IJPSR (2023), Volume 14, Issue 10 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 22 February 2023; received in revised form, 02 May 2023; accepted 31 May 2023; published 01 October 2023

SCIENTIFIC CORROBORATIVE EVIDENCE OF ANTHELMINTIC ACTIVITY OF *CITRUS MAXIMA* LEAF: A TRADITIONALLY USED HERB OF NORTHEAST INDIA

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Keywords:

Citrus maxima, Anthelmintic activity, Ethanolic extract, *Pheretima posthuma*, Albendazole

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ABSTRACT: *Citrus maxima*, a well-known citrus species, is an important Assam plant used to treat various disorders by many communities in Northeast India. The local tribes of Northeast India have been using the leaves of C. maxima to eradicate worm infestations since time immemorial. Till date, no scientific research has been conducted on this plant to determine its anthelmintic potential. As a result, an extensive screening was conducted on the anthelmintic potential of ethanol extract of *Citrus maxima* leaves against *Pheretima posthuma* using albendazole as the reference standard. Fifty-five Indian adult earthworms were collected and divided into eleven groups of five worms each. This essay considered three concentrations of the standard drug albendazole and the ethanolic extract of *C. maxima* leaves. This involved keeping track of the time required for the worms' paralysis and death after being exposed to the drugs. Results showed a dose-dependent increase in the anthelmintic activity with ethanolic extract of *Citrus maxima* showed the highest activity at the dose of 200 mg/mL. When compared with the standard drug albendazole, the results produced by the extract implied that the leaves of *C. maxima* have high anthelmintic activity. This study scientifically validated the traditional anthelmintic of the leaves of *Citrus maxima*.

INTRODUCTION: A third of the world's population suffers from helminthiasis. The primary factor contributing to this disease's higher prevalence in impoverished nations is worse personal and environmental hygiene [1]. Worm infestations are seen in cattle and crops, in addition to humans, which causes serious health and socioeconomic issues.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.14(10).5070-73

This article can be accessed online on www.ijpsr.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.14(10).5070-73

Helminth infections have been linked to illnesses like pneumonia, eosinophilia, anaemia, and malnutrition [2]. The side effects of currently available anthelminthic medications include nausea, vomiting, headaches, abdominal discomfort, and diarrhoea.

Even the well-tolerated medication albendazole has been known to cause gastrointestinal side effects and dizziness in a small percentage of people [3]. Traditional herbal therapies and home cures used to treat worm infections in tropical developing nations like India may hold the key to developing newer, superior anthelmintic medications with fewer or no adverse effects.

The issue of helminthes developing resistance to traditional anthelmintic medicines can also be resolved using herbal anthelmintic agents [4]. In these countries, it is known that helminth infection can be prevented by employing a variety of herbal remedies, which frequently serve as a natural, affordable alternative to expensive medications. Apart from evidence of their historical use, their efficacy does not appear to have any conclusive scientific evidence [5]. In order to assess the anthelmintic properties of *Citrus maxima*, mature earthworms were utilized in this assay due to their wide availability and morphological similarity to intestinal worms.

Citrus maxima is a member of the Rutaceae family and genus Citrus. Its common names include shaddock, papanus, pummelo, and chakotra. According to the botanical classification system, this plant is categorized scientifically into a number of class, divisions and families [6].

Plant Profile:
Botanical Name: *Citrus maxima*

Taxonomical Classification:

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Rosidae

Family: Rutaceae

Citrus (Linn) of Rutaceae, an evergreen aromatic shrub and small tree, plays an important role in Indian medicine and the fruit economy. *Aurantium maximum Burm*is is another scientific name for it. Some another common names are *Citrus aurantium L., Var grandis L., Citrus Decumana L., Citrus grandis Osbeck,* and *Citrus pamplemos*. *Citrus grandis (Linn)* is a crop plant found in India, China, Indonesia, America, and Thailand, among other places [7]. The pummelo tree grows to be about 16 to 50 feet tall. Pomelo is a Malayu island and East India native plant. It is prevalent in China, Japan, the Philippines, Indonesia, the United States, and Thailand [8]. *Citrus maxima*, also known as Papanus, is a perennial shrub found throughout India. *Citrus maxima*'s bark and root contain -sitosterol, an acridone alkaloid. Limonin, nerolol, nerolyl acetate, and geraniol are all found in the essential oil extracted from the leaves and unripe fruits [9]. Pommelos, like other citrus plants, are high in Vitamin C. They are commonly consumed as fruit. It has been used as a sedative in indigenous medicine to treat nervous affections, convulsive cough, hemorrhagic diseases, and epilepsy. It is said to have palatable, cardiac stimulant, and antitoxic properties [10]. *Citrus maxima* fruits are also high in polyphenolic compounds such as hesperidin, naringin, caffeic acid, P-Coumaric acid, Ferulic acid, and vanillic acid [11]. It demonstrates various pharmacological activities that have been studied.

MATERIALS AND METHODS: The leaves of *Citrus maxima* were collected from the Mirza, Assam India (26.0983° N, 91.5354° E). The leaves were identified and authenticated by Dr. Souravjyoti Borah, curator at the GUBH Department of Botany Gauhati University, Assam. vide letter reference no. Herb./GUBH/2022/022. The collected sample used for identification is shown in **Fig. 1.**

FIG. 1: LEAF OF *CITRUS MAXIMA*

The leaves were washed thoroughly with water. The leaves were then dried partially under sunlight and partially under the shade for a week. The dried leaves were then ground in mechanical grinder and stored in airtight, moisture-free containers.

Preparation of Extract: 250 g of powdered crude drug of *Citrus maxima* leaves were subjected to Soxhelation (Continuous hot extraction) with 1000

mL of ethanol. The extracts were concentrated, and preliminary phytochemical tests were carried out with all the extracts in order to evaluate for the presence of different phytochemical constituents. The ethanolic extract of *Citrus maxima* showed the presence of major phytoconstituents like amino acids, carbohydrates, flavonoids, coumarin, and carotenoids.

Test Organism: The test organism Indian Adult Earthworms (*Pheretimaposthuma*) were chosen for this assay due to their anatomical and physiological similarity to the intestinal roundworm parasites found in humans and their abundance and ease of availability. The worms were collected from damp soil and washed with 0.9% w/v normal saline to remove dirt and feces. The worms chosen were uniform in size, ranging from 4-6 cm in length to 0.3-0.6 cm in width.

Drugs and Chemicals used in the assay:

- Freshly prepared 0.9% w/v of normal saline.
- Standard drug Albendazole was prepared at different concentrations of in distilled water.
- imilarly ethanol extract was prepared at the concentration in distilled water and these were used as test drugs for the activity.

Anthelmintic Activity Evaluation: The anthelmintic assay was carried out as per the method of *Ishnava et al.*, 2022 with trivial modifications in the process [12]. Fifty-five Indian Adult Earthworms were collected, and groups were divided into eleven, containing five earthworms in each group. Five groups of the standard drug (Albendazole) treated at doses of 12.5, 25, 50, 100 and 200 mg/mL; and five groups of the extract treated at doses of 12.5, 25, 50, 100, and 200 mg/mL. After being washed with 0.9% w/v normal saline solution, the earthworms were placed in petri dishes. The worms' motility was observed, and the times for paralysis and death were recorded. When the worms were shaken vigorously, the time for paralysis was recorded. When the worms showed no movement even when dipped in warm water at 50- 60° C temperatures and when their body colour faded, the time for death was recorded.

RESULTS: The results of the *in-vitro* athelmintic activity of the ethanol extracts of *C. maxima* are tabulated in **Table 1**. It is observed that the activity demonstrated by the extract was comparable with the standard drug albendazole. The standard and the extract displayed a dose-dependent increase in the anthelmintic activity. However, it was observed that the time taken for paralysis and death were much higher of the extract when compared with the standard drug. It was observed that for the worms treated with the highest dose of albendazole (200 mg/mL), the time for paralysis and time of death was only one minute apart. However, in the extract-treated group, the time for paralysis and time of death were separated by an interval of 4.33 min. The results suggested that the ethanolic extract of the leaf of *Citrus maxima* had tremendous potential to be used as an anthelminitc herbal remedy.

TABLE 1: *IN VITRO* ANTHELMINTIC ACTIVITY OF LEAF EXTRACTS OF *CITRUS MAXIMA*

Test sample	Concentration (mg/ml)	Time taken for paralysis(min) (Mean ± SD)	Time taken for death(min) (Mean ± SD)
Control group	-	-	-
Albendazole	12.5	14.33 ± 0.76	15.16 ± 0.76
	25	12.50 ± 1.32	14.16 ± 1.60
	50	11.50 ± 1.32	13.00 ± 2.78
	100	9.66 ± 0.76	11.66 ± 2.30
	200	9.33 ± 0.81	10.33 ± 1.52
Ethanol extract	12.5	16.60 ± 0.79	18.50 ± 0.5
	25	14.33 ± 2.08	18.16 ± 1.04
	50	14.50 ± 0.50	17.16 ± 0.76
	100	12.00 ± 1.00	16.33 ± 1.25
	200	11.33 ± 1.52	13.83 ± 2.36

DISCUSSION: Various worm species, including *Ascaris, Nippostrongylus* have been used in the screening of anthelmintic agents. Earthworms, however, have been widely accepted for *in-vitro* studies due to their resemblance to intestinal roundworms in response to anthelmintics and their ease of availability. It has been discovered that all toxic anthelmintics to earthworms are worthy of

further research as potent anthelmintic agents. This study looked at the antihelmintic activity of different concentrations of *Citrus maxima* extracts. The findings are summarised in **Table 1**. Based on the findings, it can be said that *Citrus maxima* ethanol extracts exhibit dose-dependent anthelmintic action when compared to albendazole. The effectiveness of the ethanolic extract of the leaf of *Citrus maxima* as an anthelmintic has been confirmed by the study's findings because it showed resistance to the worm employed in the experiment. The time it took for paralysis to occur and the time the worms died were shown to be inversely correlated with the potency. The less time it took for the earthworms to paralyze and die, the higher the drug's efficacy. The results provided a scientific explanation to the fact that many tribal communities of Northeast India have been using these leaves in raw, or decoction form to treat worm infestation in children and adults. Based on our current findings, the potential mechanism underlying Citrus maxima leaf extract anthelminthic effects cannot be accounted for. However, more research is required to determine the compound that is the active component in anthelmintic activity.

CONCLUSION: The present study sheds scientific light on the traditional use of Citrus maxima leaves as an anthelmintic herbal remedy for treating worm infestations. Till date, no scientific study has been made on this topic. The study demonstrated that the leaves had potent anthelmintic activity compared to a standard drug. The species used in this study were common earthworms *Pheretima posthuma*. Therefore, further studies with other species of worms are required to obtain a broader overview of the topic. Further research activities are also required to be carried out to determine the exact phytochemical and mechanism responsible for the anthelmintic activities of the leaves of *Citrus maxima*. This study established the leaves of *Citrus maxima* as an anthelmintic agent.

ACKNOWLEDGEMENT: The authors are thankful to the management of NEMCARE Group of Institutions for providing the necessary facilities to conduct the research work.

CONFLICT OF INTEREST: The authors declare no conflict of interest.

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How to cite this article:
Deka B, Patowary P, Bharali D, Sahariah BJ, Talukdar A, Sarma B, Lahkar C, Sharmah H and Bora NS: Scientific corroborative evidence of anthelminitc activity of *Citrus maxima* leaf: a traditionally used herb of Northeast India. Int J Pharm Sci & Res 2023; 14(10): 5070-73. doi: 10.13040/IJPSR.0975-8232.14(10).5070-73.