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# Assessment of Anthelmintic Activity of *Elaeagnus latifolia* L. against Earthworms

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**ABSTRACT**

Human infection with parasites is still one of the big problems worldwide. Medicinal plants succeeded in overcoming a variety of protozoan and helminthic parasites. Ethanolic and aqueous extracts of *Elaeagnus latifolia* Linn fruits were taken for *in vitro* anthelmintic activity against Indian earthworm, *Pheretima posthuma*. Different concentrations (25, 50, and 100 mg/ml) of both the extracts were used for the activity. Varying albendazole concentrations (25, 50, 100 mg/ml) were used as a reference standard and normal saline (0.9% NaCl) was used for the control treatment. The results were expressed in terms of time in minutes to report the paralysis and time of death of the earthworms. The results obtained from the study indicate toward anthelmintic activity, supporting folk use of the plants when compared with the standard.

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## INTRODUCTION

Helminths infections are among the most common infections in human beings in whichhuman intestinal parasitic worms are vectored through air, food, and water, which causes disease state, secretes toxins, and steals vital nutrients from host bodies. [1]

The incidence of helminth infections is a global human health concern. The majority of infections due to helminth cause enormous hazard to health, contributing to the prevalence of under nourishment, anemia, eosinophilia and pneumonia. [2]

It is a disease in which a part of the body is infected with worms such as pinworm, roundworm or tap worm. [3]

The World Health Organization reports that 35% of diseases are because of roundworm, which is a typical parasitic worm. More than 1.5 billion individuals or 24% of the total population are tainted with soil-transmitted helminths contaminations around the world (WHO, 2016). [4]

Anthelmintics are drugs that either kill or expel infesting helminthes and the gastrointestinal tract is the abode of many helminths. [5]

Albendazole, mebendazole, thiabendazole, niridazole, diethylcarbamazine, ivermectin, praziquantel, are widely used anthelmintic drugs to control helminthiasis. [6]

In order to eliminate the harmful side effects of synthetic anthelmintic drugs, it isimportant 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] The synthetic anthelmintic drugs have too much adverse effect and toxicity. To eliminate side effects various plants are used. [8]

*Elaeagnus latifolia L.* has traditionally been known for centuries as one of the most potential underutilized fruit crops among the tribal habitat of North Eastern Himalayan region,India. [9] *Elaeagnus* has high nutritional value as the plant is contains high amount of microelements such as nitrogen, phosphorus, potassium, calcium, magnesium, sodium and also contains microelements which includes ferric, zinc, copper and manganese. [10]

When raw they are astringent, but when fully ripe they taste sweet with hints of sourness. As

the berries ripen, the soluble sugars, organic acids, lycopene, and total protein undergo variation in contents. [11]

The fruit is considered to be a very rich source of vitamins and minerals and other bioactive compounds. It is also a fairly good source of essential fatty acids. [12] 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. However, development of resistance in helminthes against conventional anthelmintics is a foremost problem in treatment of helminthes diseases. [13]

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. [14]

*Elaeagnus latifolia* L, the vernacular name is Sohshang in Khasi Hills and Slangi in Jaintia Hills of Meghalaya. The fruit has traditionally been used for centuries as one of the most potential underutilized fruit crops among the tribal habitat of the North Eastern Himalayan region,India. [15]

The fruit is edible and is rich in vitamins, sugars and minerals. Besides the edible use of the fruit, the plant *E. latifoliais* used traditionally for the management of ulcers, pulmonary complaints, heart pain, fever, sores and others. [16]

The flowers are astringent and cardiac. The fruit is astringent. The fruit of many members of this genus is a very rich source of vitamins and minerals, especially in vitamins A, C and E, flavanoids and other bio-active compounds. It is being investigated as a food that is capable of reducing the incidence of cancer and also as a means of halting or reversing the growth of cancers. The wood is a good fuel. [17]

The diseases by helminthesare neglected tropical diseases because they usually have insidious effects on growth and development. Also, the study of these diseases receives less than 1% of the global research budget. [18]

Approximately 20 helminth species are considered to be clinically significant and thesefall into two main categories those in which the worm lives in the host's alimentary canal, andthose in which the worm lives in other tissues of the host's body. [19]

The anthelmintics used against intestinal helminths (vermifuges), kill or sterilize the worms, or paralyses them so that they lose hold on the intestinal mucosa and are expelled via the faeces. When treating intestinal parasitism, 12-24 hours fast or light meals, prior to drug administration are advisable. [20]

## MATERIALS AND METHODS

### 1. Plant material

The fresh fruits of plant of *Elaeagnus latifolia* were collected from Shahuwadi region of Kolhapur district of Maharashtra state, India, in the month of March 2022. 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 fruits were washed under running tape water, dried under shade and course powdered in a mechanical grinder. The dried powder (200 gm) was extracted in a Soxhlet extractor with ethanol and a total of 50 cycles were run to obtain thick slurry. 200 gm of powder was macerated successively with 1000 ml of distilled water for seven days with intermitted stirring and then it was filtered and concentrated to get think slurry. This slurry was then vacuum evaporated to yield solid extract. The percentage yield of ethanolic extract was found to be 18.54%w/w and that of aqueous extract was 16.80%w/w. The dried extracts were stored in a well-closed, air-tight and light-resistant borosil glass container.

### 3. Phytochemical Screening

Test for alkaloids, glycosides, phytosterols, tannins, flavonoids, amino acids and fats are performed. [21]

### 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.

### 5. Drugs and Chemicals

Albendazole (Zentel, GlaxoSmithKline 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*. [22] Albendazole, the standard drug, was diluted with normal saline to obtain 25, 50 and 100 mg/ml concentrations and was poured into Petri dishes. Ethanolic and aqueous extractsof fruits of plant were diluted with normal saline to obtain 25, 50 and 100 mg/ml concentrations.Normal saline (0.9% NaCl) alone served as the negative control.

All these dilutions were poured into the Petri dishes accordingly. Six groups ofearthworms (*n* = 6) were taken for the study. Earthworms, of 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.Time of death for worms was recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water (50$^{o}$C). The paralysis time and lethal time were recorded in terms of minutes.[23]

## RESULT

The percentage yield of ethanolic and aqueous extracts was found to be 18.54%w/w and 16.80%w/w, respectively. Preliminary phytochemical screening of both the extracts showed the presence of glycosides, flavonoids, alkaloids, steroids and tannins. From the observations made, both the extracts of fruits of *Elaeagnus latifolia* were found to show a potent anthelmintic activity when compared to the standard drug (Table 3). It causes paralysis followed by death of the worms at all tested concentrations. As the concentration of both extracts was increased there was an increase in anthelmintic activity. The potency of the extracts wasinversely proportional to the time taken for paralysis and death of the worms.

## DISCUSSION

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. [24]

The anthelmintic activity of ethanolic and aqueous extracts of *Elagnus latifolia* fruits and albendazole were performed in laboratory conditions. *Pheretima posthuma* worms can beused successfully for the anthelmintic activity study as it is easy, prominent, an adaptable to laboratory conditions and reproducible method in all aspects such as equal age, size and weight of the worms.

Tannins are responsible to produce anthelmintic activities. Tannins can bind to free proteins in the gastrointestinal tract of host animal or glycoproteins on the cuticle of the parasite (earthworms) and may cause death. [25]

It is possible that, tannins present in the ethanolic and aqueous extracts of *Elaeagnus latifolia* fruits produce similar effect.

## CONCLUSION

From the above discussion, it may be concluded that both the extracts of *Elaeagnus latifolia* fruits have potent anthelmintic activity when compared with the conventionally used drugs and are equipotent to standard anthelmintic drugs. Further studies using *in vivo* models are required to carry out and establish the effectiveness and pharmacological rationale for the use of *Elaeagnus latifolia* fruits as an anthelmintic drug. Further studies to isolate and reveal the active compound (s) contained in the crude extracts of *Elaeagnus latifolia* fruits and to establish the mechanism (s) of action are required.

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**Tables -**

**Table 1. Qualitative chemical tests of ethanolic extract of *Elaeagnus latifolia* fruits. [21]**

| 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 color |
| | | Lead acetate test | + | Bulky white precipitate |
| 8 | Detection of flavonoids | Alkaline reagent test | + | Formation of intense yellow colour |
| | | $NH_4OH$ 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. Qualitative chemical tests of aqueous extracts of *Elaeagnus latifolia* fruits. [21]**

| 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 colour in the lower layer |
| 6 | Detection of triterpenoids | Salkowski test | - | Golden yellow colour |
| 7 | Detection of tannins | Ferric chloride test | + | Formation of violet color |
| | | Lead acetate test | + | Bulky white precipitate |
| 8 | Detection of flavonoids | Alkaline reagent test | + | Formation of intense yellow colour |
| | | $NH_4OH$ 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 3. Anthelmintic activity of ethanolic and aqueous extracts of *Elaeagnus latifolia* Fruits.**

| Sr. No. | Groups | Concentration (mg/ml) | Time (min) | |
|---|---|---|---|---|
| | | | Paralysis | Death |
| 1. | Control (Normal Saline, 0.9% NaCl) | - | - | - |
| 2. | Standard (Albendazole) | 25 | 15.33 ± 0.65 | 23.83 ± 0.75 |
| | | 50 | 13.17 ± 0.37 | 21.17 ± 0.60 |
| | | 100 | 7.50 ± 0.35 | 14.17 ± 0.52 |
| 3. | EEEL | 25 | 13.83 ± 0.78 | 20.67 ± 0.53 |
| | | 50 | 11.33 ± 0.67 | 17.17 ± 0.67 |
| | | 100 | 5.83 ± 0.29 | 9.17 ± 0.68 |
| 4. | AEEL | 25 | 14.83 ± 0.63 | 21.67 ± 0.49 |
| | | 50 | 12.67 ± 0.47 | 18.17 ± 0.59 |
| | | 100 | 6.83 ± 0.38 | 10.33 ± 0.49 |

Vales are expressed as a mean ± SEM, n = 6.

**EEEL:** Ethanolic Extract of *Elaeagnus latifolia*. **AEEL:** Aqueous Extract of *Elaeagnus latifolia*.