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Research Article

Phytochemical and Antioxidant Studies on Methanolic Extract of *Gmelina asiatica* Linn Stem

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ABSTRACT
To evaluate the phytochemical constituents and antioxidant activities of methanolic extract of *Gmelina asiatica* stem which is locally used for the treatment of various diseases. The antioxidant and free radical scavenging activity of methanolic extract of the plant was assessed against 1,1-diphenyl-2-picrylhydrazyl (DPPH), nitric oxide (NO), the ferric reducing agent. Total phenolics, flavonoids were also determined to assess their corresponding effect on the antioxidant activity of this plant. Phytochemical analysis revealed the presence of tannin, alkaloid, steroids, glycosides and saponins. The results exhibited a positive linear correlation between these polyphenols and the free radical scavenging activities. The activities of plant extract against DPPH, NO radicals were concentration dependent with IC_{50} value of 18.38 and 78.18µg/mL respectively. The reducing power of the extract was 84.15 µg/mL. The total phenolics content of the methanolic extract of stem was 4800±24.53 and the total flavonoid content was 28.54 ± 0.18 QE/g. Our findings provide evidence that the crude methanolic extract of *Gmelina asiatica* is a potential source of natural antioxidants and this justifies its uses in folkloric medicine.

Key words: Antioxidant, flavonoid, *Gmelina asiatica*, phenolics

INTRODUCTION

Antioxidants are the vital substances which possess the ability to protect the body from damage caused by the free radical induced oxidative stress.[1] It was reported that some medicinal plants contain a wide variety of natural antioxidants, such as phenolic acids, flavonoids and tannins, which possess more potent antioxidant activity than dietary plants.[2-5] Many investigations indicate that these compounds are of great value in preventing the onset and/or progression of many human diseases.[6] The health promoting effect of antioxidants from plants is thought to arise from their protective effects by counteracting reactive oxygen species (ROS).[2-4] Free radicals are chemically unstable atoms that cause damage to lipid cells, proteins and DNA as a result of imbalance between the generation of reactive oxygen species (ROS) and the antioxidant enzymes.[7] They are known to be the underlying cause of oxidative stress which is grossly implicated in the pathogenesis of various diseases such as cancer, diabetes, cardiovascular diseases, aging and metabolic syndrome..[8-9] Recently, there has been a worldwide trend towards the use and ingestion of natural antioxidants present in different parts of plants due to their phytochemical constituents.[10-11]

Gmelina asiatica L (Verbenaceae) popularly known as Asian Bushbeach in English, *Gopabhandra* in Sanskrit and Adavi Gummudu in Telugu is a large straggling shrub found in South India. After phylogenetic studies, it is now being classified under the family Lamiaceae. It is found in dry lands, wastelands, as a live fence in agricultural lands and also on road sides. Leaves and young shoots are traditionally used in medicine.[12] It was reported that leaves and aerial parts are used in the treatment of jaundice and other hepatic diseases by some tribes in Tamil Nadu [13] and another tribe used it for body heat.[14] Roots, bark, fruit, leaves, and young shoots are used in various medicines in Sri Lanka.[15] It is also reported that this is one of the 'Anukta Dravya' drugs.[16] Present investigation was undertaken to examine the total phenolic content and antioxidant activities of the methanolic extract of *Gmelina asiatica* stem through various in vitro assay models. The possible relationship between phenolic content and antioxidant activity was also found out which supports its traditional uses because of the presence of phenolic compounds and flavonoids. Therefore, the present study was aimed to provide information on the quantitative compositions of the phytochemicals and antioxidant activities of the methanolic extract of *Gmelina asiatica* stem in order to provide scientific basis to justify its therapeutic usage.

MATERIALS AND METHODS

Plant collection: The plants of *Gmelina asiatica* were collected from Zoo park of Visakhapatnam. The plant was authenticated by Prof. M. Venkaiah of Botany Department, Andhra University. The specimen voucher (135C) was

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Table 1: Summary of Preliminary phytochemical analysis

S. No	Phytoconstituent	Extract			
		Hexane	Chloroform	Methanol	Aqueous
1	Carbohydrates				
	a. Molisch's test	-	-	+	+
	b. Fehling's test	-	-	+	+
2	Amino acids				
	a. Ninhydrin test	-	-	-	+
3	Proteins				
	a. Biuret test	+	+	+	+
	b. Million's test	+	-	+	-
4	Fats and oils				
	a. Spot test	+	+	-	-
5	Alkaloids				
	a. Mayer's test	-	-	+	-
	b. Dragendorff's test	-	-	+	-
6	Triterpenoids				
	a. Salkowski reaction	-	+	+	-
	b.Liebermann-Burchard test	-	+	+	-
7	Flavonoids				
	a. Ferric chloride test	+	+	+	-
	b. Shinoda test	-	-	+	-
	c. Filter paper test	+	+	+	-
8	Glycosides				
	a. General test	-	-	+	-
	b. Legal's test	-	-	+	-
9	Tannins and phenolic compounds				
	a.Ferric chloride test	-	-	+	+
	b. Lead acetate test	-	-	+	+

+ Present - Absent

Table 2 : *In vitro* antioxidant studies by DPPH, NO, Ferric reducing methods

Concentration (µg/ml)	% Inhibition DPPH		% Nitric oxide scavenged		% Reducing power	
	Ascorbic acid	*Gmelina asiatica*	Ascorbic acid	*Gmelina asiatica*	Ascorbic acid	*Gmelina asiatica*
5	44.30± 0.38	13.99± 0.12	10.6± 0.44	9.43± 0.41	15.92± 0.52	11.55± 0.45
10	53.38± 0.36	42.74± 0.31	21.8± 0.89	17.96± 0.53	28.75± 0.22	19.4± 0.17
20	74.83± 0.15	59.34± 0.45	44.3±0.31	36.28± 0.62	35.49± 0.19	31.56± 0.45
50	91.28± 0.18	65.27± 0.68	47.9±0.90	45.86± 0.96	41.36± 0.22	39.63± 0.07
75	93.31± 0.12	78.36± 0.79	54.2± 0.40	48.80± 0.67	51.7± 0.32	47.47± 0.27
100	98.71± 0.14	88.92± 0.37	63.4±0.98	57.19± 0.81	68.5± 0.09	62.81± 0.29

Data are expressed as means ± standard deviation of triplicate samples. Values with different rows are significantly (P< 0.05).

deposited at the University herbarium. The plant material was dried under shade for 20 days and pulverized in a plant mill and stored in an airtight container for further use.

Preparation of extract

The powdered plant material was extracted with methanol on a Soxhlet apparatus (Borosil Glass Works Ltd, Worli, Mumbai) for 48 h. The extract was filtered using a Buchner funnel and Whatman No. 1 filter paper and sterile cotton wool. The filtrate of the extract was concentrated on Rotary Flash Evaporator (Roteva Equitron.Medica Instrument Manufacturing company, Mumbai) and dried in a desiccators for 1 wk gave the yield of 2.3 g and later reconstituted in distilled water to give the required concentrations needed in this study.

Chemicals

Folin–Ciocalteau reagents, Gallic acid, Quercetin, Aluminium chloride, 1,1-Diphenyl-1-picrylhydrazyl (DPPH), Ascorbic acid, Sodium nitroprusside were collected from Sigma-Aldrich Co. St. Louis, MO, USA. sulphanilamide, Orthophosphoric acid, N-(1- naphthyl) ethylenediamine, methanol, chloroform, H_2SO_4, Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), potassium acetate, phosphate buffer, Potassium ferricyanide ($K_3Fe(CN)_6$), trichloroacetic acid (TCA), ferrous chloride are purchased from Merck, USA. All the chemicals used in this study were of analytical grade.

Determination of total phenolics

Folin-Ciocalteu reagent was used to determine total phenolics content[17-18] in methanolic extract of *Gmelina asiatica* stem. An aliquot (20µl) of 15mg/ml of selected plant extracts or standard solution of Gallic acid was added into a cuvette, containing 1.58ml of water and 100µl of 0.2 N Folin-Ciocalteu reagents. After 5 minutes, 300µl of sodium carbonate (Na_2CO_3) solution was added into the mixture. The cuvette was incubated for 1 hour at room temperature, the absorbance against prepared reagent blank was determined at 765nm with an UV-Vis Spectrophotometer. The total phenolic content was expressed as Gallic acid equivalents (GAE) in mg/g of dried plant extract. All samples were analysed in triplicates. A range of Gallic acid concentrations from 0.3 to 0.7 mg/ml were used for calibration curve. The equation obtained from the standard Gallic acid curve was: $Y= 70.7481x – 0.0595$, $R^2 = 0.9991$, where R^2 is the regression coefficient. The experiment was conducted in triplicate and the results were expressed as mean plus standard deviation (mean $\pm$ SD).

Determination of total flavonoids

Total flavonoid content was determined by using aluminium chloride colourimetric method[19-20] on the formation of a complex flavonoid aluminium. 200µl of the standard solutions of different concentrations and 15mg/ml of extracts of selected plants were separately mixed with 600µl of 95% ethanol, 40µl of 10% (w/v) aluminium chloride, 40µl of 1M sodium acetate and 1120µl of distilled water. After incubation at room temperature for 30 minutes, the absorbance of the reaction mixture was measured at 415nm with a spectrophotometer. The amount of 10% (w/v) aluminium chloride was substituted by the same amount of distilled water for blank. The test compounds were similarly reacted with aluminium chloride for determination of flavonoid content. All experiments were done in triplicate and the results were expressed as mean plus standard deviation (mean $\pm$ SD). Total flavonoid content was expressed as Quercetin equivalents (QE) in mg/g of dried extract from the calibration curve of Quercetin standard solution. A range of Quercetin concentrations from 10 to 50µg/ml was used to prepare the calibration curve. The equation obtained from the standard Quercetin curve was: $Y = 0.0329 x – 0.0207$, $R^2= 0.9924$, where R^2 is the regression coefficient.

DPPH scavenging assay[21]

The reaction was performed in 1ml of solution containing 0.1mM freshly prepared DPPH in 95% ethanol and various concentrations of test samples. An aliquot of 3ml of DPPH solution in ethanol and 0.1ml of plant extract at various concentrations were mixed. The mixture was shaken vigorously and allowed to reach a steady state at room temperature for 30 minutes. Decolourisation of DPPH was determined by measuring the absorbance spectrophotometrically at 517nm. A control was prepared using 0.1ml respective vehicle in place of plant extract/ascorbic acid. All experiments were done in triplicates. The IC_{50} value was determined, to know the concentration of test sample required to scavenge 50% of DPPH in the reaction mixture. The scavenging ability of the plant on DPPH was calculated using the equation: DPPH scavenging activity (%) = [(Abs control - Abs sample)]/(Abs control)]x100, where Abs control is the absorbance of DPPH + methanol; Abs sample is the absorbance of DPPH radical + sample extract or standard.

Determination of reducing power[22]

A volume of 1ml of the extract was prepared in distilled water (0.2-1.0 mg/ml) and mixed thoroughly with the mixture of 2.5ml of 0.2 mM phosphate buffer (pH 7.4) and 2.5ml of potassium ferricyanide (1% w/v). The resulting mixture was incubated at 50 ºC for 20 min, followed by the addition of 2.5ml of TCA (10% w/v) and the mixture was centrifuged at 3000 rpm for 10 min. The upper layer of the solution was collected and mixed with 2.5 ml of distilled water and later with 0.5ml of ferrous chloride (0.1% w/v). The absorbance was measured at 700nm against a blank sample. Increased absorbance of the reaction mixture indicated higher reducing power of the plant extract.

Nitric oxide scavenging activity[23]

A volume of 2ml of sodium nitroprusside prepared in 0.5 mM phosphate buffer saline (pH 7.4) was mixed with 0.5ml of plant extract at various concentrations (0.2-1.0mg/ml). The mixture was incubated at 25 ºC for 150 min. An aliquot of 0.5ml of the solution was added to 0.5ml of Griess reagents [1ml of sulfanilic acid reagent (0.33% prepared in 20% glacial acetic acid at room temperature for 5 min with 1ml of naphthyethylenediamine chloride (0.1% w/v)]. The mixture was incubated at room temperature for 30 min. The absorbance was then measured at 540 nm. The amount of nitric oxide radical was calculated using the equation: NO radical scavenging activity = [(Abs control- Abs sample)/ (Abs control)]x100, where Abs control is the

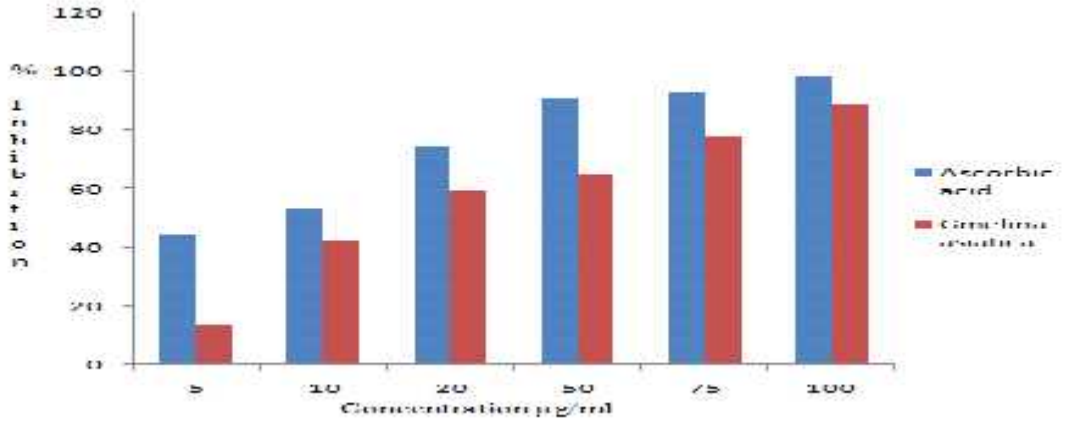

Fig. 1 : In vitro Antioxidant activity of Ascorbic acid and methanolic extract of Gmelina asiatica by DPPH method

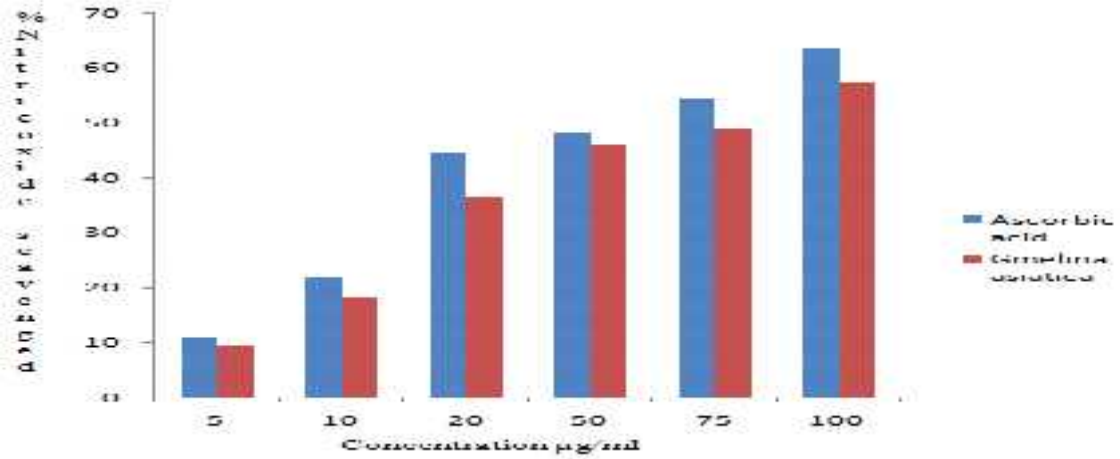

Fig. 2 : In vitro Antioxidant activity of Ascorbic acid and methanolic extract of Gmelina asiatica by Nitric oxide scavenging method

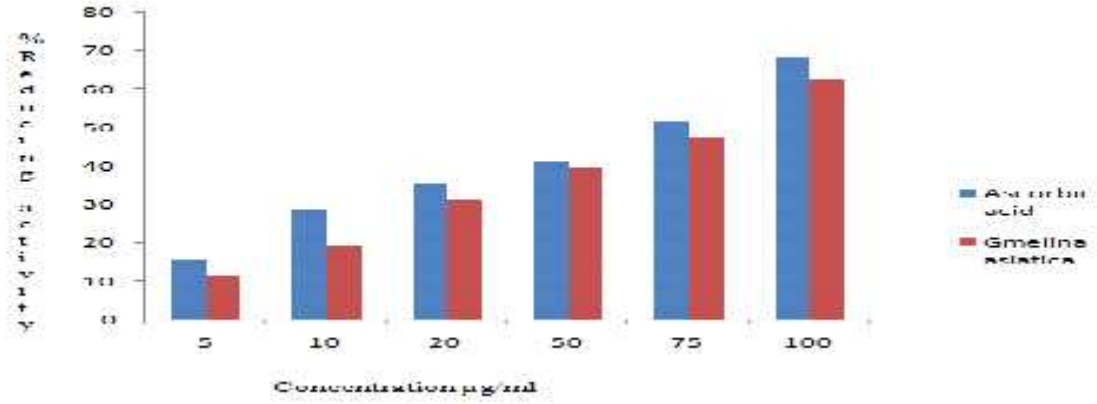

Fig. 3 : In vitro Antioxidant activity of Ascorbic acid and methanolic extract of Gmelina asiatica by reducing power method

absorbance of NO radical + methanol; Abs sample is the absorbance of NO radical + sample extract or standard.

Statistical analysis: Statistical analysis was carried out and the values were reported as the average of the values from three individual experiments in each case plus Standard deviation (Mean ± S.D). Statistical significance was estimated using One Way ANOVA, Tukey Multiple Comparison Test by SPSS 11.5 for windows Software and Regression analysis was carried out for determining IC_{50} for each sample.

RESULTS AND DISCUSSION

Preliminary phytochemical analysis of different extracts revealed the presence of carbohydrates, proteins, triterpenoids, alkaloids, glycosides, flavonoids and phenolic compounds. The results are summarised in Table

1. By the quantitative phytochemical analysis the total phenolic content as gallic acid equivalent was found to be 4800±24.537, and the total flavonoid content as quercetin equivalent were found to be 28.54 ± 0.18. The results of in vitro antioxidant studies by different methods were summarised in Table 2. The results of DPPH radical scavenging activity of the extract and the standard drug, ascorbic acid were presented in Figure 1. The percentage inhibitory activity of free radicals by 50% has been used widely as a parameter to measure antioxidant activity. In this study, both plant extract and standard drugs significantly reduced the DPPH radical with increasing concentrations. The IC_{50} values of the Ascorbic acid and methanolic extracts of *Gmelina asiatica* (stem) were found to be 5.14 and 18.38 µg/ml respectively. The scavenging activity of the plant extract against nitric oxide released by sodium nitroprusside was investigated and the results were represented in Figure 2. The inhibitory effect of the extract was comparable to the standard drugs used in this study. The IC_{50} values of the Ascorbic acid and methanolic extract of *Gmelina asiatica* (stem) were found to be 63.86 and 78.18 µg/ml respectively. The results of the antioxidant activity of *Gmelina asiatica* methanolic stem extract determined by measuring its ability to transform Fe^{3+} to Fe^{2+} was represented in the Figure 3. The reducing power was confirmed by the changes of yellow colour of the test solution to various shades of green and blue depending on the concentration of the plant extract. The reducing power of the extract and the standard drugs increased with an increase in concentration though the plant extract had lesser antioxidant activity than the ascorbic acid, which is used as reference drug. The IC_{50} values of the Ascorbic acid and methanolic extract of *Gmelina asiatica* (stem) were found to be 64.47 and 84.15 µg/ml respectively.

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