

Computation of Antioxidant Properties and Phytochemical Composition of *Gmelina Arborea*

G. Satyanarayana Goud¹, P. Bheemeshwaramma², and N. Venu Gopal³

^{1, 2, 3} Department of Chemistry, MVS Govt. Arts & Science College (A), Mahabubnagar-509001,
Telangana State, India

Abstract:

The current study focuses on analyzing the secondary metabolites and antioxidant properties of various extracts derived from the leaf and stem of *Gmelina arborea*. Phytochemical analysis confirmed the presence of several bioactive secondary metabolites, including alkaloids, saponins, phenolic compounds, tannins, phenols, and steroids, which are known for their therapeutic and pharmacological significance. The antioxidant potential of the extracts was evaluated through free radical scavenging assays, and the results indicated that both leaf and stem extracts exhibited notable antioxidant activity, effectively neutralizing free radicals. Among the different solvent extracts tested, the ethanol and petroleum ether extracts demonstrated the most significant antioxidant potential, suggesting that these solvents are more effective in extracting active compounds responsible for antioxidant activity. These findings support the traditional medicinal use of *Gmelina arborea* and highlight its potential as a natural source of antioxidants, which could be further explored for pharmaceutical and nutraceutical applications.

Index terms: *Gmelina Arborea*, Antioxidant Activity, DPPH, Phytochemical Screening

INTRODUCTION

Plants not only supply the essential nutrients required for sustaining life but also offer a wide range of bioactive compounds that contribute to health enhancement and the prevention of various diseases. They are invaluable to human well-being, as they serve as natural sources of medicinal compounds that play a crucial role in maintaining and promoting health. In this context, the present study was undertaken to evaluate the antioxidant potential and conduct phytochemical screening of crude extracts from *Gmelina arborea* (Roxb), a fast-growing deciduous tree belonging to the family Verbenaceae and widely distributed across India (Cook, 1966). This plant holds significant medicinal value, with various parts being traditionally used in folk medicine. It is believed to enhance digestive function, boost memory, and aid in the treatment of conditions such as fever, cardiovascular ailments, nervous disorders, and piles (Banu et al., 2013). Given its rich ethnomedicinal background, *Gmelina arborea* is considered a valuable candidate for further pharmacological and phytochemical investigations.

MATERIALS AND METHODS

Preparation of Plant Extracts

The plant extracts were prepared using the Soxhlet extraction method as described by Tiwari et al. (2011). Fresh leaves and stems of *Gmelina arborea* were collected from Nagpur, Maharashtra (Plate-I). The plant materials were thoroughly washed under running tap water to remove any dirt or impurities, then dried in the shade to preserve their bioactive compounds. Once dried, the materials were ground into a fine powder and stored in airtight bottles to maintain their quality. The powdered leaves and stems were placed in a Soxhlet thimble, and extraction was carried out using various solvents, including petroleum ether, ethyl acetate, acetone, ethanol, and water. After the extraction process, the residues were collected, allowed to air dry, and the resulting crude extracts were stored in a refrigerator for further experimental analysis. This method ensures efficient extraction of phytochemicals from the plant material while maintaining the integrity of the compounds for subsequent evaluations.



Plate I - *Gmelina arborea* Roxb.

Qualitative Phytochemical Tests

The solvent-free extract obtained as described above was subjected to preliminary qualitative phytochemical screening to identify various plant constituents, following the methodologies of Harborne (1998) and Kokate (2001).

1. Test for Alkaloids

A 50 mg portion of the solvent-free extract was stirred with a few milliliters of dilute HCl and filtered. The filtrate was then tested with several alkaloidal reagents:

- **Mayer's Test:** A drop or two of Mayer's reagent was added to the filtrate. The formation of a white or creamy precipitate indicated the presence of alkaloids.
- **Mayer's Reagent:** Mercuric chloride (1.358g) was dissolved in 60 ml of water, and potassium iodide (5.0g) was dissolved in 10 ml of water. The two solutions were mixed, and the volume was made up to 100 ml with water.
- **Wagner's Test:** A few drops of Wagner's reagent were added to the filtrate. The appearance of a reddish-brown precipitate confirmed a positive result.

- **Wagner’s Reagent:** Iodine (1.27g) and potassium iodide (2g) were dissolved in 5 ml of water, and the volume was made up to 100 ml with distilled water.
- **Hager’s Test:** 1 or 2 ml of Hager’s reagent (saturated aqueous solution of picric acid) were added to the filtrate. The formation of a prominent yellow precipitate indicated the presence of alkaloids.

2. Detection for Phenolic Compounds

- **Lead Acetate Test:** A 50 mg sample of the extract was dissolved in distilled water, and 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicated the presence of phenolic compounds.

3. Test for Tannins

About 0.5 g of the plant extract was added to 10 ml of water in a test tube, filtered, and then a few drops of 0.1% ferric chloride were added. The appearance of brownish-green or blue-black coloration indicated the presence of tannins.

4. Test for Proteins

To 2 ml of protein solution, 1 ml of 40% NaOH solution and 1–2 drops of 1% CuSO₄ solution were added. A violet color indicated the presence of peptide linkages, confirming the presence of proteins.

5. Test for Amino Acids

To 2 ml of the sample, 2 ml of Ninhydrin reagent was added and the mixture was heated in a water bath for 20 minutes. The appearance of a purple color indicated the presence of amino acids.

6. Test for Reducing Sugars

2 ml of the extract was mixed with 2 drops of Molisch’s reagent and shaken well. Concentrated H₂SO₄ (2 ml) was added carefully to the side of the test tube. The appearance of a reddish-violet ring at the junction of two layers immediately indicated the presence of reducing sugars.

7. Test for Glycosides

Each extract was hydrolyzed with HCl and neutralized with NaOH solution. A few drops of Fehling’s solution A and B were added to each mixture. The formation of a red precipitate indicated the presence of glycosides.

8. Tests for Flavonoids

- **Alkaline Test:** 0.2 g of each extract was dissolved in diluted NaOH, and a few drops of HCl were added. A yellow solution that turned colorless indicated the presence of flavonoids.
- **Magnesium Test:** To 2 ml of the test solution, 0.5 ml of alcohol was added, followed by a bit of magnesium and 1–2 drops of concentrated HCl. Heating the mixture resulted in a color change that indicated the presence of flavonoids.

9. Test for Phenols

To 2 ml of the test solution, alcohol was added, followed by a few drops of neutral ferric chloride solution. The formation of a colored reaction indicated the presence of phenols.

10. Tests for Coumarins

3 ml of 10% NaOH was added to 2 ml of the aqueous extract. The appearance of a yellow color indicated the presence of coumarins.

11. Test for Resins

To 0.2 g of each extract, 10 ml of glacial acetic acid was added, heated, and cooled. A drop of concentrated H_2SO_4 was added. The appearance of a purplish-red color indicated the presence of resins.

12. Test for Steroids/Terpenoids

1 ml of the extract was dissolved in 10 ml of chloroform, and an equal volume of concentrated H_2SO_4 was added carefully by the side of the test tube. The appearance of a red upper layer and a yellow color with green fluorescence in the H_2SO_4 layer indicated the presence of steroids or terpenoids.

13. Saponins Test (Foam Test)

5 mg of plant extract powder was added to a test tube containing water and a pinch of sodium bicarbonate. The mixture was shaken vigorously, and the formation of stable, characteristic honeycomb-like froth indicated the presence of saponins

14. Saponins Test (Foam Test)

A 5 mg portion of the plant extract powder was weighed and placed in a test tube. The extract was then shaken vigorously along with a pinch of sodium bicarbonate and a small amount of water. The formation of a stable, characteristic honeycomb-like froth indicated the presence of saponins.

ANTIOXIDANT ACTIVITY

Antioxidant Activity with DPPH Assay

The antioxidant activity of the plant extract, was estimated utilizing 2, 2-diphenyl-1-picrylhydrazyl (DPPH) (Blois,1958). Five concentrations (22, 40, 60, 80 & 100ug/ml) of each sample were prepared. 0.1 Mm solution of DPPH in methanol was prepared and 180 μ l of this solution was added to 20 μ l of different plant extracts in 96 well plates and incubated for 30 min at room temperature in the dark. Ascorbic acid was used as a positive control. The DPPH radical-scavenging activity was determined by measuring the absorbance at 490nm and calculated using the equation (Badami and Gupta 2005).

$$I\% = \frac{(Ac - As)}{Ac} \times 100 \dots\dots (1)$$

Where, Ac – absorbance of the control

As – absorbance of the sample

RESULT AND DISCUSSION

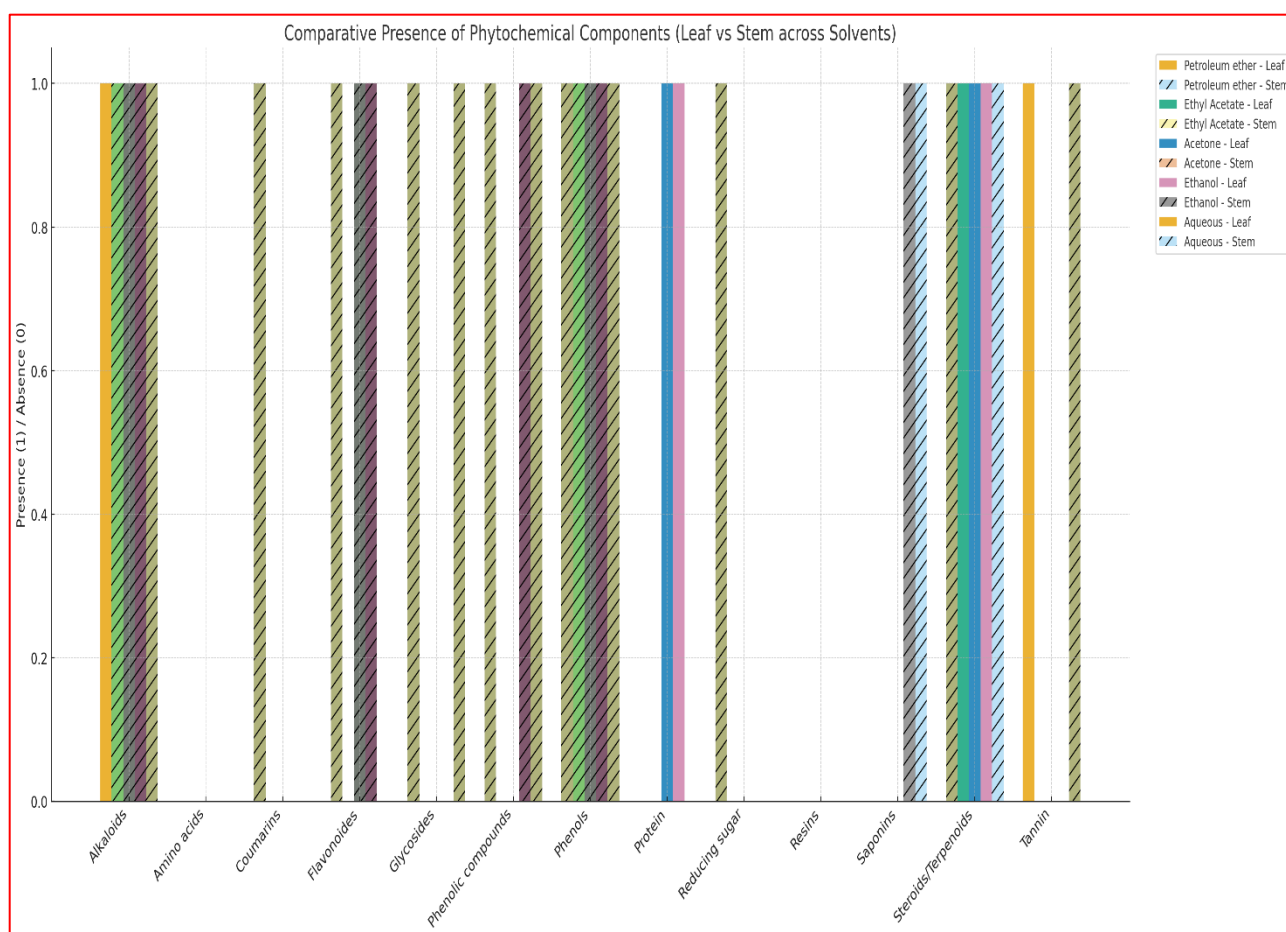
The phytochemical screening of the leaves and stems of *Gmelina arborea* was conducted and the results are summarized in Table 1. The qualitative analysis revealed the presence of various bioactive compounds, including alkaloids, saponins, phenolic compounds, tannins, reducing sugars, glycosides, flavonoids, coumarins, and steroids. These findings are consistent with previous studies by Akyala et al. (2013), Chotani and Patel (2012), Kaswala et al. (2012), EI-Mahmood et al. (2010), and Banu et al. (2013), all of which also reported the presence of phenols, saponins, glycosides, and tannins in the phytochemical composition of *Gmelina arborea*.

Table 1 : Phytochemical analysis of various extracts of *G.arborea* (leaf and stem)

Phytochemical Components	Petroleum ether		Ethyl Acetate		Acetone		Ethanol		Aqueous	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
Alkaloids	+	-	+	+	+	+	+	+	+	+
Amino acids	-	-	-	-	-	-	-	-	-	-
Coumarins	+	+	-	-	-	-	-	-	-	-
Flavonoides	+	+	-	-	+	+	+	+	-	-
Glycosides	+	+	-	-	-	-	-	-	+	+
Phenolic compounds	+	+	-	-	-	-	+	+	+	+
Phenols	+	+	+	+	+	+	+	+	+	+
Protein	-	-	-	-	+	-	+	-	-	-
Reducing sugar	+	+	-	-	-	-	-	-	-	-
Resins	-	-	-	-	-	-	-	-	-	-
Saponins	-	-	-	-	-	+	-	+	+	+
Steroids/Terpenoids	+	+	+	-	+	-	+	-	-	+
Tannin	+	-	-	-	-	-	-	-	+	+

Keys: (+) (-) = indicates absent , Ania= indicates present,

The antioxidant activity of the leaves and stems of *Gmelina arborea* was evaluated by measuring the reducing ability and free radical scavenging activity using various extracts (petroleum ether, ethyl acetate, acetone, ethanol, and water) through the DPPH assay (Fig. 1-5). Ascorbic acid was used as the standard control. Among the extracts, ethanol extracts from both the leaf and stem demonstrated the highest activity, with the lowest IC₅₀ values of 13.89 µg/ml and 18.03 µg/ml, respectively (Fig. 6). These results align with those of Hutke and Naswale (2020). For the leaf, petroleum ether extract (IC₅₀ 15.47 µg/ml) was the second most active, while for the stem, the aqueous extract (IC₅₀ 28.9 µg/ml) showed significant antioxidant activity. In comparison, Attanayake et al. (2015) reported an IC₅₀ of 36.89 ± 1.23 µg/ml for the aqueous stem bark extract of *Gmelina arborea*. Patil et al. (2009) found that the methanol extract, rich in phenolic compounds, exhibited high antioxidant and free radical scavenging activities. Additionally, flavonoids isolated from *Gmelina arborea* demonstrated moderate antioxidant activity, as noted by Shoeb et al. (2014).



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