



Phytochemical investigation and anthelmintic activity of various root extracts of *Gmelina arborea* Roxb.

^{1*}Sangram K. Panda, ²Debajyoti Das and ³Niraj K. Tripathy

¹Jeypore College of Pharmacy, Rondapalli, Jeypore, Koraput, Odisha, India

²School of Pharmaceutical Sciences, SOA University, Bhubaneswar, Odisha, India

³Department of Zoology, Berhampur University, Berhampur, Odisha, India

ABSTRACT

Helminthes have been common cause of concern and pose many problems to human beings and animals. Many medicinal plants claimed to possess anthelmintic activity. *Gmelina arborea* belonging to Verbenaceae locally named as Gambhari (Oriya) was traditionally used to treat Helminthia. The objective of present work was to phytochemical investigation and evaluate the anthelmintic activity of ethanol, ethyl acetate, n-butanol and petroleum ether extract of root of *Gmelina arborea* using Indian earthworms *Pheretima posthuma* as test worm. Various concentrations of each extracts were tested. which involved determination of paralysis time and death time of the worm. albendazole. (60mg/ml) was used as a reference standard. The result of present study indicates that. Among all the extract, ethanol and petroleum ether showed dose dependent & significant anthelmintic activity. petroleum ether showed better activity as compared to reference drug albendazole. The data were verified as statistically significant by using One way ANOVA followed by Dunnet's test. Values are mean \pm S.E.M. n = 6 in each group. *P < 0.05 when compared to standard.

Key words: *Gmelina arborea* Roxb.; anthelmintic activity; albendazole.

INTRODUCTION

The half of world suffering from bacterial and helminthes infection, due to poor sanitation, poor family hygiene, malnutrition, and crowded living conditions.[1] Helminthes infections are among the most widespread infections in humans, distressing a huge population of the world. The gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases. Hence there is an increasing demand towards natural anthelmintics.[2] In the last few decades there has been an exponential growth in the field of herbal medicine. It is getting popularized in developing and developed countries owing to its natural origin and lesser side effects [3]. Nature has provided a complete storehouse of remedies to cure all ailments of mankind. About 80% of the world population depending on herbal based alternative system of medicine (Ayurveda, Unani medicine & Chinese traditional medicine). Herbal drugs have played a vital role in curing diseases throughout history of mankind. *Gmelina arborea* Roxb. (Verbenaceae) belongs to a genus of trees and shrubs distributed chiefly in South East Asia, tropical Australia and tropical Costa Rica. [4,5]. *Gmelina arborea* Roxb. belonging to family Verbenaceae locally named as Gambhari(Oriya), Gambhar (Hindi), Gambhar (Bengali), Sriparni (Sanskrit) and Gummadi (Telgu).[6]. Flowering takes place during February to April when the tree is more or less leafless whereas fruiting starts from May onwards up to June. Flowers occur in narrow branching clusters at the end of branches. 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.[7]. The root of this plant has been used in traditional Indian systems of medicines as a demulscient, stomachic, bitter tonic, refrigerant, laxative, and galactagogue. The tender leaves are used as demulscient, in headache, fevers, gonorrhoea, cough etc. The whole plant is used in snake bite and scorpion sting throughout India [8]. As per the folkore medicine the root decoction is used in folk remedies for, demulcent, stomachic, and tonic, diarrhea, dropsy, dyspepsia, epilepsy, fever, gout, headache, hemorrhage, rheumatism, smallpox, snakebite, sores, sore throat, stomachic and urticaria. Ayurvedics prescribe them for alopecia, anemia, consumption, leprosy, thirst, and vaginal discharges; the flowers for blood disorders and leprosy; the root, deemed anthelmintic, laxative and stomachic, for abdominal pains, burning sensations, fever, hallucinations, piles and urinary discharges.[9,10]. According to scientific studies, the root decoction is used as a folk remedy for abdominal tumors. The roots are useful in hallucination, piles, abdominal pains, fevers, 'tridosha' and urinary discharge.[11,12]. Traditional people are using to get relieve from Post delivery weakness. They are using half glass of boiled root extract. The extract is prepared by boiling roots with one glass of water till it gets reduced to half a glass. The plant has also been reported to have anti-inflammatory activity hypoglycaemic and anti-viral activities against Ranikhet disease virus [13].

MATERIALS AND METHODS

Drugs and chemicals

Albendazole was procured as gift sample from Sri Pharmicare, Mumbai, India. The ethanol AR and ethyl acetate AR 60-80°C (Emsure® ACS) were procured from Merck Pvt. Ltd., Navi Mumbai, Maharashtra, India. n-butanol GR 80°C, petroleum ether AR 40-60°C, Loba Chemie Pvt. Ltd., Mumbai, India. All other chemicals reagents used in present work were procured from authorized dealer.

Collection of Plant Material

The root of *Gmelina arborea* were collected from the tribal belts of the local area of Baipariguda of Koraput district. (India) in the month of November 2011. The plant was identified, confirmed 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/SS/P-198/11, dated (16.12.2011)). After authentication root were collected in bulk and washed under running tap water to remove adhering dirt. Then leaves were shade dried. The dried materials were made into coarse powder by grinding in mechanical grinder. and stored in a closed air tight container for further use.

Preparation of Extracts

The coarse powder was taken in Soxhlet apparatus and extracted successively with ethanol, ethyl acetate, n-butanol and petroleum ether as solvent. A total amount of 750 g coarse powder was extracted with 1200 ml of each solvent. For each solvent, 10 cycles were run to obtain thick slurry. Each slurry was then concentrated under reduced pressure to obtain crude extract. All crude extracts were kept in closed air tight containers under cool and dark place for further study [14,15].

Preliminary phytochemical investigation

The crude ethanol, ethyl acetate, n-butanol and petroleum ether extracts of the root of *Gmelina arborea* were subjected to preliminary phytochemical analysis in order to detect the presence of various groups of phyto constituents such as Alkaloids, Carbohydrates, Flavonoids, Phenolic compounds, Cardiac glycosides, Anthraquinone glycosides, Gums and mucilages, Proteins and amino acids, Tannins, Steroids and sterols, Saponins, Triterpenoid etc by chemical analysis [15,16,17]

Anthelmintic activity

Worm collection and authentication

The anthelmintic activity was evaluated on adult Indian earthworm *Pheretima posthuma*. because It resembles anatomically and physiologically with the intestinal round worm parasite of human being. Indian earthworms were obtained from vermiculture area and were identified by the V.D, College (Autonomous), Dept. of Zoology, Jeypore, Koraput, Odisha, India

Preparation of Test sample

The test samples were prepared by dissolving and suspending 2.5 grams of each extract in 25 ml of distilled water to obtain a stock solution of 100 mg/ml. From this stock solution, different dilutions were prepared to get concentration range of 20, 40 and 60 mg/ml.

Anthelmintic Assay

The anthelmintic activity of root extract of *Gmelina arborea* was evaluated by using method of Ajayieoba E.O. et al on adult Indian earthworms by the reported methods with minor modification. The assay was performed on adult Indian earthworm *Pheretima posthuma* its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings. And also easy availability earthworms have been used widely for the initial evaluation of anthelmintic activity. The in-vitro anthelmintic activity was determined by released into 10 ml of desired formulation containing three different concentration, each of crude extract i.e. pet. ether extract, ethyl acetate, n-butanol and ethanol extract (20, 40 and 60 mg/ml in distilled water) were prepared. Albendazole (10 mg/ml) was used as reference standard while distilled water used as control and six worms (same type) were placed in it. Observations were made for the time taken to paralysis and/or death of individual worms. Paralysis was said to occur when the worms do not revive even in normal saline water. Death was concluded when the worms lose their motility followed with fading away of their body color or not moved when dipped in warm water at 50°C [18, 19, 20].

Statistical analysis

All data were calculated statistically by standard error mean (n=6) and statistically significant were verified by applying one way ANOVA at 5 % level of significance where $p < 0.05$ [21]

Table 1: Phytochemical constituents of Root extracts of *Gmelina arborea*

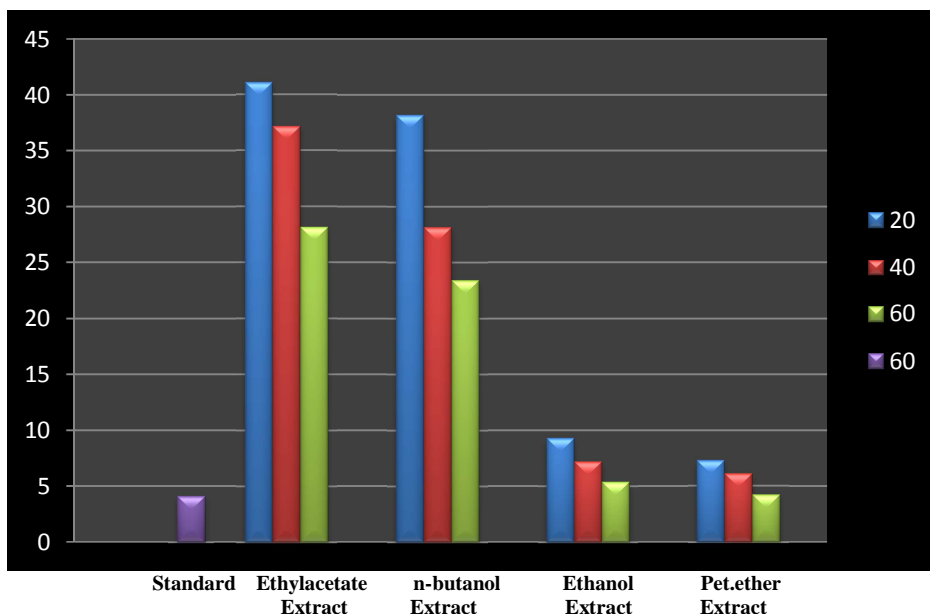
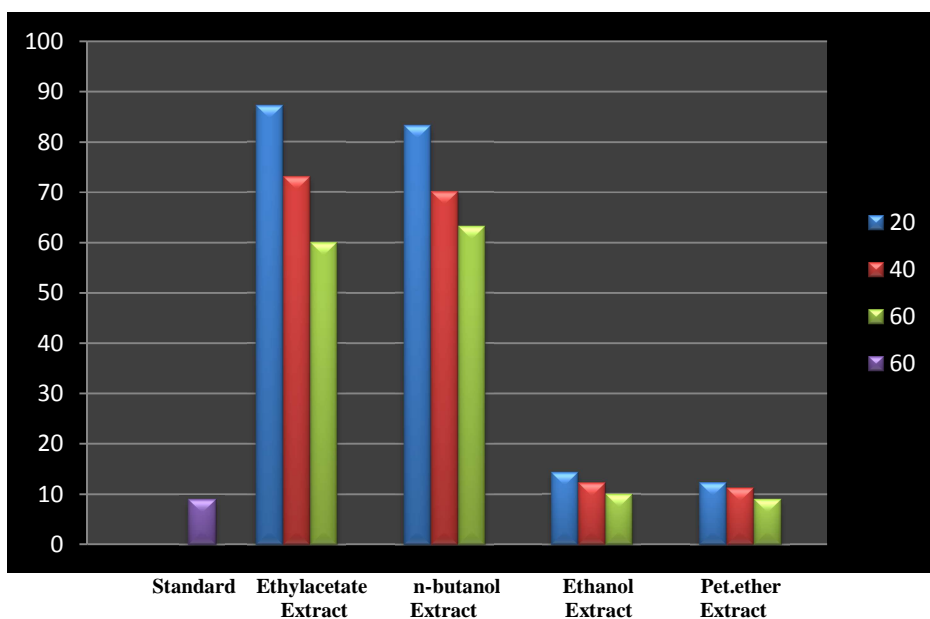
Phytochemicals	Ethanol extract	Ethyl acetate extract	n-butanol extract	Petroleum ether extract
Alkaloids	+	+	+	+
Carbohydrates	+	--	+	--
Cardiac glycosides	+	+	+	+
Anthraquinone glycosides	+	-	+	+
Proteins and amino acids	--	+	--	+
Triterpenoid	--	+	--	+
Phenolic compounds	+	--	+	+
Steroids and sterols	+	+	--	+
Tannins	+	+	+	+
Saponins	+	--	+	+
Flavonoids	+	--	+	--

+ = present, -- = absent

Table 2 : Anthelmintic Activity of Root extracts of *Gmelina arborea*

Treatment vehicle	Concentration used	Time taken (in min.)	
		For paralysis	For death
Ethyl acetate extract	20mg/ml	41.11 ± 0.31	87.21 ± 0.42
	40mg/ml	37.18 ± 0.36	73.11 ± 0.27
	60mg/ml	28.17 ± 0.07	60.03 ± 0.26
n-butanol extract	20mg/ml	38.21 ± 0.09	83.25 ± 0.22
	40mg/ml	28.17 ± 0.21	70.14 ± 0.17
	60mg/ml	23.43 ± 0.15	63.27 ± 0.24
Ethanol extract	20mg/ml	9.31 ± 0.12	14.27 ± 0.17
	40mg/ml	7.22 ± 0.14	12.23 ± 0.18
	60mg/ml	5.40 ± 0.17	10.03 ± 0.07
Pet. ether extract	20mg/ml	7.28 ± 0.11	12.37 ± 0.21
	40mg/ml	6.12 ± 0.28	11.33 ± 0.06
	60mg/ml	4.23 ± 0.27	9.08 ± 0.23
Albendazole	60mg/ml	4.12 ± 0.21	9.01 ± 0.14
Vehicle	-----	-----	-----

One way ANOVA followed by Dunnet's test. Values are mean ± S.E.M. n = 6 in each group. *P < 0.05 when compared to standard

Graph 3 : Anthelmintic activity of Root extracts of *Gmelina arborea* (Paralysis Time)Graph 4 : Anthelmintic activity of Root extracts of *Gmelina arborea* (Death Time)

RESULTS AND DISCUSSION

The preliminary phytochemical screening of *G.arborea* root extracts showed that alkaloids were present in all the extract, carbohydrate present in ethanol and n-butanol extract flavonoids were present in ethanol and n-butanol extract, terpenoids present in ethyl acetate and petether extract, saponins present in all three extract except ethyl acetate, Phenolic compounds present in ethanol,n-butanol and pet ether extract, Steroids and sterols present in ethanol,n-butanol and pet ether extract, Cardiac glycosides were present in all the extract, Anthraquinone glycosides were present in all three extract except ethyl acetate, Proteins and amino acids were present in pet ether and ethyl acetate extract and tannins were present in all the solvent extract. which were shown in (Table no.1) The ethanolic and pet ether extract of root of *G.arborea* showed significant anthelmintic activity at higher

concentration.(60mg/ml) The extract showed a dose dependent activity like shortest time of paralysis and death with (60mg/ml) concentration. The ethanol extract of *G.arborea* root caused paralysis in 5.40 min. and death at 10.03.min. while pet. ether extract showed paralysis in 4.23min. and death at 9.08 min.as compared to the reference drug albendazole showed the same at 4.12min. and 9.08 min. respectively. which were shown in (Table no.2).

CONCLUSION

It was concluded that roots of *G.arborea* showed potential anthelmintic activity. Among all the extracts. pet. ether and ethanol extract showed dose dependant & significant anthelmintic activity. Pet. ether extract showed better activity as compared to reference drug albendazole. From Phytochemical analysis, the presence of tannins as one of the chemical constituent said to possess anthelmintic activity. The folklore claim of root of *G.arborea* as an anthelmintic have been confirmed as the root extracts showed activity against the earthworms used in the study. Further studies to isolate and reveal the active compound present in the crude extract of *G.arborea* root and to establish the MOA of anthelmintic activity.

Acknowledgements

Authors wish to thank to local people of south eastern Odisha and the Biju Patnaik Medicinal Plants Garden and Research Centre, Dr M. S. Swami Nathan Research Foundation, Jeypore, Koraput (District), Orissa, India. for providing valuable information about the plant and its identification.The author wish to express their gratitude to Jeypore College of Pharmacy, Rondapalli, Jeypore, Koraput, Odisha.

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