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Phytochemical Investigation and *in vitro* Evaluation of Anthelmintic Activity of *Gmnelina arborea roxb*. Fruit Extracts

BHABANI SHANKAR NAYAK[1,*], PRABHAT KUMAR JENA[1], SUBAS CHANDRA DINDA[1] and P. ELLAIAH[2]

[1]Department of Pharmaceutical Technology, School of Pharmaceutical Education and Research, Berhampur University, Berhampur-760 007, India
[2]Department of Pharmaceutical Technology, Jeypore College of Pharmacy, Rondapalli, Jeypore-764 002, India

*Corresponding author: Fax: +91 6854 246602; E-mail: bhabani143@yahoo.co.in

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Zmelina arborea Roxb. is found in the tribal areas of Koraput and Ganjam district of Odisha, India. It is extensively used traditionally by the tribal people as anthelmintic, antimicrobial, antifungal, antidiabetic and hepatoprotective. The present study is an attempt for the preliminary investigation of phytochemical constituents and to explore the anthelmintic activity of different extracts of unripe fruits of plant *Z. arborea* using ethanol, ethyl acetate, *n*-butanol and petroleum ether as solvents. The extracts were screened for phytochemical constituents and evaluated for their anthelmintic activity on adult Indian earthworms, *Pheretima posthuma*. The tests for cardiac glycosides and steroids were positive for all the extracts. The ethanol and *n*-butanol extracts were containing most phytochemicals where as ethyl acetate extracts was containing least number of phytochemicals. All extracts were able to show anthelmintic activity at 10 mg/mL concentration. The activities are comparable with the standard drugs such as piperazine citrate and albendazole. All the doses of ethanol, ethyl acetate and petroleum ether extracts of *Z. arborea* showed lesser anthelmintic activity than the standard drugs piperazine citrate and albendazole except ethanol extract at 25 mg/mL of concentration which showed better anthelmintic activity than the both standard drugs. When the dose of such extract is increased, a gradual increase in anthelmintic activity was observed. Among all the solvent extracts the *n*-butanol extract showed better anthelmintic activity even in comparison with both the standard drugs. The data were verified as statistically significant by using one way ANOVA at 5 % level of significance ($p < 0.05$).

Key Words: *Zmelina arborea*, Anthelmintic, Piperazine citrate, Albendazole, *Pheretime posthuma*.

INTRODUCTION

Helminthes infections are among the most widespread infections in humans, distressing a huge population of the world. Although the majority of the infections due to helminthes are generally restricted to tropical regions, cause enormous hazard to health and contribute to the prevalence of undernourishment, anaemia, eosinophilia and pneumonia[1]. Parasitic diseases cause ruthless morbidity affecting principally population in endemic areas[2]. The gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases[3]. Hence there is an increasing demand towards natural anthelmintics.

Gmelina arborea Roxb. belonging to family Verbenaceae locally named as Gambhari (Oriya), Gambhar (Hindi), Gambhar (Bengali), Sriparni (Sanskrit) and Gummadi (Telugu)[4]. Bark light grey colored exfoliating in light colored patches when old, blaze thick, a chlorophyll layer just under the outer bark, pale yellow white inside[7]. The yellow flower, tinged with brown, is trumpet shaped, 3-4 cm long. The trumpets flare open into a gaping mouth with 5 distinct lobes[5]. The fruit is oval in shape, 3/4 inches in length and is yellow in color. The fruit taste sweet and astringent. Leaves are 4-9 inches in length and 2 ½ inches in breadth. These are of heart shape, petioles is 2-6 inches in length[6].

The fruits alleviate pitta dosa and possess heavy and oily attributes. Fruits are used traditionally for heart diseases, leprosy, vomiting and burning sensations. Its fruits have alterative, aphrodisiac, astringent, diuretic and tonic characteristics that some have prescribed them for alopecia, anaemia, strangury, thirst and vaginal discharges[7].

The literature survey reveals that there were no reports scientifically on the anthelmintic activity of the fruit extracts of *G. arborea*. This prompted us to investigate the anthelmintic activity of *G. arborea* unripe fruit extracts.

EXPERIMENTAL

Albendazole (Micro Lab. Ltd., Goa, India) and piperazine citrate (Burroughs Wellcome Ltd., Mumbai, India) were

procured as gift sample. The AR ethanol and AR ethyl acetate 60-80 ºC (Emsure® ACS) were procured from Merck Pvt. Ltd., Navi Mumbai, Maharastra, India. *n*-Butanol GR 80 ºC and petroleum ether AR 40-60 ºC were procured from Loba Chemie Pvt. Ltd., Mumbai, India. All other chemicals and reagents were procured from authorized dealer.

Collection of plant materials, identification and size reduction: The unripe fruits of *Z. arborea* were collected from local area of Koraput district (India) in the month of April and May 2008. The plant was identified and authenticated by the Biju Patnaik Medicinal Plants Garden and Research Centre, Dr. M.S. Swami Nathan Research Foundation, Jeypore, Koraput (District), Orissa (Letter No. MJ/DBT (08)/1067, dated 05.09.2008). The unripe fruits were shade dried under normal environmental condition. The dried fruits were pulverized to from coarse powder by using electrical grinder and stored in a closed air tight container for further use.

Worm collection and authentication: The Indian earthworm *Pheretima posthuma* (Annelida) were collected near the swampy water lodge area along Jeypore road, Koraput, Odisha and authenticated from the Department of Zoology, B.D. College, Jeypore, India.

Preparation of extract: The coarse powder form of dried fruits was extracted by soxhlation method by using ethanol, ethyl acetate, *n*-butanol and petroleum ether as solvents. In this extraction process, a total amount of 1500 g powdered leaves were extracted with 1200 mL of each solvent. For each solvent, 10 cycles were run to obtained thick slurry. The thick slurry was then concentrated under reduced pressure to obtained crude extract. All crude extracts were kept in closed air tight container under cool and dark place for further study.

Phytochemical analysis: For the detection of the presence of carbohydrates and reducing sugars the standard tests Molisch's tests for carbohydrate and reduction of Fehling's solution for reducing sugars were done. In short, in Molisch's test, the gum was treated with a-naphthol and concentrated sulphuric acid, which gave violet ring at the junction of two layers. In case of the detection of reducing sugars to the *Z. arborea* fruit mucilage, equal quantity of Fehling's solution. The presence of tannin was tested upon treating the gum with ferric chloride solution. There was no black precipitation for tannin with ferric chloride solution. The presence of mucilage was tested by treating the mucilage with ruthenium red solution and benzidine solution, formation of pink colour with ruthenium red and blue colour with benzidine solution indicate the presence of mucilage. The phytochemical properties such as presence of protein, flavanoids, sterols, alkaloids, saponins, glycoside, resin, phenol and terpenoids were determined[8,9].

Animals: Healthy adult Indian earthworm, *Pheretima posthuma* (Annelida, Megascolecidae) was used for evaluating the anthelmintic activity due to its anatomical and physiological resembles with the intestinal roundworm parasites of human beings[10-12]. All earthworms were of approximately equal weight and size (3-5 cm in length and 0.1-0.2 cm in width). They were collected from local place, washed and kept in water.

***In vitro* anthelmintic activity:** The *in vitro* anthelmintic activity of ethanol, ethyl acetate, *n*-butanol and petroleum ether solvent fruit extracts of *Zmelina arborea* was evaluated on adult Indian earthworms *Pheretima posthuma* by the reported methods with slight modification[13,14]. Eleven groups of approximately equal sized Indian earthworms consisting of six earthworms in each group were released into 10 mL of desired formulation in petridish. Group I received vehicle (normal saline water), group II received standard drug 1 (piperazine citrate 10 mg/mL), group III received standard drug 2 (albendazole 15 mg/mL), groups IV and V received ethanol extracts (10 and 25 mg/mL), groups VI and VII received ethyl acetate extracts (10 and 25 mg/mL), groups VIII and IX received *n*-butanol extract (10 and 25 mg/mL) and groups X and XI received petroleum ether extract (10 and 25 mg/mL) respectively. Observations were made for the time taken to paralysis and/or 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 lose their motility followed with fading away of their body color.

Statistical analysis: Each value is expressed as mean ± standard deviation (n = 6). For determining the statistical significance, standard error mean and one way analysis of variance (ANOVA) at 5 % level significance was employed. p values < 0.05 were considered significant[15].

RESULTS AND DISCUSSION

The soxhlation method was found to be efficient for extraction of phytochemicals from fruit coarse powder by using ethanol, ethyl acetate, *n*-butanol and petroleum ether as solvents. The percentage yield of all the solvent crude extracts were found in the order of ethanol > *n*-butanol > ethyl acetate > petroleum ether.

Phytochemical analysis: Table-1 shows the phytochemicals detected in *Z. arborea* fruit extracts. The test for cardiac glycosides and steroids were positive for all the extracts. The tests for all phytochemicals were found to be positive for ethanol extract except proteins, amino acids triterpenoids and saponins. The tests for cardiac glycosides, proteins, amino acids, gums, mucilages, steroids and sterols were found to be positive ethyl acetate extract. The tests for all phytochemicals were found to be positive for *n*-butanol extract except carbohydrate, proteins and amino acids. The tests for all phytochemicals were found to be positive for petroleum ether extract except gums, mucilages, tannins, phenolic compounds and flavonoids.

***In vitro* anthelmintic activity:** The unripe fruit extracts of *Z. arborea* produced a significant anthelmintic activity in dose dependent manner as shown in Table-2. The anthelmintic activities of all extracts were comparable with that of standard drugs piperazine citrate and albendazole. The normal saline water was used as a control. No symptoms of paralysis and death of earth worm were observed in normal saline water. All extracts were able to show anthelmintic activity at 10 mg/mL concentration. The activities are comparable with the standard drugs, piperazine citrate and albendazole. All the doses of *n*-butanol extracts of *Z. arborea* showed greater anthelmintic activity than the standard drugs piperazine citrate and albendazole. The extracts of ethanol at concentration of 25 mg/mL showed greater anthelmintic activity than both the standard drugs where as at concentration 10 mg/mL showed

TABLE-1
PHYTOCHEMICAL CONSTITUENTS DETECTED IN FRUIT EXTRACTS OF *Zmelina arborea*

Phytochemicals	Ethanol extract	Ethyl acetate extract	*n*-Butanol extract	Petroleum ether extract
Alkaloids	+	–	+	+
Carbohydrates	+	–	–	+
Cardiac glycosides	+	+	+	+
Anthraquinone glycosides	+	–	+	+
Gums and mucilages	+	+	+	–
Proteins and amino acids	–	+	–	+
Tannins	+	–	+	–
Phenolic compounds	+	–	+	–
Steroids and sterols	+	+	+	+
Triterpenoids	–	–	+	+
Saponins	–	–	+	+
Flavonoids	+	–	+	–

(+) Sign indicates present and (–) sign indicates absent.

TABLE-2
ANTHELMINTIC ACTIVITY OF FRUIT EXTRACTS OF *G. arborea* AGAINST *P. posthuma*

I	Vehicle (NSW)	–	No paralysis	No death
II	Standard drug 1 (piperizine citrate)	10	23.50 ± 1.88	41.23 ± 1.93
III	Standard drug 2 (albendazole)	15	34.36 ± 1.78	63.53 ± 1.87
IV	Ethanol extract	10	34.56 ± 1.99	233.57 ± 1.78
V	Ethanol extract	25	15.45 ± 1.65	33.28 ± 1.91
VI	Ethyl acetate extract	10	85.41 ± 1.87	335.26 ± 1.91
VII	Ethyl acetate extract	25	69.22 ± 0.96	308.22 ± 1.61
VIII	*n*-Butanol extract	10	22.31 ± 1.51	53.52 ± 1.37
IX	*n*-Butanol extract	25	7.42 ± 1.95	18.33 ± 1.62
X	Petroleum ether extract	10	103.54 ± 1.79	370.19 ± 1.73
XI	Petroleum ether extract	25	89.16 ± 1.42	342.32 ± 1.98

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between groups	100489	1	100489	4.6001	0.0125	8.2001
Within groups	171564	14	12254.6	–	–	–
Total	272053	15	–	–	–	–

Each values is represented as mean ± standard deviation ($n = 6$). NSW – Normal saline water. Standard error of mean < 0.812. Data are found to be significant (F value < F crit) by testing through one way ANOVA at 5 % level of significance ($p < 0.05$ that is $p = 0.0125$).

lesser anthelmintic activity than the standard drugs. All the doses of ethyl acetate and petroleum ether extracts of *Z. arborea* showed lesser anthelmintic activity than the standard drugs piperazine citrate and albendazole. When the dose of the extract is increased, a gradual increase in anthelmintic activity was observed. From the above results, it was concluded that the *n*-butanol extract showed better anthelmintic activity in comparison with ethanol, ethyl acetate and petroleum ether extracts as well as more potent than standard drugs piperazine citrate and albendazole as shown in Fig. 1. The activities revealed the concentration dependence nature of the different extracts. Potency of the extracts was found to be inversely proportional to the time taken for paralysis/death of the worms. The anthelmintic activity of all the extracts were found in the order of *n*-butanol > ethanol > ethyl acetate > petroleum ether.

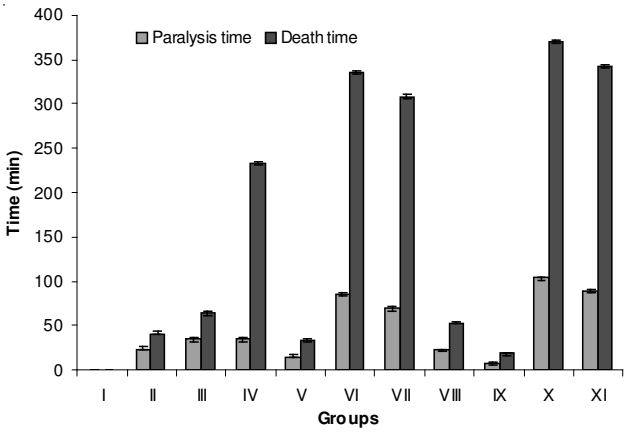

Fig. 1. Anthelmintic activities of fruit extracts of *Zmelina arborea* on Indian Earthworm *Pheretima posthuma*. Each bar is represented as mean ± standard deviation (n = 6). Group I - Control (normal saline water), group II- standard - 1 (piperazine citrate-10 mg/mL), group III - standard - 2 (albendazole-15 mg/mL), groups IV and V - ethanol extracts at 10 and 25 mg/mL, groups VI and VII - Ethyl acetate extracts at 10 and 25 mg/mL, groups VIII and IX - *n*-butanol extracts at 10 and 25 mg/mL and groups X and XI - Petroleum ether extracts at 10 and 25 mg/mL

Conclusion

It is confirmed that *Zmelina arborea* fruits do possess anthelmintic activity. It is concluded that the *n*-butanol extract of *Z. arborea* fruits showed most potent anthelmintic activity. Further studies are required to identify the actual chemical constituents that are present in the crude extracts of this plant which are responsible for anthelmintic activity and to establish the effectiveness and pharmacological rationale for the use of *Z. arborea* as an anthelmintic drug. It is, however, suggested that further research on large scale be carried out on higher

doses than those used in the current study, standardization of dose for drug development.

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