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Original Article

PHARMACOGNOSTICAL, PHYTOCHEMICAL AND *IN VIVO* GASTRO-PROTECTIVE INVESTIGATION OF *GMELINA ARBOREA*

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ABSTRACT

Objective: *Gmelina arborea* Roxb, (Family: Verbenaceae) known as "Gambhari", is a fast growing tree found throughout India. Traditionally, the various parts of tree have been used as diuretic, aphrodisiac and also finds use in the treatment of anaemia, alopecia, leprosy and ulcers. Therefore the present study was planned to screen pharmacognostical, antioxidant and antiulcer potentials of *Gmelina arborea* aerial part.

Methods: In the present study, microscopic studies on *G. arborea* leaves and stems were performed. Physico-chemical constants, preliminary phytochemical and *in vitro* antioxidant assay were performed using ABTS assay. In addition, light is also thrown on the toxicity profile and antiulcer potential of aerial part hydro-alcoholic extract.

Results: Preliminary phytochemical screening revealed the presence of tannin, saponin, glycosides, alkaloids, phytosterols, flavonoids and terpenoids. The extract when screened for antioxidant potential revealed encouraging results. Acute oral toxicity was performed and found to be safe upto 2g/kg, b. wt. The hydro-alcoholic extract showed significant gastroprotective efficacy at the dose 250 mg/kg against ethanol induced gastric ulcer model in experimental animals.

Conclusion: In the present study, preliminary phytochemical, antioxidant and preclinical studies were conducted and confirmed that hydro-alcoholic extract of *G. arborea* showed significant gastroprotection in rat model.

Keywords: *Gmelina arborea*, Verbenaceae, Microscopy, Acute oral toxicity, Peptic ulcer

INTRODUCTION

Gmelina arborea Roxb. (Family: Verbenaceae) is a deciduous tree grows to a height of 12 to 30 m and grows preferably in moist fertile area. In Indian folk medicine, the root decoction is used to treat abdominal tumors. In addition, it is also a folk remedy for anthrax, bites, blood disorders, cholera, colic, convulsions, diarrhea, dyspepsia, fever, gout, smallpox, snakebite, swelling and urticarial [1]. Ayurvedic physicians prescribe *Gmelina arborea* for alopecia, anemia, leprosy, thirst and vaginal discharges; the flowers for blood disorders and leprosy.

The root of the plant is used as laxative, stomachic, colic and in urinary discharges. The plant has been used as an ingredient in formulations such as Brahat Panchmool, Elanirkujambu, Kutajarista and Chyawanprasha. The extract form of different parts of *G. arborea* has been proven scientifically for its Analgesic [2], Antidiabetic [3], Antimicrobial [4], Antioxidant [5] and wound healing activities [6].

Even though there are many antiulcer drugs available in market, due to their high cost and adverse effects such as impotence, gynaecomastia, arrhythmia and changes in haematopoietic system the alternate drug should be developed [7]. Keeping this in view and to develop an alternate and efficacious herbal antiulcer drug, the present study is conducted and data of the results obtained were reported.

MATERIALS AND METHODS

Collection of plant materials

The aerial parts of the plant were collected from Courtallam, Thirunelveli District, Tamil Nadu. The plant specimens were identified and authenticated by Botanical Survey of India, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India (Ref. BSI./SC/5/23/07-08/Tech.1226, Dt.: 28-11-2007.). The herbarium of the plant has been preserved at CARISM, SASTRA University, Thanjavur, Tamil Nadu, India. (Voucher No. G0097).

Before conducting animal experiments standards were also determined for the test drug as per Ayurvedic Pharmacopoeia.

Macroscopical and Microscopical Evaluation

Transverse section of stem and leaf were taken and staining was carried out as described by O'Brien et al and Esau, 1964 [8, 9].

Physicochemical evaluation

The ash values and extractive values were determined as per official methods [10-12]. The fluorescence analysis of plant powder was performed as per the procedure [13]. The powdered plant sample was subjected to preliminary phytochemical analysis to detect the presence of various phytoconstituents described by Harborne (1998), Evans (2003) and Kokate et al. (2002) [14-16].

ABTS free radical scavenging assay

ABTS free radical scavenging assay was performed using the method of Re et al., 1999 [17]. The generation of chromophore $ABTS^{\bullet+}$ with maximum absorbtion at 734 nm is achieved by reacting ABTS with potassium persulfate. The test substance with antioxidant property reduces preformed radical cation to ABTS. Briefly equal volume of ABTS (7 mM) and sodium persulfate (2.4 mM) was mixed and diluted with methanol to get absorbance of 0.70±0.01 at 734 nm. The different concentration of plant extracts were treated with $ABTS^{\bullet+}$ (1 ml) and allowed to react for 5 minutes. The absorbance of resultant solution was measured at 734 nm using spectrophotometer and the % inhibition was calculated.

Preparation of plant extracts

The powdered aerial part (200 g) of the plant was subjected to dynamic maceration using 70% v/v ethanol for 4 h. This process was repeated thrice and the filtered extract was dried under reduced pressure, yielding a dark green semisolid. The yield of hydro-alcoholic extract was 12.17% w/w and stored in refrigerator for further use.

Experimental animals

Albino Wistar rats of age 8 to 10 weeks were utilized and were housed in polypropylene cages under standard laboratory conditions. The experimental animals were fed with standard pellets and reverse osmosis water *ad libitum*. The rats were acclimatized for 7 days before the start of experiment. All the experimental procedures involving laboratory animal use were in compliance to the Institute Animal Ethics Committee (IAEC) regulations approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals. The IAEC approval number for this study is 44/SASTRA/IAEC/RPP.

Acute oral toxicity test

Acute oral toxicity study was performed using three female rats as per OECD-423 guidelines [18]. A single oral dose of *G. arborea* extract was treated to three female rats at the dose of 2 g/kg, b. wt. The extract treated rats were observed for the first 5 min after loading for signs of regurgitation and then kept in individual polypropylene rat cage. The cage-side observation was done for every 15 min in the first 4 h after dosing, then every 30 min for the successive 6 h and then daily for 14 days. The test substance treated animals were observed for behavioral signs such as repetitive circling, convulsions, tremors, piloerection, diarrohea, depression, hyperactivity, salivation, and mortality. The daily feed intake and weekly body weight changes were measured during the observation period of 14 days. On Day 14, all the surviving animals were sacrificed and macroscopically examined for any pathological changes.

Ethanol induced gastric ulcer model

The gastroprotective activity of the test substance was evaluated by using ethanol induced gastric lesions model according to Chun-Ying Li et al., (2008) [19]. All the fasted Albino wistar rats were divided into 6 groups each containing six animals. Group 1 animals received 1% tween 80 and considered as vehicle control group. Group 2 animals were treated orally with sucralfate at the dose 400 mg/kg, B. wt. Group 3 to 6 animals were administered orally with hydro-alcoholic extract of *G. arborea* aerial part at the dose of 125, 250, 500 and 1000 mg/kg respectively. One hour after the test substance treatment, all the experimental animals were challenged with 1 ml of absolute alcohol. All the rats were sacrificed after one hour of alcohol challenge. The stomach was removed, cut along with greater curvature and stretched on board for macroscopic examination. The scoring of gastric mucosal lesions was performed using illuminated magnifying hand lens [20]. The ulcer area (UA) was measured using Image Tool 3.0 and the ulcer index (UI) was calculated as follow:

$$UI = 10/X$$

Where, X is the ratio of total mucosal area to total ulcerated area

Statistical analysis

The results were expressed in mean±SD. The statistical analysis was performed using GraphPad Prism 6.0 software for windows, Inc. the difference in significance of mean values for the various treatment groups were calculated using one way analysis of variance (ANOVA) followed by Dunnett's test. The p values less than 0.05 were considered as significant [21].

RESULTS

The plant grows in the hills above 350-500 m. The plant is unarmed deciduous tree and is upto 15 m height. The leaf and young branches are tomentose. The leaves are broadly ovate-cordate 8-15 cm wide and 9-14 cm long. The flowers are brownish yellow. Fruits become yellow on ripening. (Fig.1).

Microscopic features of leaf

T. S of leaf through midrib

The leaf has thick and wide abaxial midrib and densely trichomatous lamina (Fig. 2A). The adaxial side of the midrib is flat and prominently projecting on the abaxial side. The midrib is roughly circular in sectional view and bears dense epidermal trichomes. The midrib is 600 µm thick. Midrib consists of prominent epidermal layer of papillate thick walled cells. The ground tissue in the adaxial part of the midrib is collenchymatous. Remaining portion of the midrib is homogeneous and parenchymatous; the cells are wide, circular and fairly thick walled (Fig. 2C&2D).The vascular system is a thick shallow are of vascular strands. The xylem elements are in short lines; they are wide, thick walled and angular in outline (Fig. 2D). The xylem elements are up to 40 µm wide. Phloem elements occur in small groups along the basal part of the xylem elements.

Fig. 1: Macroscopy of aerial parts of *Gmelina arborea*Roxb.

Lamina

The lamina has smooth and even adaxial side and ridged and furrowed abaxial side. Dense covering types as well as glandular trichomes areabundant on the abaxial surface. The adaxial epidermis is thick and the cells are vertically oblong and have prominent cuticle. The epidermis is about 20 µm in thickness. The abaxial epidermis is narrow with tabular cells. The epidermal trichomes have 9 stalk cells, a basal cell and a terminal cell. The terminal cell may be glandular head producing mucilage (Fig. 2B). The mesophyll tissue consists of two layers, long, narrow cylindrical palisade cells and four or five layers of small lobed cells forming reticulate aerenchyma. The palisade zone is 50 µm in height. The lateral veins occur in the ridged part of the lamina. It has a small cluster of xylem element and a group of phloem elements. The vascular strand is surrounded by parenchymatous bundle sheath which extends abaxially into wide based part and adaxially as a thin vertical pillar. The lamina is 120 µm to 160 µm thick.

Stem

The stem has circular cross sectional outline. It consists of discontinuous periderm, intact epidermis, wide cortex, thick continuous vascular cylinder and wide pith (Fig. 2E). Periderm is superficial in position; it has narrow fairly deep vertical tissues. The epidermis remains intact even in the region of the periderm. The periderm is 5 -7 layered and is 70 µm wide. The cortex is 250 µm wide. It comprises outer zone of thick walled smaller cells (collenchymatous) and inner zone of lightly layered thin walled cells. The inner boundary of the cortex is marked by discrete, spherical masses of thick walled sclerenchyma elements (Fig. 2F). The cells have lignified walls and reduced lumen. Secondary phloem is 150 µm wide. It consists of narrow, angular thin walled cells arranged in radial rows. Phloem rays are not prominent (Fig. 2F)

Secondary xylem cylinder is 600 µm thick. It includes wide, circular, thin walled, mostly solitary vessels, narrow straight xylem rays and librified fibres. The vessels are 70 µm wide. The pith consists of thin walled compact parenchyma cells.

The powdered microscopic preparation (Fig. 3) and macerated specimen exhibit the following inclusions. (i) Libriform fibres: Narrow as well as wide fibres are abundant in the powder. They are long with tapering ends. The wide fibres have their walls and wide lumen; the narrow fibres are thick walled with narrow lumen. The fibres have no lateral wall pits (Fig. 3A&B). They are 500 to 600 µm

long and the narrow fibres are 10 µm wide and the wider ones are 20 µm. (ii) Vessel elements are narrow, long and cylindrical measuring 350 µm in length.

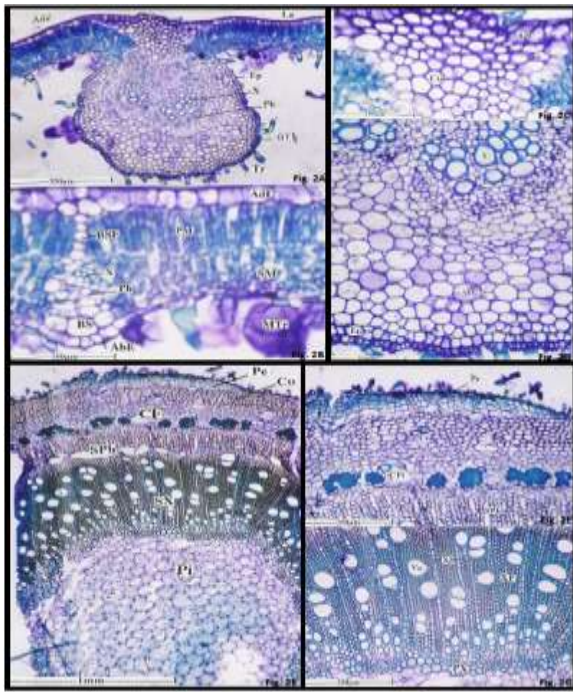

Fig. 2: Microscopy of Leaf and Stems of *Gmelina arborea* Roxb. 2A: Transversal section (TS) of leaf through midrib (10×).2B: T. S of lamina (40×). 2C & 2D: T. S of the midrib, a portion enlarged (40×). 2E: T. S of stem, a sector (4×). 2F: T. S. of stem: cortex and phloem portions enlarged (10×). 2G: T. S. of stem: secondary xylem enlarged (10×).Secondary xylem

They have simple perforation plates which is horizontal or oblique in orientation (Fig. 3A&B). The lateral wall pits are elliptical, dense, multiseriate and alternate (Fig. 3D) some of the vessels members have short conical tail. (iii) Epidermal cells (Fig. 3E) In surface view, the epidermal cells are polygonal, thick and straight walled. The epidermal cells surround the cell from which a trichome originates, form radiating rosette. (iv) Epidermal trichomes Two types of epidermal trichomes are seen in abundance in the powder. (a) Non-glandular trichomes (Fig. 3E, F&G) non-glandular or covering type of trichomes was dense on the lower surface of the lamina. They are 1-3 celled, uniseriate and unbranched. They are uniform in thickness and the cells are narrow, long and cylindrical. The walls are thick and lignified. The length of the trichomes range from 150-400 µm and the thickness is 10 µm. (b) Glandular trichomes (Fig. 3G&H) A unique type of glandular trichomes is also abundant in the powder. These trichomes have short, thick stalk cell bearing four vertically standing, free glandular cells which resemble the wings of the butterfly (Fig. 3H). The glandular body cells are hemispherical so that the gland will appear capitate in shape. The glands are 35×40 µm in size. The body cells have their walls and dense contents.

Table 1: Ash values of *Gmelina arborea* Roxb. aerial parts

Sl. No.	Ash values	Results (% w/w) Vales are in Mean±SEM
1	Total ash	6.35±0.4
2	Sulphated ash	6.34±0.3
3	Water insoluble ash	5.74±0.4
4	Acid insoluble	5.08±0.2

The importance of performing ash values of any crude drug is to check the presence of inorganic or earthy materials and other impurities. The physicochemical standards were performed and their results are tabulated (Table: 1).

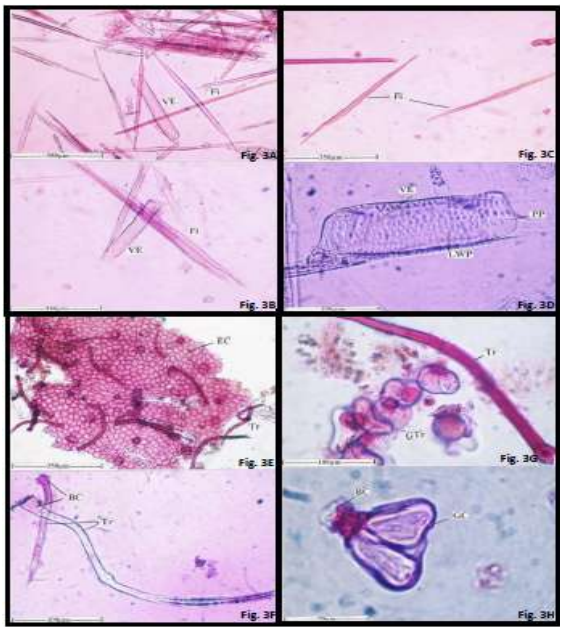

Fig. 3: Powder microscopic observation of *Gmelina arborea*Roxb. 3A. Macerated elements of fibres and vessel element. 3B. Wide fibres and a vessel element. 3C. Macerated fibres. 3D. A vessel element enlarged showing perforation plate and lateral wall pits. 3E. Adaxial epidermal fragment with non-glandular trichomes. 3F. Short and other long trichomes enlarged. 3G: A cluster of glandular trichomes. 3H. single trichome enlarged.

The percentage extractive values and fluorescence analysis were carried out their results are tabulated in Table: 2 and 3 respectively. The preliminary qualitative phytochemical screening revealed the presence of glycosides, alkaloids, saponin, tannin, phytosterols, flavonoids and terpenoids and were quantified and tabulated. (Table 4)

Acute oral toxicity study was performed using hydro-alcoholic extract of *Gmelina arborea* as per OECD 425 guidelines and no mortality was observed upto 2g/kg. The test substance showed no abnormal behavioral and physiological signs of symptoms in the entire drug treated animals and this concludes that the test substance is nontoxic and the maximum tolerated dose was found to be 2g/kg, b. wt. by oral route.

The dose ranging gastro-protective activity of *G. arborea* extract was performed using ethanol induced gastric lesions in rats. The Hydro-alcoholic extract of *G. arborea* extract (250 mg/kg) showed significant ($P<0.01$) gastro-protective activity when compared with diseased control. The maximum percentage protective index (65.05%) was observed at 500 mg/kg b. wt. of test substance treated groups.

Table 2: Percentage extractive values of *Gmelina arborea* Roxb.aerial parts

Sl. No.	Solvent	Extractive value (% w/w) Values are in Mean±SEM
1	Hexane	10.00±0.5
2	Petroleum ether (60-80ºC)	1.10±0.2
3	Dichloromethane	4.20±0.3
4	Chloroform	2.66±0.2
5	Acetone	3.43±0.3
6	Ethanol	7.66±0.4
7	Distilled Water	5.42±0.3

Table 3: Fluorescence analysis of *Gmelina arborea* Roxb.aerial parts

S. No	Treatment	Observation under	
		Visible light	UV light
1	Powder as such	Brown	Light green
2	Powdered drug + conc. Hydrochloric acid	Dark brown	Dark green
3	Powder + 80% Sulphuric acid	Blackish red	Black
4	Powder + Glacial acetic acid	Pale brown	Green
5	Powder + saturated solution of picric acid	Yellow	Parrot green
6	Powder + 5N sodium hydroxide	Dark brown	Blackish green
7	Powder + 5% Ferric chloride solution	Green	Blackish green
8	Powder + Aqueous Iodine solution	Reddish Brown	Dark green

Table 4: Quantitative analysis of Aerial parts of *Gmelina arborea* for its primary and secondary metabolites

Sl. No	Parameter	Mg/kg
1	Total Alkaloids	1.28
2	Total flavonoids	2.30
3	Tannins	0.58
4	Lignin	0.36
5	Glycosides	0.06
6	Terpenoids	0.03
7	Saponins	0.02
8	Total carbohydrates	1.28
9	Total protein	0.25
10	Total fats	0.03

The hydro-alcoholic extract was subjected to ABTS assay and the percentage inhibition at 150µg/ml for ethyl acetate, water and ethanol extracts was found to be 63.28, 72.14 and 88.33 respectively.

Table 5: ABTS free radical scavenging activity of different extracts of *Gmelina arborea* Roxb.aerial parts

Extract	% inhibition (Average value)		
	50 µg/ml	100 µg/ml	150 µg/ml
Pet. Ether	5.24	1.67	9.81
Chloroform	11.67	22.38	33.10
Ethyl acetate	40.71	50.00	63.28
Ethanol	39.52	70.48	88.33
Water	30.71	52.14	72.14

Table 6: Effect of hydroalcholic extract of *Gmelina arborea* aerial parts on Ulcer Index and Protective Index (%)

Treatment	Total glandular area (sq. mm)	Total ulcerated area (sq. mm)	Ulcer index	Protective index (%)
Diseased control	909.95±93.89	245.07±17.34$^{\#}$	2.71±0.47$^{\#}$	-
Sucralfate (400 mg/kg, p.o)	897.93±36.06	112.53±8.90*	1.25±0.04*	54.08
HAGA (125 mg/kg, p.o)	842.53±46.67	217.11±36.96$^{\#}$	2.56±0.29$^{\#}$	11.41
HAGA (250 mg/kg, p.o)	942.00±86.76	113.84±19.58*	1.22±0.32*	53.54
HAGA (500 mg/kg, p.o)	931.01±11.99	85.64±14.45*	0.92±0.16*	65.05
HAGA(1000 mg/kg, p.o)	932.89±41.48	92.47±6.97*	0.99±0.11*	62.26

Values are expressed as mean ± SE, (n=6). *P<0.01 as compared with diseased control, $^{\#}$P<0.01 as compared with standard control (Sucralfate)

DISCUSSION

The importance of pharmacognostic study is to check the uniqueness and to evaluate the purity, strength and quality of the crude drugs. The Macroscopic evaluation is performed on sensory and morphological profile of crude drug, whereas the microscopical evaluation is done to identify the salient anatomical features of herbs either in crude or powder forms [16]. The botanical identification and standardization of crude drug is achieved by determining macroscopical, microscopical and physicochemical standards as established in this present study [22, 23]. The salient features observed microscopically were densely trichomatous lamina, thick walled and angular xylem elements, glandular trichomes, reticulate aerenchyma, discontinuous periderm, spherical masses of thick walled sclerenchyma elements and libriform fibres. The fluorescence analysis is used to determine the chemical nature of drug after treating with different reagents and observed under ultraviolet light [24].

The ABTS assay is used to evaluate the antioxidant potential of both water-soluble and lipid-soluble antioxidants, food extracts and pure compounds [17]. The antioxidant potential was evaluated using ABTS assay and the maximum percentage inhibition was noticed in ethanol (88.33%) and aqueous (72.14%) extracts. Syamsul et al reported that 3,4,5-trimethoxyphenol has been isolated from *Gmelina arborea* bark extract and showed maximum antioxidant potential by DPPH assay [25]. The presence of saponin, tannin, flavanoids and terpenoidsalso contribute the antioxidant potential of the extract. Nayak et al reported that ethanol extract of *Gmelina arborea* fruits showed maximum antioxidant activity and is evident from our present study [3]. Acute oral toxicity was performed as per OECD guidelines and revealed no significant change in weekly body weight and daily feed intake thus confirmed that the hydro-alcoholic extract of *Gmelina arborea* is safe upto 2 g/kg b. wt. The previous toxicity studies conducted with bark extract also confirmed that the plant is safe in both acute and sub-acute toxicity studies [26].

The hydro-alcoholic extract of *G. arborea* extract showed gastroprotecive effect at doses of 250, 500 and 1000 mg/kg against ethanol induced gastric lesions, which is probably due to the presence of potent antioxidant phytoconstituents in the extract. There are many studies which confirmed the antiulcer properties of flavonoids [7, 27, 28]. In addition, flavonoids are also known to scavenge the free radicals generated by reactive oxygen species thus prevents the experimentally induced gastric mucosal injuries [29]. Tannin at low concentration protects the outermost gastric mucosal layer and is less permeable and more resistant to gastric juice [30]. The administration of tannins to experimental animals showed significantly decreased reactive oxygen species (ROS) level in stomach [31]. Researcher has reported the antiulcer potential of saponins in various experimental ulcer models [32, 33]. The phytochemical analysis also confirmed the presence of tannin, saponin, glycosides, alkaloids, phytosterols, flavonoids and terpenoids.

CONCLUSION

The data obtained from the present study evidently suggested that experimental rats treated with hydro-alcoholic extract of *G. arborea* showed significant gastroprotection. These experimental results have established a pharmacological evidence for the folklore claim of *G. arborea* to be used as an anti-ulcer agent.

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