	16.00
3.	HAEEL	25	15.33	21.16
		50	14.00	18.16
		100	11.05	14.33

HAEEL - Hydroalcoholic Extract of *Elaeagnus latifolia* Juss.

