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## TRADITIONAL USE, TOXICITY STUDY AND PHYTOCHEMICAL SCREENING OF *Gmelina arborea* ROXB FRUITS

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### ABSTRACT

*Gmelina arborea roxb.* is found in the tribal areas of Koraput, Keonjhar and Ganjam districts. The present study is an attempt for review on tribal use and the preliminary investigation of phytochemical constituents of different fruit extracts of plant *G. arborea* using ethanol, ethyl acetate, n-butanol and petroleum ether as solvents. The non toxic property of plant extracts was examined by heavy metal study in soil and acute toxicity study using Albino mice as animal model. The survey from tribal people reveals that plant *G. arborea* used against fever due to cold, as hypoglycemic, to relieve headache, for washing of ulcer, abortion in early stage of pregnancy, blood diseases, aphrodisiac, antimicrobials, in snake bite, post delivery weakness, blood in cough and hepatoprotective. The heavy metal was absent in soil and the fruit extracts of *G. arborea* was non toxic for living body. The ethanol and n-butanol extracts were containing most phytochemicals where as ethyl acetate extracts was containing least number of phytochemicals.

**Keywords:** *Gmelina arborea*, Verbenaceae, Traditional use, Acute toxicity study, Heavy metal, Phytochemicals.

### INTRODUCTION

For centuries, the medicinal plants are the basis for treatment of various diseases [1]. Nearly 80% of peoples living in developing countries still depend on plant based traditional medicine for their preliminary health care and almost three-fourths of the herbal drugs used world wide is derived from medicinal plants [2]. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects [3].

*G. arborea* belonging to family *Verbenaceae* locally named as Gambhari (Oriya), Gambhar (Hindi), Gambhar (Bengali), Sriparni (Sanskrit) and Gummadi (Telgu) [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 [5]. 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 [6]. The fruit is oval in shape, ¾ inches in length and is yellow in color. The fruit taste sweet and astringent. Leaves are 4 to 9 inches in length and 2½ inches in breadth. These are of heart shape, petioles is 2 to 6 inches in length [7].

The wood is used planking, paneling, carriages, furniture, carpentry of all kinds, in Myanmar for carving images and canoes, match manufacture, packing cases, in all ornamental work, for making quality toys and picture frames [8].

### MATERIALS AND METHODS

#### Chemicals

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 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.

#### Tribal people use of *G. arborea*: Survey in Odisha

Leaf paste is applied to relieve headache and juice is used as wash for ulcers. As per traditional use, if body temperature of a patient suffering from fever increases excessively, there will be severe headache. By using leaf paste of Gambhari on the forehead, headache gets relieved [9].

Traditional people get cured from headache due to convulsion and weakness and head riling by using half glass of bark extract prepared by boiling 20 g of bark in one glass of water. As per traditional people view, blood disorder and disease like Tuberculosis (Blood coming in cough) can be cured by taking ripe fruits extract twice a day for 10 to 15 days. This extract is prepared by boiling one fistful of ripe fruits with one glass of milk and one glass of water.

Also traditional people were using for fetal

growth by using half glass of fruit extract by boiling one fistful of gambhari fruits with 20 g of honey, two glasses of milk and two glasses of water till it gets reduced to one glass. This preparation (half glass) can be taken twice a day in empty stomach during morning and night time. Tribal peoples were using ripe fruits juice as antidiabetics.

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. Root paste is used against poison in snake bite.

Pound the ripe fruit of *Gmelina arborea* and extract the juice. Take 10 ml of this juice orally each time twice daily for a month. Grind the bark of *Gmelina arborea* into a paste. Mix this paste in a glass of water and take this glass of water only once. To cure from fever due to cold, boil *Gmelina arborea* bark, stem and fruits in 500 ml water. Take 20 ml of this decoction each time orally twice daily for three to four days.

### Heavy metals study in soil

The heavy metal study in soil, from where plant being collected was studied to assure the absence of heavy metal in soil, as presence of heavy metal in soil may lead to the presence of heavy metal in plant extract, which may cause toxicity in living body. The heavy metal study was done as per the standard procedure for limit test of lead and arsenic [10].

### Collection of plant materials, identification and size reduction

The fruits of *G. arborea* were collected from local area of Koraput district (India) in the month of April and May 2013. 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 (13)/ 1067, dated 14.08.2013). The fruits were shade dried under normal environmental condition. The dried fruits were pulverized to form coarse powder by using electrical grinder and stored in a closed air tight container for further use.

### Preparation of extracts

The coarse powder form of dried fruits was extracted by Soxhlation method using ethanol, ethyl acetate, n-butanol and petroleum ether as solvents [11]. In this extraction process, a total amount of 1500 g coarse powdered fruits 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  $\alpha$ -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 [12-14].

### Acute toxicity studies

To study the toxic effect (if any) of *G. arborea* extracts, Albino mice of either sex (20-25 g) were used. The animals were kept in the standard polypropylene cages at  $25\pm 2^\circ\text{C}$ / 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 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 [15, 16]. 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 body weight (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 1 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 for further higher doses such are 100, 500, 1000 and 2000 mg/kg b.w.

**Table 1. Phytochemical constituents detected in different solvents fruit extracts of *Gmelina arborea* Roxb.**

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.

## RESULTS AND DISCUSSION

No such significant amount of heavy metals like lead and arsenic was found in the soil, which assured absence of heavy metal in plant extracts, demonstrated preliminarily that plant extract could be non toxic for living body which was further confirmed by acute toxicity study.

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.

Table 1 shows the phytochemicals detected in *G. 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 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.

Acute toxicity study revealed that no mortality was found in any solvent extract at any dose in Swiss albino mice, which confirmed that *G. arborea* fruit extract would be non toxic in living body.

## CONCLUSIONS

The study revealed that *G. arborea* is a potential plant for treatment of various ailments and also safe for living boy. The plant (Fruit) is containing glycosides, alkaloids, gums, mucilages, sterols and steroids, as phytoconstituents. Further study has to be carried out scientifically to evaluate the pharmacological activities of this plant.

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