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Research Article

EXTRACTION AND CHARACTERIZATION OF *FICUS GLOMERATA* FOR ANTIOXIDANT ACTIVITY

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ABSTRACT

Objective: This study is focused on the evaluation of phytochemical constituents and antioxidant properties contained in *Ficus glomerata*. Various phytochemical constituents such as alkaloid, glycoside, total phenolic content, flavonoid, and carbohydrate constituent content were evaluated.

Methods: Extraction of plant *F. glomerata* was obtained using methanol as solvent using soxhlation. Antioxidant properties in *F. glomerata* extract were evaluated using free radical scavenging activity using 2, 2-diphenyl-1-picrylhydrazyl method. Antioxidant activity of the extract of *F. glomerata* was expressed as gallic acid equivalent.

Results: The result obtained from the study was in support of the use of *F. glomerata* as a natural antioxidant against free radicals that are formed in the pathogenesis of many metabolic disorders.

Conclusion: The results obtained from the research found to conclude that antioxidant properties derived from natural extracts may be a promising alternative to synthetic oxidants and may aid in avoiding the side effects associated with the use of synthetic antioxidants. Other than the antioxidant properties, a correlation was observed between the total phenolic content and the antioxidant properties.

Keywords: Free radicals, Phytochemicals, Karkatshringi, Antioxidants, *Ficus glomerata,* Reactive oxygen species.

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INTRODUCTION

Ficus glomerata Roxb. also known as *Ficus racemosa* belongs to the Moraceae family. It is a moderate to a large-sized plant found in the larger parts of India and Sri Lanka. These are also known as the Cluster Fig tree or Goolar (Gular). Cultivation of these plants is generally done for their edible fruit and barks that have astringent and antidiarrheal activity [1]. *F. glomerata* have a great value in Ayurveda and Unani systems due to their various therapeutic activity. Other than fruits and barks, their other parts are useful like root sap that possesses antidiabetic and antipyretic activity, alcoholic extracts of stem bark are used for antiprotozoal activity against *Entamoeba histolytica*. These plants are generally planted near temples and known to be a member of "ksirivrkas" the plants that are valuable to human health. *F. glomerata* consist of gall-like excrescences generally known as Karkatshringi in Sanskrit that is formed by insects like *Pauropsylla depressa* (Homoptera). This Karkatshringi is commonly used in Ayurveda, Unani, and Siddha for the treatment of respiratory and liver disorders along with cough and asthma [2]. Fruits of *F. glomerata* contain lupeol acetate, glycol, and glycol acetate. Other major constituents of aerial parts of plants are b-sitosterol, lupeol, and quercetin. The previous studies on the fruits of *F. glomerata* were found to show that the plant possesses significant activity toward gastric ulcers as seen in rats [3].

F. glomerata is 40–70 feet high evergreen tree having a young glabrous, pubescent shoot. Leaves of these species are 1–2.5 in long, glabrous in nature with 0.75 inch long stipules [4]. Recent research on *F. glomerata* has proved to possess activity against respiratory, cardiovascular, and central nervous system disorders. The plant also has a therapeutic activity for the treatment of cystitis and diabetes mellitus [4]. Ethanolic extract from *F. glomerata* has been reported to inhibit the growth of bacteria during *in vitro* study, namely, *Enterococcus faecalis*, *Shigella flexneri*, and *Staphylococcus saprophyticus* with a minimum inhibitory concentration ranging from 250 to 500 µg/ml [5].

Cellular metabolism produces highly reactive toxic species, known as reactive oxygen species (ROS). ROS are free radicals involving oxygen molecules that have proved to be causative reasons of many chronic diseases due to their oxidizing nature toward lipids, nucleic acids, and proteins [3]. Damage caused by these ROS is avoided by our normal body immune system to protect us from oxidative damageother than aging some other external unfavorable factors also leads to metabolic distress and thus induce formation of ROS in body which play a major role in pathophysiology of disease like diabetes, heart problem, acquired immunodeficiency syndrome, rheumatoid arthritis, cancer etc. [1]. To overcome this problem, external antioxidants are highly recommended, that might be natural or synthetic in nature. Synthetic antioxidants such as butylhydroxyanisole and butylhydroxytoluene having great antioxidant properties are now been not in use due to their toxic and carcinogenic effects. Therefore, considerable researches have been conducted toward the utilization of naturally occurring antioxidants that can prevent oxidative stress from ROS molecules. Our research is currently based on the persistence of antioxidant properties from the extracts of *F. glomerata* [6,7].

METHODS

Gallic acid, quercetin, L-ascorbic acid, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma-Aldrich Co. All the chemicals used were of analytical grade.

Collection and authentication of plant

The leaves of *F. glomerata* were collected locally during the month of February from Sambalpur (Odisha). Herbarium file of plant part was prepared and authenticated by Dr. A. K. Panigrahi (Professor, Department of Botany), G.M. University, Sambalpur (Odisha), and the specimen voucher no. assigned was 459/Bot/GMU/14.

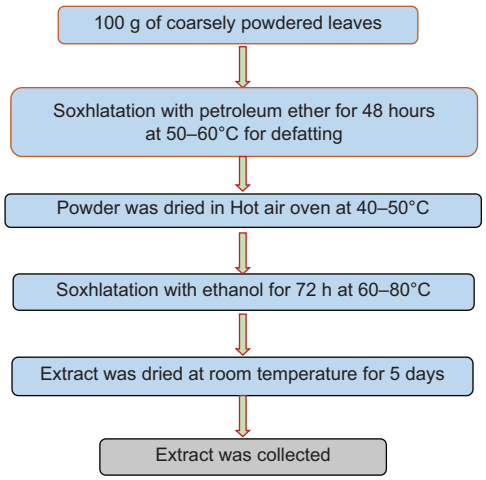
Drying and size reduction of plant material

The leaves of *F. glomerata* were dried under shade in the laboratory. It was pulverized into a coarse powder. To preserve uniformity, the coarse powder of leaves was sieved No.18 and stored in a cool, dry place as given in Fig 1.

Extraction of plant

Extraction of plants was continued by the approach of Soxhlation (Jensen, 1914). Soxhlet apparatus was used for the extraction using

ethyl alcohol and petroleum ether as solvent. A 100 g of coarsely dried powdered leaves were Soxhlet using petroleum ether for the principle of defatting for 48 h (Fig. 2). The obtained powder was dried under a hot air oven at 40–50°C and soxhlation was continued using ethyl alcohol at 60–80°C for 72 h. After extraction, petroleum ether was used for defatting of waxy materials. After completion of soxhlation, the extract was dried at room temperature for 5 days to obtain a dried extract.



Phytochemical analysis of crude extract

The obtained dried crude extract of *F. glomerata* was subjected to various qualitative and quantitative tests to detect the existence of common phytochemical constituents. All the chemicals and reagents used in phytochemical testing were of analytical grade.

Tests for alkaloids

Preparation of Dragendorff solution

Stock Dragendorff's solution was prepared according to a literature procedure [8]:

Solution A.

a) 8.0 g bismuth(III) nitrate was dissolved in 25 mL 25% nitric acid (bismuth solution);
b) 20 g potassium iodide was used to make a slurry in 1 mL 6 N HCl and 5 mL water (i.e., KI does not completely dissolve);
c) The bismuth solution was added to the slurry slowly while maintaining stirring;
d) The resulting solution was diluted with 100 mL water and any solid present was removed by filtration.

Dragendorff's stock solution: In a solution containing 20 mL water and 5 mL, 6 N HCl has added 2 mL of solution A, followed by 6 mL 6 N NaOH, and the resulting mixture was shaken. If residual bismuth hydroxide solid could not be dissolved, an additional 6 N HCl (typically 5–20 drops) were added until a yellow-orange translucent solution was obtained.

Dragendorff's test

In a pipette, 1 ml of ethanolic extract of sample and 1 ml of Dragendorff's reagent (potassium bismuth iodide solution) were added. An orange-red precipitate appeared which designated as the presence of alkaloids.

Wagner's test

Wagner's reagent was prepared by dissolving 1.27 g of iodine and 2 g of potassium iodide in 5 mL of water and volume was made up to 100 mL with distilled water.

With the help of a pipette, 1 ml of ethanolic extract of the sample was taken in a test tube and to it, 1 ml of Wagner's reagent (iodine potassium iodide solution) were added through the side of the test tube. Yellow-colored precipitate indicated the presence of alkaloids [9].

Tests for glycosides

Killer-Killani's test (for cardiac glycoside)

First, 2 ml of extract was dissolved in 1 ml of glacial acetic acid and then one drop of 5% $FeCl_3$ (ferric chloride solution) and conc. H_2SO_4 was added. The junction of the two liquid layers was reddish-brown, and the upper layer was bluish-green, indicating the presence of glycosides [10].

Foam test

A 0.5 g extract of the sample was taken and vigorously shaken with water. The arrangement of a layer of foam indicated the presence of glycoside.

Sulfuric acid test

A 2 ml of the extract was taken and added to a small quantity of 80% sulfuric acid to which a deep yellow color indicated the presence of glycoside.

Tests for carbohydrate

Iodine test

To 2 ml of extract added 1 drop of iodine solution. Formation dark blue-black/red or brown color was found indicated presence of sugars.

Molisch's test

A small fraction of the ethanolic extract was taken separately in ethanol and a few drops of a 20% w/v solution of α-naphthol in ethanol (90%) were added to it. After thoroughly shaking, 1 ml of concentrated sulfuric acid was carefully allowed to flow by the side of the test tube. A reddish-

Fig. 1: Coarsely powdered leaves of *Ficus glomerata*

Fig. 2: Soxhlet extraction of *Ficus glomerata* leaves

violet ring at the interface of the two layers revealed the presence of carbohydrates.

Test for proteins

To confirm the existence of proteins, Biuret test was conducted. A 1 ml of 40% Na0H solution was added to two drops of 1% CuSO4 solution till a blue color was produced, and then, it was further added to the 1 ml of the ethanolic extract. The formation of pinkish or purple-violet color indicated the presence of proteins.

Tests for saponins

A little fraction from the extract was boiled with about 1 ml of distilled water and shaken. The appearance of a characteristic foam formation indicated the presence of saponins. The aqueous and alcoholic extracts were tested directly.

Tests for flavonoids

Alkaline reagent test

To the extract add a few drops of sodium hydroxide solution, intense yellow color is formed which turns colorless on the addition of a few drops of dilute acid indicating the presence of Flavonoids.

Quantitative estimation

Estimation of total polyphenol content (TPC)

The TPC of the extract was estimated using the Folin–Ciocalteu reagent-based assay. A 5–50 µg/ml methanolic gallic acid solutions were used as standards and methanol was used as a blank. The mixture was administered with 1 ml of Folin–Ciocalteu's phenol reagent was shaken for 5 min. The Folin–Ciocalteu reagent is a phosphotungstic ($H_3PW_{12}O_{40}$) and phosphomolybdic ($H_3PMO12O_{40}$) acid mixture that is reduced to blue tungsten (W_8O_{23}) and molybdenum (Mo_8O_{23}) oxides during polyphenol oxidation [11]. To the above-obtained mixture, 10 ml of 7% Na_2CO_2 solution was added and the volume was diluted up to 25 ml with methanol. The absorbance of the developed color was recorded at 765 nm using a UV–Vis spectrophotometer. All gallic acid and plant extract determinations were performed in triplicate. The data are represented as the mean of the three determinations. A calibrated gallic acid standard curve was created using these readings. Based on the measured absorbance of the plant extract, the concentration of phenolic was estimated (µg/ml) from the calibration line. The content of polyphenols in the extract was calculated and expressed in terms of gallic acid equivalent (mg of GAE/g of dry weight material) [12].

Fig. 3: Reagents and chemicals used in phytochemical screening

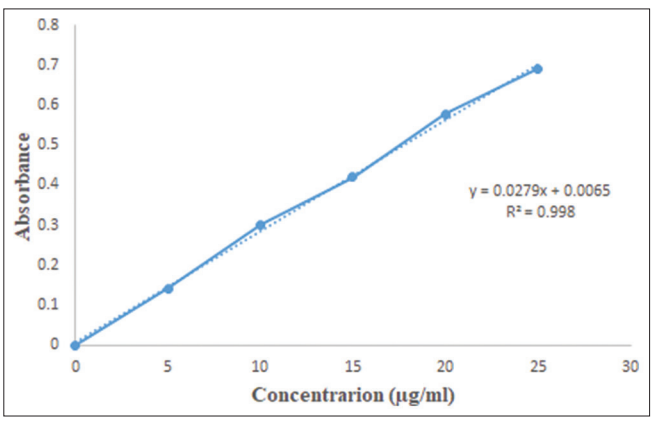


Fig. 4: Total phenolic content for standard gallic acid

Estimation of total flavonoids content

Total flavonoid content estimation was done with the help of a colorimetric assay based on the aluminum chloride method. The 10 mg quercetin was dissolved in 10 mL of methanol, and aliquots of 5, 10, 15, 20, and 25 g/ml of methanol were prepared. In addition, 10 mg of dried extract was dissolved in 10 mL of methanol and filtered. A 3 ml (1 mg/ml) of this solution was used for the evaluation of flavonoids. Furthermore, 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or normal and allowed to stand at room temperature for 15 min. At 420 nm, absorption was measured [12].

Antioxidant activity by DPPH method

A spectrophotometric assay was performed to determine the antioxidant property in the extract using the stable radical, DPPH as a reagent. Because of the delocalization of the spare electron over the entire molecule, DPPH acts as a stable free radical, and the molecules do not dimerize, as most other free radicals. DPPH solution possesses purple color, characterized by absorption at 520 nm. The hydrogen atom or electron-donating abilities of the compounds and some untainted compounds can be measured from the bleaching of the purple-colored ethanol solution of DPPH [11].

The antioxidant properties of the extract were assayed with standard solutions of ascorbic acid. Solution of plant extract was made in

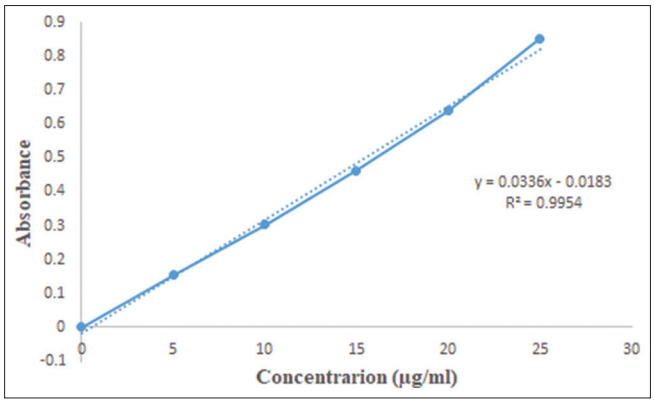


Fig. 5: Total flavonoid content for standard quercetin

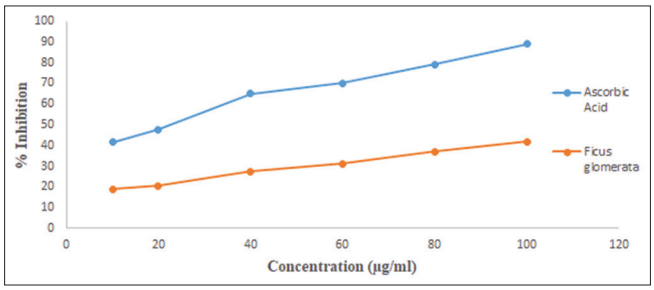


Fig. 6: Antioxidant estimation by DPPH of *Ficus glomerata*

ethanol and 0.3 mM solution of DPPH was made in 100% ethanol. DPPH and ethanolic solution of extract were mixed in a 1:3 ratio. A control reaction mixture was made with DPPH and blank ethanol solvent but with standard phosphate buffer solution. After allowing the reaction to continue for 30 min, their absorbance was observed at 517 nm to determine the % scavenging activity at different concentrations.

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

RESULTS AND DISCUSSION

Phytochemical analysis of crude extract

Various phytochemical analyses performed to determine the presence of various biological components in the extract of *F. glomerata* proved the presence of components such as alkaloids, glycosides, steroids, phenolic compounds, and flavonoids, and absence of components like carbohydrate was found, as shown in Table 1.

Table 1: Qualitative analysis of *Ficus glomerata* ethanolic extract of the presence of different phytoconstituents

Test	Observation	Inference
Alkaloid		
Wagner's reagent	Reddish-brown ppt	+ve
Dragendorff's reagent	Reddish-brown ppt	+ve
Mayer's reagent	Cream color ppt	−ve
Glycoside		
Killer-Killani test	The appearance of reddish-brown-colored ring at the junction of two layers	+ve
Conc. sulfuric acid test	Reddish color precipitate	+ve
Molisch's test	Formation of the reddish-purple-colored ring at the junction of two layers.	−ve
Steroid		
Salkowski test	Brown or red-colored ring on the sulfuric acid layer given the confirmatory test.	−ve
Liebermann–Burchard's test	Translucent green color was given the confirmatory test.	−ve
Carbohydrates		
Molisch's test	The presence of carbohydrates is indicated by the formation of a red-violet ring at the solution's junction and its disappearance on the addition of excess alkali solution.	−ve
Benedict's test	The amount and color of the precipitate produced varied depending on the concentration of the reducing sugar. Benedict's test results that are positive appear green, yellow, orange, or red.	−ve
Phenolic compounds		
Ferric chloride test	The formation of blue, green, or violet color indicates the presence of phenolic compounds.	+ve
Lead acetate test	The formation of a white precipitate indicates the presence of phenolic	+ve
Flavonoids test		
Alkaline reagent test	Yellow color is formed which turns colorless with the addition of a few drops of dilute acid indicating the presence of flavonoids.	+ve

Quantitative estimation of TPC

Total phenolic content estimation was performed using the Folin–Ciocalteu reagent. The polyphenolic content was expressed in the terms of gallic acid equivalent and was found to be 0.185 GAE mg/100 mg Fig 4. describes the graph plotted between absorbance vs concentration with data given in Table 2 describes the total phenolic content for standard gallic acid.

Quantitative estimation of flavonoid content

Total flavonoid content estimation was performed using colorimetric assay by aluminum chloride method. Flavonoid content was expressed in terms of standard quercetin and was found to be 0.274 QE mg/100 mg. The graph in Fig. 5 describes the total flavonoid content for standard quercetin as data provided in Table 3.

Antioxidant activity

The antioxidant activity of extract from *F. glomerata* was determined using the DPPH method. The antioxidant property is expressed as the % inhibition of the free radicals present in DPPH which was used as a reagent. Percentage inhibition of free radicals by the extracts of *F. glomerata* as compared with standard ascorbic acid is shown in Table 4. It was observed that the methanol extract showed an IC_{50} value of 130.99 µg/ml as compared to standard ascorbic acid 21.74 µg/ml. The graph in Fig 6 describes the antioxidant property by DPPH in *Ficus glomerata* with

Table 2: Estimation of total phenolic content

Concentration µg/ml	Absorbance
0	0
5	0.142±0.001
10	0.301±0.003
15	0.419±0.002
20	0.578±0.002
25	0.691±0.003

Table 3: Estimation of total flavonoid content

Concentration, µg/ml	Absorbance
0	0
5	0.153±0.002
10	0.303±0.003
15	0.463±0.002
20	0.638±0.003
25	0.853±0.001

Table 4: Estimation of total phenols and flavonoid content

Extract	Total phenols content (GAE mg/100 mg)	Total flavonoid content (QE mg/100 mg)
Hydroalcoholic extract of *Ficus glomerata*	0.185	0.274

Table 5: Antioxidant estimation by DPPH of *Ficus glomerata*

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	*Ficus glomerata*
1.	10	41.53	18.86
2.	20	47.65	20.43
3.	40	64.94	27.50
4.	60	70.10	31.23
5.	80	79.23	36.93
6.	100	88.91	41.84
	IC_{50}	21.74	130.99

data provided in Table 5. The extracts demonstrated proton-donating capacity, which might function as free radical inhibitors or scavengers, perhaps acting as major antioxidants. As a result, the methanol extract of this plant has the greatest potential to scavenge DPPH radicals.

CONCLUSION

Antioxidant properties obtained from natural extracts can be a promising alternative toward the use of synthetic oxidants and could help in avoiding the side effects that occur due to the use of synthetic antioxidants. The data obtained from this research prove to support the view that alcoholic extract obtained from *F. glomerata* showed effective free radical scavenging activity. Besides antioxidant properties found in extracts of *F. glomerata*, a correlation can be observed between total phenolic content and flavonoid content with that of antioxidant activity. Further, the result obtained in this research supports the use of an alcoholic extract of *F. glomerata* as a preventive agent in the pathogenesis of some metabolic disorders. Proceeding further with more extensive research on chemical composition and understanding of the mechanism of action could help in the development of the drug for its therapeutic application.

AUTHORS' CONTRIBUTIONS

All the authors have equally contributed toward the completion of the paper, including data collection, and compilation of data.

CONFLICTS OF INTEREST

Authors have none to declare.

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