



PHYTOCHEMICAL INVESTIGATION, ANTI-OXIDANT ASSAY OF FICUS GLOMERATA AND AEGLE MARMELOS EXTRACTS

Pankaj Kumar Shankhdhar^{*1}, Dr. Pankaj Mishra², Dr. Pushpendra Kannoja³ and Rohit Kumar Bijauliya¹

Ph.D. Scholar¹, Professor and Principal³, BIU College of Pharmacy, Bareilly International University, Bareilly (U.P.)

Professor and Principal², Rohilkhand College of Pharmacy, Bareilly International University, Bareilly (U.P.)

Corresponding Author: Pankaj Kumar Shankhdhar, pankajsharmarbmi@gmail.com, Orchid id 0009-0007-7980-4763

ABSTRACT

Medicinal plants are a rich source of bioactive compounds with significant therapeutic potential, particularly as natural antioxidants. This study evaluated the phytochemical composition, antioxidant content, and antioxidant activity of methanolic and chloroform extracts of *Ficus glomerata* and *Aegle marmelos* leaves. The plants were collected, authenticated, shade-dried, powdered, and subjected to Soxhlet extraction using methanol and chloroform. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, reducing sugars, and phenolic compounds in both plant extracts, with methanol extracts generally exhibiting a richer profile. Antioxidant activity was assessed using DPPH radical scavenging, total flavonoid content, reducing power, and total polyphenolic content assays. Methanolic extracts showed higher DPPH scavenging activity with IC₅₀ values of 98.78 µg/mL (*F. glomerata*) and 101.23 µg/mL (*A. marmelos*), whereas chloroform extracts demonstrated slightly lower activity. Total flavonoid content ranged from 0.268–0.342 QE mg/100 mg, and reducing power and polyphenolic content were higher in methanolic extracts. These findings highlight the significant antioxidant potential of both plants, supporting their traditional use and potential development as natural antioxidant sources.

KEYWORDS: Medicinal plants, *Ficus glomerata*, *Aegle marmelos*, Anti-oxidant assay

INTRODUCTION

Medicinal plants have long been recognized as valuable sources of bioactive compounds with therapeutic potential. Among these, phytochemicals such as phenolics, flavonoids, tannins, alkaloids, and terpenoids play a crucial role in protecting the human body against oxidative stress. [1] Oxidative stress, caused by an imbalance between free radicals and antioxidant defense systems, is implicated in the pathogenesis of various chronic diseases including cancer, cardiovascular disorders, diabetes, and neurodegenerative conditions. [2-4] Consequently, there is growing scientific interest in exploring plant-based antioxidants as safer and effective alternatives to synthetic antioxidants. [5] *Ficus glomerata* and *Aegle marmelos* are well-known medicinal plants extensively used in traditional systems of medicine such as Ayurveda, Unani, and Siddha. *F. glomerata* is traditionally employed for the treatment of diabetes, inflammation, liver disorders, and gastrointestinal ailments, while *A. marmelos* is valued for its antidiarrheal, antimicrobial, hepatoprotective, and anti-inflammatory

properties. [6, 7] These plants are reported to be rich in diverse phytoconstituents, including polyphenols and flavonoids, which are known for their strong antioxidant potential. However, the type and concentration of these compounds may vary depending on the extraction solvent used. [8-10]

In this context, the present study focuses on the phytochemical investigation, antioxidant assay of *Ficus glomerata* and *Aegle marmelos* extracts prepared using methanol and chloroform solvents. Methanol is known to extract polar bioactive compounds, whereas chloroform extracts relatively non-polar constituents, allowing a comparative evaluation of antioxidant potential. Assessing and comparing these extracts will contribute to a better understanding of the antioxidant properties of these medicinal plants and support their potential use in the development of natural antioxidant formulations.

Collection and Authentication of plants: Leaves of *Ficus glomerata* and *Aegle marmelos* were collected from the local region of Bareilly District, Uttar Pradesh, India, in January 2022. The plants were authenticated by Dr. Alok Srivastava, Associate Professor, Department of Plant Science, MJPRU, Bareilly. A voucher specimen (MJPRU/PS/01/22/01) was deposited in the herbarium. The collected leaves were shade-dried at room temperature (25–30 °C), powdered using a dry grinder, and sieved through a 40-mesh sieve. [11]

Extraction: The leaves of *Ficus glomerata* and *Aegle marmelos* were collected separately, chopped with a knife, shade-dried, and powdered using a mechanical grinder. The powder was passed through sieve no. 40 and stored in labeled airtight containers. About 100 g of powdered leaves were extracted by Soxhlet extraction using 500 ml of chloroform and methanol for 24 hours. The obtained extracts were concentrated by evaporation to dryness. [12, 13]

Phytochemical Screening: The presence of primary and secondary metabolites in an extract is determined by primary assays called phytochemical screens. [14] To find alkaloids, flavonoids, saponins, tannins, steroids, reducing sugar, and phenols, a number of qualitative analyses. [15]

Antioxidant Assay

Hydrogen-Donating Assay: The antioxidant activity of the plant extracts was evaluated using the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) through a spectrophotometric assay. DPPH is a stable radical due to the delocalization of its unpaired electron over the molecule, preventing dimerization. It exhibits a deep violet color with a strong absorption maximum at 517 nm. [16] The antioxidant potential of the extracts was determined based on their ability to reduce DPPH by donating hydrogen atoms or electrons, resulting in discoloration of the purple DPPH solution. Ascorbic acid was used as a reference standard for comparison of antioxidant activity. A 0.3 mM DPPH solution was prepared in absolute ethanol, and plant extracts were also dissolved in ethanol. The extract solution was mixed with DPPH in a 1:3 ratio. A control solution containing DPPH, ethanol, and phosphate buffer was prepared simultaneously. After incubation in the dark for 30 minutes at room temperature, the absorbance of each sample was measured at 517 nm. [17] The percentage of DPPH radical scavenging activity was calculated at different concentrations of the extracts.

$$\text{Calculation of \% inhibition} = \frac{\text{Control absorbance} - \text{Test absorbance}}{\text{Control absorbance}} \times 100$$

Total Flavonoids Content: The total flavonoid content of the plant extracts was estimated using a colorimetric aluminium chloride assay. Quercetin was used as the standard compound for calibration. A stock solution was prepared by dissolving 10 mg of quercetin in 10 mL of methanol, from which standard solutions of 5, 10, 15, 20, and 25 µg/mL were prepared. Similarly, 10 mg of the dried plant extract was dissolved in 10 mL of methanol and filtered to obtain a clear solution. For flavonoid estimation, 3 mL of the extract solution (1 mg/mL) or standard solution was mixed with 1 mL of 2% aluminium chloride solution prepared in methanol. The reaction mixture was incubated at room temperature for 15 minutes to allow complex formation. The absorbance of the resulting yellow-

colored complex was measured at 420 nm using a UV–visible spectrophotometer. The total flavonoid content of the extracts was expressed as quercetin equivalents based on the calibration curve. [18]

Reducing Power Assay: The assay is based on the ability of antioxidants to reduce ferric (Fe^{3+}) ions to ferrous (Fe^{2+}) ions. Briefly, 1 mL of extract at different concentrations (5, 10, 15, 20, and 25 $\mu\text{g/mL}$) was mixed with 2.5 mL of phosphate buffer (200 mM, pH 6.6) and 2.5 mL of 1% potassium hexacyanoferrate. The reaction mixture was incubated at 50 °C for 20 minutes. After incubation, 2.5 mL of 10% trichloroacetic acid (TCA) was added to terminate the reaction, followed by centrifugation at 3000 rpm for 10 minutes. Subsequently, 2.5 mL of the supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride solution. The absorbance was measured at 700 nm using a UV–visible spectrophotometer. Ascorbic acid was used as the positive control, and the reducing power of the extracts was expressed as μg ascorbic acid equivalents per gram of sample ($\mu\text{g/g}$). [19]

Total Poly Phenols Content: The total phenolic content (TPC) of the plant extracts was determined using the Folin–Ciocalteu reagent method. Methanol was used as the blank, and gallic acid served as the standard. Standard solutions of gallic acid (5–25 $\mu\text{g/mL}$) were prepared in methanol. To the extract or standard solution, 1 mL of Folin–Ciocalteu’s phenol reagent was added and the mixture was allowed to react for 5 minutes. The Folin–Ciocalteu reagent, consisting of phosphotungstic and phosphomolybdic acids, undergoes reduction during the oxidation of phenolic compounds, forming blue-colored tungsten and molybdenum oxides. Subsequently, 10 mL of 7% sodium carbonate (Na_2CO_3) solution was added, and the final volume was adjusted to 25 mL with methanol. The reaction mixture was incubated at room temperature, and the absorbance was measured at 765 nm using a UV–visible spectrophotometer. All determinations for both gallic acid standards and plant extracts were performed in triplicate, and results were expressed as the mean of three readings. [20] A calibration curve was generated using gallic acid, and the phenolic content of the extracts was calculated from the calibration line. The results were expressed as milligrams of gallic acid equivalents per gram of dry weight of the sample (mg GAE/g).

RESULTS AND DISCUSSION

Extraction and Percentage Yield of Extract: Methanol and chloroform extracts of *Ficus glomerata* and *Aegle marmelos* leaves showed varying crude extract yields. Methanol produced the highest yield for *F. glomerata* (7.70%) and *A. marmelos* (6.23%), while chloroform yielded lower amounts, indicating the presence of solvent-specific phytoconstituents in the leaves.

Phytochemical Analysis of Crude Extract: Preliminary phytochemical screening of the powdered leaves of *Ficus glomerata* extracted with methanol and chloroform revealed the presence of various bioactive constituents, as summarized in Table 1. Alkaloids were prominently detected in both methanolic and chloroform extracts, as confirmed by Dragendorff’s and Wagner’s tests, indicating their broad solubility in both polar and non-polar solvents. However, Mayer’s and Hager’s tests showed positive results only in the methanolic extract, suggesting a higher abundance of alkaloids soluble in polar solvents.

Flavonoids exhibited varied responses depending on the test and solvent used. The methanolic extract showed positive results in Shinoda’s and alkaline reagent tests, whereas the chloroform extract responded positively to lead acetate and alkaline reagent tests. This variation indicates the presence of different classes of flavonoids with differing solubility profiles. Saponins were detected in both extracts by the foam test, although the haemolysis test was negative, suggesting low haemolytic activity. Tests for tannins and steroids were negative in both extracts, indicating their absence or presence in negligible amounts.

Reducing sugars were detected by the Molisch test in both extracts, while Fehling’s test was negative, suggesting the presence of non-reducing carbohydrates. Phenolic compounds were confirmed in both extracts through the ferric chloride test, with additional positivity in the chloroform extract by the lead acetate test. Overall, the methanolic extract exhibited a richer phytochemical profile compared to the chloroform extract, which may account for its higher antioxidant potential observed in subsequent assays.

Table 1: Data for the phytochemical screening of powdered leaves of methanolic and chloroform extract of *Ficus glomerata* Linn.

S. No.	Test to be performed	Result	
		Methanol	Chloroform
1.	Test for Alkaloids		
a	Dragendorff's Test	+ve	+ve
b	Wagner's test	+ve	+ve
c	Mayer's test	+ve	-ve
d	Hager's test	+ve	-ve
2.	Test for flavonoids		
a	Shinoda's Test.	+ve	-ve
b	Lead Acetate Test	-ve	+ve
c	Alkaline Reagent Test	+ve	+ve
3.	Test for Saponin		
a	Foam Test	+ve	+ve
b	Haemolysis Test	-ve	-ve
4.	Test For Tannin		
a	Gold Beater's Skin Test	-ve	-ve
b	Gelatin's Test	-ve	-ve
5.	Test for Steroids		
a	Liebermann Burchard's Test	-ve	-ve
b	Salkowski's Test	-ve	-ve
6.	Tests for Reducing Sugars		
a	Molisch test	+ve	+ve
b	Fehling test	-ve	-ve
7.	Test for Phenols		
a	Ferric chloride test.	+ve	+ve
b	Lead acetate test.	-ve	+ve
c	Gelatin test	-ve	-ve
d	Mayer's reagent test	+ve	-ve

Phytochemical screening of the powdered leaves of *Aegle marmelos* extracted with methanol and chloroform demonstrated the presence of diverse bioactive compounds, as presented in Table 2. Alkaloids were detected in both extracts by Dragendorff's and Wagner's tests, while Mayer's and Hager's tests were positive only in the methanolic extract, indicating a higher concentration of polar alkaloids. Flavonoids were present in both extracts, with the methanolic extract showing positivity in Shinoda's, lead acetate, and alkaline reagent tests, while the chloroform extract was positive in Shinoda's test only. This suggests the presence of flavonoid compounds with different solubility profiles. Saponins were detected in both extracts by foam and haemolysis tests, indicating their strong presence and potential bioactivity.

Tannins and steroids were also present in both methanolic and chloroform extracts, as indicated by positive results in all respective tests, highlighting the rich phytochemical composition of *A. marmelos*. Reducing sugars showed a variable pattern: the methanolic extract tested positive with Fehling's test but negative with Molisch, while the chloroform extract was positive with Molisch but negative with Fehling's test, suggesting different types of carbohydrates. Phenolic compounds were confirmed in both extracts by ferric chloride and Mayer's reagent tests, indicating antioxidant potential. Overall, the methanolic extract contained a broader range of phytochemicals, which may correlate with higher biological activities.

Table 2: Data for the phytochemical screening of powdered leaves of methanolic and chloroform extract of *Aegle marmelos* Linn.

S. No.	Test to be performed	Result	
		Methanol	Chloroform
1.	Test for Alkaloids		
a	Dragendorff's Test	+ve	+ve
b	Wagner's test	+ve	+ve
c	Mayer's test	+ve	-ve
d	Hager's test	+ve	-ve
2.	Test for flavonoids		
a	Shinoda's Test.	+ve	+ve
b	Lead Acetate Test	+ve	-ve
c	Alkaline Reagent Test	+ve	-ve
3.	Test for Saponin		
a	Foam Test	+ve	+ve
b	Haemolysis Test	+ve	+ve
4.	Test For Tannin		
a	Gold Beater's Skin Test	+ve	+ve
b	Gelatin's Test	+ve	+ve
5.	Test for Steroids		
a	Liebermann Burchard's Test	+ve	+ve
b	Salkowski's Test	+ve	+ve
6.	Tests for Reducing Sugars		
a	Molisch test	-ve	+ve
b	Fehling test	+ve	-ve
7.	Test for Phenols		
a	Ferric chloride test.	+ve	+ve
b	Lead acetate test.	+ve	-ve
c	Gelatin test	-ve	-ve
d	Mayer's reagent test	+ve	+ve

Antioxidant Assay

Hydrogen Donating Assay: Table 3 displays the extracts of *F. glomerata*'s percentage suppression of free radicals in comparison to normal ascorbic acid. In comparison to normal ascorbic acid, which has an IC₅₀ value of 22.75 µg/ml and 21.64 µg/ml, respectively, the methanol and chloroform extracts were found to have IC₅₀ values of 98.78 µg/ml and 86.65 µg/ml. The extracts showed the ability to donate protons, which could operate as scavengers or inhibitors of free radicals, potentially serving as powerful antioxidants. As a result, the methanol and chloroform extract of this plant has the greatest potential to scavenge DPPH radicals.

Table 3: Hydrogen donating assay of methanolic and chloroform extract of *Ficus glomerata*

Concentration in mg/ml	Absorbance at 517 nm (% Inhibition)			
	Methanol		Chloroform	
	Ascorbic acid	<i>F. glomerata</i>	Ascorbic acid	<i>F. glomerata</i>
5	25.03 ± 0.1	11.03 ± 0.2	23.56 ± 0.1	8.03 ± 0.12
10	30.03 ± 0.2	15.34 ± 0.1	27.98 ± 0.2	13.03 ± 0.22
15	38.85 ± 0.2	21.11 ± 0.2	34.12 ± 0.2	17.05 ± 0.21
20	45.54 ± 2.29	24.78 ± 0.1	39.27 ± 0.2	22.54 ± 0.19
25	51.99 ± 2.15	30.44 ± 0.2	46.43 ± 0.2	27.01 ± 0.09
IC ₅₀ (µg/ml)	22.75	98.78	21.64	85.65

Values are expressed as mean ± in standard deviation (N=6).

Table 4 displays the percentage reduction of free radicals caused by *Aegle marmelos* extracts in comparison to regular ascorbic acid. The IC₅₀ values of the methanol and chloroform extracts were found to be 101.23 µg/ml and 97.47 µg/ml, respectively, in contrast to the standard ascorbic acid concentrations of 20.32 µg/ml and 19.49 µg/ml. The extracts showed the ability to donate protons, which could operate as scavengers or inhibitors of free radicals, potentially serving as powerful antioxidants. Consequently, this plant's methanol extract has the highest capacity to scavenge DPPH radicals.

Table 4: Hydrogen donating assay of methanolic and chloroform extract of *Aegle marmelos*

Concentration in mg/ml	Absorbance at 517 nm (% Inhibition)			
	Methanol		Chloroform	
	Ascorbic acid	<i>A. marmelos</i>	Ascorbic acid	<i>A. marmelos</i>
5	31.04 ± 2.14	22.87 ± 3.26	28.31 ± 1.19	18.45 ± 1.10
10	36.45 ± 2.23	28.87 ± 2.15	31.45 ± 2.09	23.63 ± 2.17
15	42.91 ± 3.27	33.61 ± 2.26	35.75 ± 1.98	27.87 ± 1.87
20	50.54 ± 3.28	37.52 ± 2.17	42.83 ± 2.17	32.56 ± 1.43
25	58.34 ± 2.14	42.52 ± 1.99	49.82 ± 3.27	37.75 ± 1.78
IC ₅₀ (µg/ml)	20.32	101.23	97.47	19.49

Values are expressed as mean ± in standard deviation (N=6).

Total Flavonoid Content: *Ficus glomerata* methanol and chloroform extract's total flavonoid content was estimated using the colorimetric test using aluminium chloride technique. In terms of standard quercetin, the flavonoid concentration was determined to be 0.273 in methanol and 0.268 in chloroform extract QEmg/100 mg. The colorimetric assay by aluminium chloride technique was used to estimate the total flavonoid content of *Aegle marmelos* methanol and chloroform extract. In terms of standard quercetin, the flavonoid concentration was determined to be 0.289 in methanol and 0.342 in chloroform extract QEmg/100 mg.

Table 5: Total Flavonoids content

Plant	Total flavonoid content (QE mg/100 mg)	
	Methanol	Chloroform
<i>Ficus glomerata</i>	0.273	0.268
<i>Aegle marmelos</i>	0.289	0.342

Reducing Power Assay: By contributing an electron, the antioxidants in the sample would cause ferric cyanide Fe³⁺ to be reduced to ferro cyanide Fe²⁺. The production of Perl's Prussian blue at 700 nm can then be used to measure the quantity of Fe²⁺ complex. The results are displayed in Tables 6. The extract's reducing power was measured in µg ascorbic acid equivalent per gramme of extract.

Table 6: Reducing Power Assay

Plant	Reducing Power Assay (µg ascorbic acid/g extract)	
	Methanol	Chloroform
<i>Ficus glomerata</i>	217.65	215.82
<i>Aegle marmelos</i>	189.76	176.53

Total Polyphenolic Content: The Folin-Ciocalteu reagent was used to estimate the total phenolic content of the *Ficus glomerata* methanol and chloroform extract. When expressed in gallic acid equivalent, the polyphenolic content was shown to be 186.54 and 176.87 GAE mg/100 mg. The total phenolic content of standard gallic acid is shown in Table 7.

Table 7: Total Polyphenolic content

Plant	Total Polyphenolic content (GAE mg/100 mg)	
	Methanol	Chloroform
<i>Ficus glomerata</i>	186.54	176.87
<i>Aegle marmelos</i>	87.19	76.23

CONCLUSION

The study demonstrates that *Ficus glomerata* and *Aegle marmelos* leaves are rich sources of bioactive phytochemicals, including alkaloids, flavonoids, saponins, reducing sugars, and phenolic compounds. Methanolic extracts generally exhibited higher yields and a more diverse phytochemical profile compared to chloroform extracts, reflecting the solvent-dependent extraction of polar compounds. Antioxidant assays, including DPPH radical scavenging, reducing power, and total flavonoid and polyphenolic content, confirmed the significant antioxidant potential of both plants, with methanolic extracts showing superior activity. These findings validate the traditional medicinal use of *F. glomerata* and *A. marmelos* and suggest their potential application as natural antioxidant sources in the development of nutraceuticals and therapeutic agents. Further studies on isolation, characterization, and in vivo evaluation of individual bioactive compounds could provide deeper insights into their mechanisms and health benefits.

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