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EVALUATION OF ANTIOXIDANT ACTIVITY AND PHYTOCHEMICAL SCREENING OF *GMELINA ARBOREA* (ROXB.)

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Abstract: The present study deals with screening of secondary metabolites and antioxidant activity of different extracts of leaf and stem of *Gmelina arborea*. Phytochemical screening revealed the presence of various secondary metabolites viz. alkaloids, saponins, phenolics compound, tannins, phenols and steroids. Both leaf and stem extracts scavenged free radicals significantly. Whereas ethanol and petroleum ether extract significantly exhibited the antioxidant activity.

Index terms - *Gmelina arborea*, antioxidant activity, DPPH, Phytochemical screening

INTRODUCTION

Plants provide not only essential nutrients needed for life but also other bioactive compounds for health promotion and disease prevention. Plants are indispensable for human health they provide medicinally important compound to human that play vital role in human health. The present study was conducted to investigate the antioxidant activity and phytochemical screening of crude extracts of *Gmelina arborea* (Roxb) belongs to the family Verbenaceae is fast growing deciduous tree found throughout India, (Cook, 1966). The whole plant is medicinally very important. Folklore states that it promotes digestive power, improve memory and is useful alteration of fever, heart diseases, nervous disorder and piles (Banu et al, 2013).

MATERIALS AND METHODS

Preparation of plants extracts

Soxhlet method was used to obtain the plant extract (Tiwari, et al, 2011). Fresh leaves and stems were collected from Nagpur, Maharashtra (Plate-I). The collected plant materials were washed under tap water, dried in shade and then homogenized to fine powder and stored in airtight bottles. The powdered material of leaves and stem were kept in thimble and petroleum ether, ethyl acetate, acetone, ethanol and water extracts were carried out using the Soxhlet apparatus. The residues were collected and left for air drying and dried crude extracts were stored in refrigerator for further experimental work.

Plate I- Gmelina arborea Roxb.

Qualitative Phytochemical tests

The solvent free extract obtained as above was then subjected to qualitative preliminary phytochemical screening for identification of various plant constituents following the methodology of Harborne (1998) and Kokate (2001).

1. Test for alkaloids

Solvent free extract, 50 mg was stirred with few ml of dilute HCL and filtered. The filtrate was tested with various alkaloidal reagents as follows.

Mayer's test

To a few ml of filtrate, a drop or two of Mayer's reagent were added by the side of the test tube. A white or creamy precipitate indicated the test as positive.

Mayer's reagent

Mercuric chloride (1.358g) was dissolved in 60 ml of water and potassium iodide (5.0g) dissolved in 10 ml of water. The two solutions were mixed and the volume was made up to100 ml with water.

Wagner's test

To a few ml of filtrate, few drops of Wagner's reagent were added by the side of the test tube. A reddish- brown precipitate confirmed the test as positive.

Wagner's reagent

Iodine (1.27g) and potassium iodide (2g) was dissolved in 5ml of water and the volume was made up to 100 ml with distilled water.

Hager's test

To a few ml of filtrate, 1 or 2 ml of Hager's reagent (saturated aqueous solution of picric acid) were added. A prominent yellow precipitate indicated the test as positive.

2. Detection for phenolic compound

Lead acetate test

The extract (50mg) was dissolved in distilled water and to this; 3ml of 10% lead acetate solution was added. A bulky white precipitate indicated the presence of phenolic compounds.

3. Test for Tannins

About (0.5g) of the plant extract was added in 10 ml of water in test tube and filtered. A few drops of 0.l%ferric chloride was added and observed for brownish green or blue-black coloration.

4. Test for proteins

2 ml of protein solution 1ml of 40% NaOH solution and 1 to 2 drops of 1% $CuSO_4$ solution was added. A violet color indicated the presence of peptide linkage of the molecule.

5. Test for amino acids

2 ml of sample was added to 2 ml of Ninhydrin reagent and kept in water bath for 20 minutes. Appearance of purple color indicated the presence of amino acid in the sample.

6. Test for reducing sugars

To 2 ml of extract 2 drops of Molisch's reagent was added and shaken well. 2ml of concentrated $H2SO_4$ was added on the sides of the test tube. A reddish violet ring appeared at the junction of two layers immediately indicated the presence of carbohydrates.

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. Formation of red precipitate indicated the presence of glycosides.

9. Tests for Flavonoids

0.2 g of each extract was dissolved in diluted NaOH and few drops of HCL were added. A yellow solution that turned colorless indicated the presence of flavonoids.

To 2 ml of test solution, 0.5ml alcohol was mixed. Then a bit of magnesium and 1 or 2 drops of conc. HCL were added and heated. The mixture was analyzed for reaction.

10. Test for Phenols

To 2 ml of test solution, alcohol was added and then few drops of neutral ferric chloride solution were added. The test result was observed.

11. Tests for Coumarins

3 ml of 10% NaOH was added to 2 ml of aqueous extract, formation of yellow color indicated the presence of coumarins.

12. Test for Resins

To the 0.2 g of each extract 10 ml of glacial acetic acid was added then heated and cooled. A drop of conc. H_2SO_4 was added. Purplish red color showed the presence of resins.

13. Test for Steroids/ Terpenoids

1 ml of the extract was dissolved in 10 ml of chloroform and equal volume of conc. H_2SO_4 was added by the side of the test tube. The upper layer turned red and H_2SO_4 layer showed yellow with green fluorescence. This indicated the presence of steroids.

14. Saponins Test

Foam test

Weighed 5 mg of plant extract powder was taken in a test tube and then shaken vigorously by adding pinch of sodium bicarbonate and little bit of water. In result, formation of stable characteristics honey comb 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 µl of this solution was added to 20µl 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% = (Ac – As) / Ac x 100 …… (1)

Where, Ac – absorbance of the control As – absorbance of the sample

RESULT AND DISCUSSION

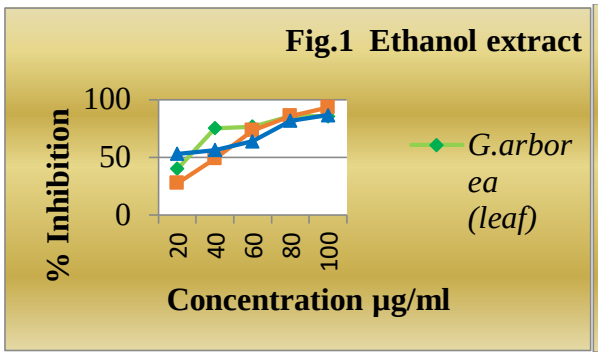
The phytochemical screening of the leaves and stem of *Gmelina arborea* were performed and presented in table–1. The qualitative phytochemical analysis of *G. arborea* leaves and stem contains alkaloids, saponins, phenolic compound, tannins, reducing sugar, glycosides, flavonoids, coumarin and steroids. Analogous to the result of a present investigation Akyala *et al*. 2013; Chotani and Patel, 2012; Kaswala *et al*., 2012; EI-Mahmood *et al*. 2010 and Banu *et al*. 2013 also reported that the phytochemical study revealed the presence of phenols, saponins, glycosides and tannins.

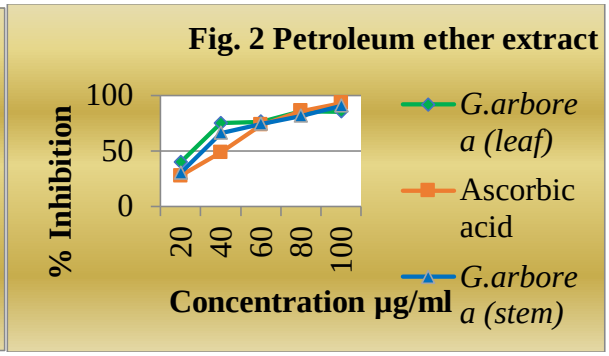
Table 1 : Phytochemical analysis of various extracts of *G.arborea* (leaf and stem)

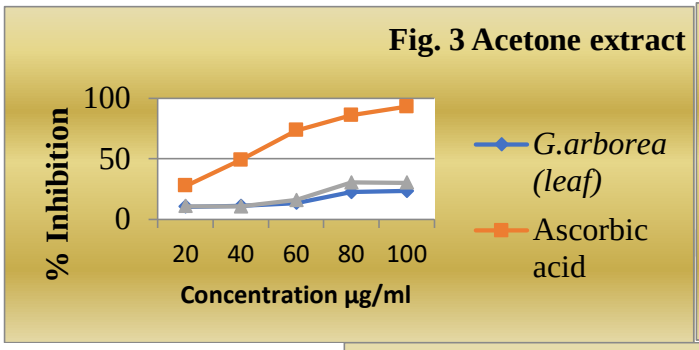
Phytochemical Components	Petroleum ether		Ethyl Acetate		Acetone		Ethanol		Aqueous	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
Alkaloids	+	-	+	+	+	+	+	+	+	+
Saponins	-	-	-	-	-	+	-	+	+	+
Phenolic compounds	+	+	-	-	-	-	+	+	+	+
Tannin	+	-	-	-	-	-	-	-	+	+
Protein	-	-	-	-	+	-	+	-	-	-
Amino acids	-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

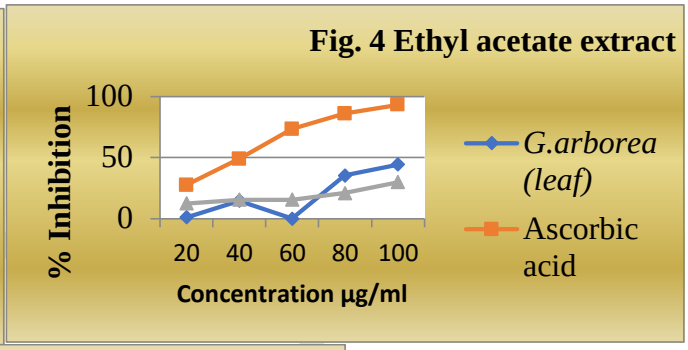
Keys: (+) = indicates present, (-) = indicates absent

The antioxidant activity of leaves and stem of *G. arborea* were carried out by measuring reducing ability free radical scavenging activity with various extracts (petroleum ether, ethyl acetate, acetone, ethanol and water) by using DPPH assay (Fig.1-5). Ascorbic acid was used as standard control. In leaf and stem, ethanol extracts were most active with low IC 50 of 13.89 and 18.03 µg/ml respectively (Fig.-6). Results of present study about the ethanol extract are in support of the findings of Hutke and Naswale, (2020). In leaf the petroleum ether (15.47 µg/ml) was second significant extract and for stem aqueous extract (28.9 µg/ml) in present study. Attanayake *et al.*, (2015) reported IC50 36.89 ±1.23 µg/ml as the antioxidant potential of aqueous stem bark extract of *G. arborea* whereas Patil *et al*. (2009) reported methanol extract which contained large amounts of phenolic compounds and exhibited high antioxidant and free radical scavenging activities. Flavonoids isolated from *G. arborea* exhibited moderate antioxidant activity, (Shoeb *et al*. 2014).









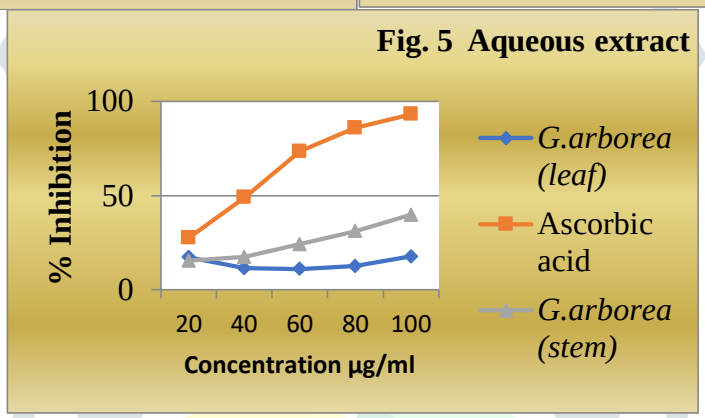


Fig. 1 - 5 DPPH radical scavenging activity (% inhibition) of *Gmelina arborea* (Leaf & Stem)

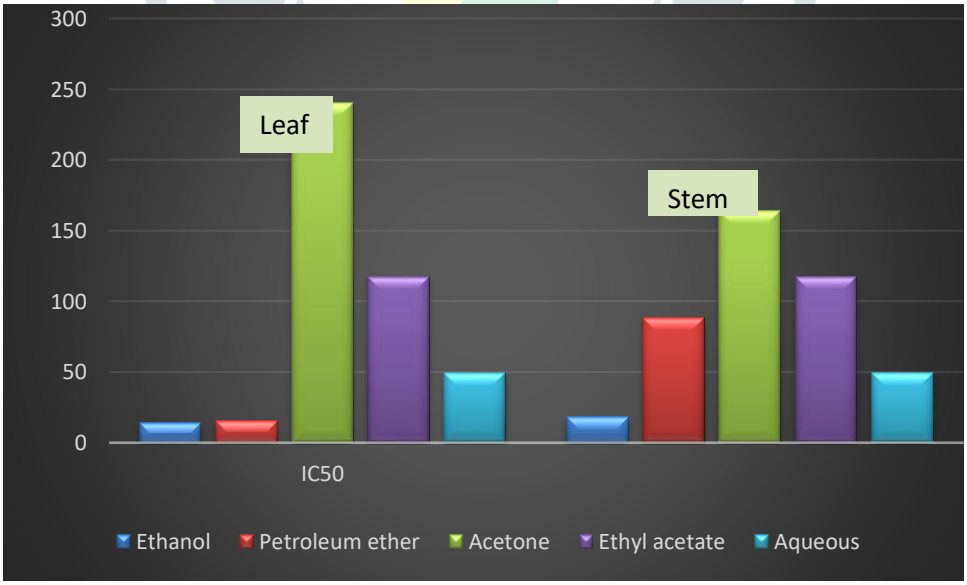


Fig.6- IC50 values of *G.arborea* Leaf and Stem

References

[1] Akyala, I. A., David, I., & Simon, I. (2013). Phytochemical screening and antibacterial activity of *Gmelina arborea* fruit extracts. *International Journal of Microbiology and Immunology Research*, *1*(2), 26-31.

[2] Attanayake, A. , Kamani Ayoma Perera Wijayawardana Jayatilaka , Chitra Pathirana , and Lakmini Kumari Boralugoda Mudduwa. (2015) Phytochemical screening and in vitro antioxidant potentials of extracts of ten medicinal plants used for the treatment of diabetes mellitus in sri lanka. Afr J Tradit Complement Altern Med. 12(4):28-33.

[3] Badami, S. and Gupta, M. K. (2005). Antioxidant activity of Grewia tiliaefolia bark. Oriental Pharmacy and Experimental Medicine, 5(1), 16-20

[4]Banu, M., Gururaja, G. M., Deepak, M., Roopashree, T., & Shashidhara, S. (2013). An overview on Phytochemistry and Pharmacological properties of *Gmelina arborea*. *J Nat Prod Plant Resour*, *3*(4), 62-71.

[5]Blois, M.S. (1985) Antioxidant determination by use of stable free radicals. Nature, 29 : 1999-1200.

[6]Chothani, D. L., & Patel, N. M. (2012). Preliminary phytochemical screening, pharmacognostic and physicochemical evalution of leaf of *Gmelina arborea*. *Asian Pacific Journal of Tropical Biomedicine*, *2*(3), S1333-S1337.

[7]Cooke, T. (1967). *The Flora Of The Presidency Of Bombay Vol-3*. Botanical Survey of India; Calcutta.

[8]El-Mahmood, A. M., Doughari, J. H., & Kiman, H. S. (2010). In vitro antimicrobial activity of crude leaf and stem bark extracts of *Gmelina arborea* (Roxb) against some pathogenic species of Enterobacteriaceae. *African Journal of Pharmacy and Pharmacology*, *4*(6), 355-361.

[9]Harborne, J. B. "Method of extraction and isolation." *Phytochemical Methods* (1998): 60-66.

[10]Kaswala, R., Patel, V., Chakraborty, M., & Kamath, J. V. (2012). Phytochemical and pharmacological profile of *Gmelina arborea*: An overview. *Int Res J Pharm*, *3*(2), 61-64.

[11]Hutke, V. D. and Naswale, M.P. (2020) Evaluation of in vitro antioxidant activity of different solvent extracts from Grewia hirsute Int. J. Adv. Res. Biol. Sci. 7(10): 110-115

[12]Kokate CK. Practical Pharmacognosy, 16 th ed., Vallabh Prakasan, Delhi, 2001, 107-111.

[13]Patil, S. M., Kadam, V. J., & Ghosh, R. (2009). In vitro antioxidant activity of methanolic extract of stem bark of *Gmelina arborea* Roxb.(Verbenaceae). *International Journal of PharmTech Research*, *1*(4), 1408-84.

[14]Shoeb, H. A., Madkour, H. M., Refahy, L. A., Mohamed, M. A., Saad, A. M., & Ghareeb, M. A. (2014). Antioxidant and cytotoxic activities of *Gmelina arborea* Roxb. leaves. *British Journal of Pharmaceutical Research*, *4*(1), 125.

[15]Tiwari, P., Kumar, B., Kaur, M., Kaur, G., & Kaur, H. (2011). Phytochemical screening and extraction: a review. *Internationale pharmaceutica sciencia*, *1*(1), 98-106.