

Research Article

ANTHELMINTIC AND ANTIDIABETIC ACTIVITIES OF *GMELENA ARBOREA* ROXB. BARK EXTRACTS

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ABSTRACT

Background: The plant *Gmelina arborea* has been traditionally used in India for several medicinal purposes like anthelmintic, diuretic, anti-inflammatory, antibacterial and antidiabetic.

Aims: The aim of the present study is to explore the anthelmintic and antidiabetic activities of *G. arborea* bark extracts using ethanol, ethyl acetate, n-butanol and petroleum ether as solvents.

Material and methods: The extracts were screened for phytochemical constituents and evaluated for their toxicity. The anthelmintic activity was evaluated on adult Indian earthworms, *Pheretima posthuma*. The antidiabetic activity of above extracts was evaluated in alloxan induced diabetic model of Wistar rats. Statistical analysis used: All data are verified for statistically significant by using one way ANOVA at 1 % level of significance ($p < 0.01$). Results and

Discussion: 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 found to be non toxic to the living body. All extracts were able to show anthelmintic activity at 10 mg/ml concentration and well are comparable with the standard drugs such as piperazine citrate and albendazole. Among all the solvent extracts the n-butanol extract showed better anthelmintic activity even in comparison with both the standard drugs. All the extracts were able to reduce sugar level in blood. Ethanol extract was found to have good antidiabetic activity in comparison to other extracts.

Conclusion: It can be concluded that the bark extracts of *G. arborea* possess anthelmintic and antidiabetic activities.

Keywords: *Gmelina arborea*, bark, anthelmintic, piperazine citrate, diabetes, alloxan.

INTRODUCTION

Helminthes infections are among the most widespread infections in humans, distressing a huge population of the world. Although the majority of 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.

Diabetes mellitus is one of the common metabolic disorders with micro- and macro vascular complications that results in significant morbidity and mortality. It is considered as one of the five leading causes of death in the world [4, 5]. In modern medical fluid (Aroma), no satisfactory effective therapy is still available to cure diabetes mellitus [6]. There is increasing demand by patients to use natural products with antidiabetic activity due to side effects associated with the use of insulin and oral hypoglycemic agents [7–9].

Gmelina arborea Roxb barks are smooth whitish grey color corky bark, warty with lenticular tubercles and the bark exfoliating in regular patches over it when old [10,11]. The bark of plant, *G. arborea* was reported to have several medicinal properties such as aphrodisiac, astringent, analgesic, antipyretic, antidiabetic, diuretic, anthelmintic and tonic characteristics [12].

MATERIALS AND METHODS

Drugs and Chemicals

Albendazole (Micro Lab. Ltd., Goa, India), piperazine citrate (Burroughs Wellcome Ltd., Mumbai, India) and Glibenclamide (Dr. Reddy lab., Hyderabad) were procured as gift sample. The Alloxan (Hydrate – CAS: 2244-11-3), 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 and petroleum ether AR 40-60°C 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 barks of *G. 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 barks were shade dried under normal environmental conditions. The dried barks were pulverized to form coarse powder by using electrical grinder and stored in a closed air tight container for further use.

Preparation of solvent extracts:

The coarse powder form of dried barks was extracted by Soxhletion method by using ethanol, ethyl acetate, n-butanol and petroleum ether as solvents. A total amount of 1500 g coarse powdered barks 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.

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 α -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 *G. 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 [13-15].

In vitro Anthelmintic activity

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, Koraput, Odisha.

Animals

Healthy adult Indian earthworm, *Pheretima posthuma* (Annelida, Megasclecoidea) was used for evaluating the anthelmintic activity due to its anatomical and physiological resembles with the intestinal roundworm parasites of human beings [16-18]. All earthworms were of approximately equal weight and size (3 to 5 cm in length and 0.1 to 0.2 cm in width). They were collected from local place, washed and kept in water.

Methodology

The *in vitro* anthelmintic activity of ethanol, ethyl acetate, n-butanol and petroleum ether solvent fruit extracts of *Gmelina arborea* was evaluated on adult Indian earthworms *Pheretima posthuma* by the reported methods with slight modification [19]. 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 & V received ethanol extracts (10 and 25 mg/ml), groups VI & VII received ethyl acetate extracts (10 and 25 mg/ml), groups VIII & IX received n-butanol extract (10 and 25 mg/ml) and groups X & 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.

Acute toxicity studies

To study the toxic effect (if any) of *G. arborea* bark extracts, Albino mice of either sex (20-25 g) were used. The animals were kept in the standard polypropylene cages at 25±2°C/60% relative humidity in normal day and night photo cycle (12: 12 h). The animals were acclimatized for a period of 14 days prior to performing the experiments. Prior to the study, the experimental protocols were approved by the Institutional Animal Ethics Committee of Gayatri College of Pharmacy, Gayatri Vihar, Jamadarpali, Sambalpur, Odisha (Ethical Committee No 1339/ac/10/CPCSEA).

Acute oral toxicity study was performed as per OECD-423 guidelines [20,21]. The animals were kept fasting overnight but allowed free access to water ad libitum. The fasted mice were divided into different groups of six animals each. Each solvent extract solution was administered orally at a dose of 10 mg/Kg b.w., using normal saline water as vehicle and mortality in each group was observed for 14 days. If mortality was observed in 2 out of 3 animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the same procedure was repeated in each group for each extract with higher doses such as 100, 300, 600, 1000, 2000 and 3000 mg/Kg b.w. One tenth of this lethal dose was selected as the therapeutic dose for the evaluation of anti-inflammatory and antipyretic activities.

Antidiabetic activity (Alloxan induced diabetic model)

Animals

Healthy Wister rats of either sex were used in the present study. They were housed under standard conditions of temperature (25±2 °C) with 12

h light per day cycle and relative humidity of 45-55 % in animal house of Gayatri College of Pharmacy, Odisha. They were kept in fasting condition for 16 h and prior to experiment they were fed with excess water ad libitum. Animals were caged and all operations on animals were done in aseptic condition.

Extracts and Drugs

The extracts of *G. arborea* were tested in single doses in each group of experimental animals (300 mg/Kg b.w.). Glibenclamide was used as the standard drug in alloxan induced diabetic model at a dose of 5 mg/Kg of body weight of rat.

Experimental protocol

Animals were selected, weighed (150-180 g) and divided into seven groups (n=3), namely normal control, diabetic control, standard drug and four groups belonging to four different extracts of *G. arborea*. Approval for the research work was obtained by the Institutional Animal Ethics Committee of Gayatri College of Pharmacy, Gayatri Vihar, Jamadarpali, Sambalpur, Odisha (Ethical Committee No. 1339/ac/10/CPCSEA).

Experimental method

The alloxan induced diabetic model was used to evaluate the blood sugar level reducing capacity of various extracts. Here the blood sugar level of rats was raised by administration of alloxan [22,23].

Wister rats were divided into seven groups of three animals in each group. The animals were fasted for 16 h with water ad libitum. The group - I was served as normal solvent control which received the normal saline water 2 ml/kg through oral route, the group - II was served as diabetic control which received alloxan (120 mg/Kg) with normal saline water subcutaneously, group - III was served as standard control which received alloxan 120 mg/Kg with glibenclamide at a dose of 5 mg/Kg orally, groups - IV to VII were served as test groups which received alloxan (120 mg/kg) along with single dose (300 mg/Kg, b.w.) of ethanol, ethyl acetate, n-butanol and petroleum ether extracts respectively.

Rats were made diabetic by a single intraperitoneal injection of alloxan monohydrate (120 mg/Kg) [24-26]. Two days after of alloxan injection, rats with plasma glucose levels of more than 200 mg/dl were included in the study and at this stage the blood glucose level of each rat was consider as basal value in each group. Treatment with plant extracts and standard drug was started after 48 h of alloxan injection. Blood samples were drawn from tip of the tail on 1st, 3rd, 5th and 7th day respectively and estimated for fasting blood glucose level using electronic digital glucometer (Counter digital glucometer) which is previously validated for correctness.

Statistical analysis

To determine the statistical significance, standard deviation, standard error mean and one way analysis of variance (ANOVA) at 1% level significance was employed followed by z-test. P values < 0.01 were considered significant [27, 28].

RESULTS

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 *G. arborea* bark 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.

Table 1: Phytochemical constituents detected in bark extracts of *Gmelina arborea*.

Phytochemicals	Ethanol extract	Ethyl acetate extract	n-butanol extract	Petroleum ether extract
Alkaloids	+	-	+	+
carbohydrates	+	-	-	+
Cardiac glycosides	+	+	+	+
Antraquinone glycosides	+	-	+	+

Gums and mucilages	+	+	+	-
Proteins and amino acids	-	+	-	+
Tannins	+	-	+	-
Phenolic compounds	+	-	+	-
Steroids and sterols	+	+	+	+
Triterpenoids	-	-	+	+
Saponins	-	-	+	+
Flavonoids	+	-	+	-

(+) sign indicates present and (-) sign indicates absent.

The tests for cardiac glycosides, proteins, amino acids, gums, mucilages, steroids, sterols and flavonoids were found to be positive for 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 bark extracts of *G. 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 worms 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 extract showed greater anthelmintic activity than the standard drugs piperazine citrate and albendazole. The ethanol extract at 25 mg/ml showed greater anthelmintic activity than the standard drugs whereas at a concentration of 10 mg/ml showed lesser anthelmintic activity than the standard drugs. All the doses of ethyl acetate and petroleum ether extracts 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. By employing one-way ANOVA, all data were found to be statistically significant (F value < F crit) at 5% level of significant (p < 0.05 that is p = 0.0125). From the above results, it is 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 activity than the standard drugs, piperazine citrate and albendazole (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 the extracts were found in the order of n-butanol > ethanol > ethyl acetate > petroleum ether. So it is concluded that *G. arborea* bark extracts do possess anthelmintic activity. Further, it is also concluded that the n-butanol extract has more potent anthelmintic activity.

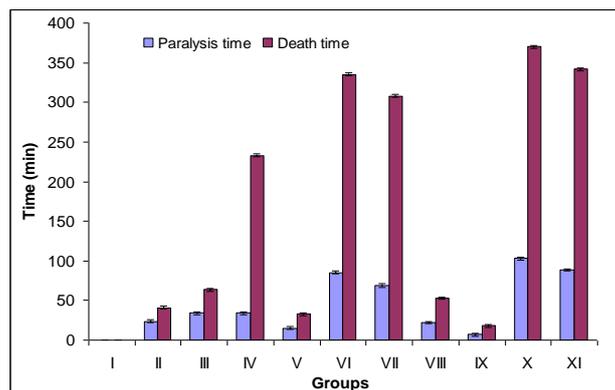


Fig 1: Anthelmintic activities of bark extracts of *Gmelina arborea* on Indian Earthworms (*Pheretima posthuma*).

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 & V – Ethanol extracts at 10 & 25 mg/ml, groups VI & VII – Ethyl acetate extracts at 10 & 25 mg/ml, groups VIII & IX – n-butanol extracts at 10 & 25 mg/ml and groups X & XI – Petroleum ether extracts at 10 & 25 mg/ml.

Table 2: Anthelmintic activities of bark extracts of *G. arborea* against *P.posthuma*.

Groups	Treatments	Concentration (mg/ml)	Paralysis time (min) (X±S.D.)	Death time (min) (X±S.D.)		
I	Vehicle (NSW)	-	No paralysis	No death		
II	Standard drug 1 (Piperazine 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	Pet. ether extract	10	103.54±1.79	370.19±1.73		
XI	Pet. 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).

Toxicity study

Acute toxicity study revealed that no mortality was found with any solvent extract at any dose in Swiss albino mice. No significant symptoms and side effects were observed with any animal.

Antidiabetic activity

The extracts produced a significant antidiabetic effect on first, third, fifth and seventh days at 300 mg/Kg body weight (Table 3). The results on antidiabetic activity revealed that all the four extracts were able to reduce

blood sugar levels in treated animals. The potency of reducing blood sugar levels shown by the extracts was many folds when compared with control (Normal Saline water) and standard drug (Glibenclamide). Among all the extracts, ethanol and n-butanol extracts showed antidiabetic activity in more significant manner when compared to the normal control. These effects are comparable with the standard drug (Glibenclamide). It will be worth to mention that although different constituents were extracted in different solvents as per their polarities, ethanol extract is more effective as compared to other solvent extracts. The activity shown by this extract is of considerable importance and

justified its use in the diabetic control in the folklore medicines. The antidiabetic activity of the extracts is in the order of ethanol > n-butanol > petroleum ether > ethyl acetate. By employing one-way ANOVA, all data were found to be statistically significant (F value < F crit) at 1% level of significant ($p < 0.01$ that is $p = 0.00417$) followed by z-test. The bark extracts of *G. arborea* have blood sugar level reducing effect and justified its ethnopharmacological use as antidiabetic. This effect may be further explored for the use of this plant in the management of diabetic diseases. Further study is required to identify the actual chemical constituents responsible for exhibiting the antidiabetic activity

Table 3: Antidiabetic activities of bark extracts of *G. arborea* by alloxan induced diabetic model.

Groups	Blood glucose level (mg/dl) (X±S.D.)					
	Basal value	1 st day	3 rd day	5 th day	7 th day	
I	77±0.34	82±0.41	80±0.52	83±0.22	81±0.19	
II	337±0.58	337±0.75	334±0.81	301±0.49	300±0.88	
III	283±0.62	251±0.92	155±0.85	131±0.95	119±1.06*	
IV	389±0.97	252±1.11	166±1.08	114±1.17	92±1.21***	
V	335±1.15	208±1.13	177±0.96	146±0.90	124±1.10*	
VI	371±1.18	258±1.06	180±1.01	160±1.14	97±0.92**	
VII	308±0.99	238±1.06	152±1.13	136±0.89	118±1.19*	
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	127568.07	4	31892.0196	1.7863	0.0041	2.689
Within Groups	199894.69	30	6663.15635			
Total	327462.76	34				

Each values is represented as mean ± standard deviation (n = 3). Standard error of mean < 0.6986. *P<0.05, **P<0.01 and ***P<0.001 (test of significance between two proportions by z-test) in comparison to control in seven days study. Data are found to be significant (F value < F crit) by testing through one way ANOVA at 1% level of significance ($p < 0.01$ that is $p = 0.00417$). Group I – Control (Normal saline water), group II – Diabetic control (Alloxan – 120 mg/Kg), group III – Standard control (Glibenclamide 5 mg/Kg), groups IV to VII - Alloxan (120 mg/Kg) with ethanol, ethyl acetate, n-butanol and petroleum ether extracts respectively (300 mg/Kg of b.w.).

CONCLUSION

It can be concluded that the extracts of *G. arborea* barks possess anthelmintic and antidiabetic activities. The ethanol extracts showed better activities. However, the components responsible for the anthelmintic and antidiabetic activities are currently unclear. Therefore, further investigation is needed to isolate and identify the constituents present in the barks extracts.

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