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Research Article

Evaluation of Total Phenolic Content and Free Radical Scavenging Activity of *Ficus glomerata* Roxb.

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ABSTRACT

In present study attempt has been made to characterize the antioxidant and phenolic content of the traditional medicinal plant *Ficus glomerata*. Fruit powder was subjected to extraction with methanol, concentrated and lyophilized. Different concentrations of extracts evaluated for their invitro antioxidant potentials using DPPH, ABTS, FRAP, NBTS, TAA tests and total phenolic content also estimated. Total phenolic content observed in this study ranges from 76.77 to 351.45 mg Gallic acid equivalents (GAE)/g. In free radical scavenging assays, Inhibitory Concentration (IC_{50}) of extract ranging from 7.23 mg/ml to 35.08 mg/ml. Correlation studies between phenolic content and free radical scavenging activity and total antioxidants shows the plant phenolics role in their antioxidant capacities. Results indicated that the methanolic extract of *F. glomerata* fruit is highly effective against free radicals due to rich source of phenolics.

Keywords: *Ficus glomerata*, Fruits, Free radicals, Total Phenolic content, DPPH, Total antioxidants.

INTRODUCTION

Production of oxygen free radicals in the biological system, leads to the functional and structural damages to the cell. Free radicals are the key factors in cancer, aging, immunosuppression, inflammation, ischemic heart disease and neurodegenerative disorders[1, 2]. In biological system, antioxidants such as phenolic compounds and vitamins are involved in promoting health also prevent aging and chronic diseases[3]. Phenolic compounds synthesized primarily from products of the Shikimic acid pathway, have several important roles in plants[4]. Ethno-medical literature contains a large number of plants those can be used against free radical mediated diseases[5]. The undesirable effects of synthetic antioxidants lead to their limited use in treating human diseases. Hence, there is much attraction is drawn towards the natural antioxidants from plant origin, which could protect cellular machinery from free radicals[6]. Plants of Moraceae family are rich sources of Phenolics, Terpinoids and Carbohydrates of interest[7]. Number of plants from Moraceae family had been used in traditional system of medicine. Literature survey has indicated that the study on Ficus spp. on bark, leaves, latex is more while the ripe fruits are rarely studied. Medicinal effect of *F. glomerata* fruits in treating wide variety of human diseases mentioned in traditional practices[8].

Due to the diversity of chemical structure and synergetic effects among different antioxidants found in food; it is difficult to relay on a single antioxidant assay to estimate the antioxidant capacity[9]. Several invitro assays have been frequently used to estimate antioxidant capacities in fresh fruits and vegetables and their products for clinical studies. 2, 2- diphenyl-1-picrylhydrazyl (DPPH) , 2, 2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS), ferric reducing antioxidant power (FRAP), NBTS, Metal chelating activity(MCA) and total antioxidant assays are said to be more relevant because, they utilizes a biologically relevant radical source[10]. Significant correlation regression between total phenolics and antioxidant activity supports the antioxidant potentiality of the bioactive drug.

The aim of this study was to evaluate the free radical scavenging activity by using different assays and to evaluate the correlation between total phenolic content and total antioxidant activity of fig fruit extract.

MATERIALS AND METHODS

Plant material: 500gm of Fruits were collected from Gulbarga region and authenticated by Department of Botany, Gulbarga University. Fruits were washed thoroughly and dried at room temperature.

Extraction: The fruit samples were ground to fine powder and passed through a sieve. The ground samples were dried at room temperature. 10g of the powdered sample was extracted with 100 ml of water at 80^0C for 30 min in a water bath shaker. After cooling, the extract was centrifuged at 5,000 rpm for 10 min and filtered. The filtrate was stored at 4^0c for further use.

Chemicals: 1,1-diphenyl-2picryhydrazyl(DPPH), trichloroaceticacid(TAC), ethylene diamine tetraacetic acid(EDTA), potassium ferricynide, ferricchloride, ferrozine, nitrobluetetrazolium(NBT), phenazine methosulfate(PMS), nicotinamide adenine dinucleotide reduced(NADH), Gallic acid were obtained from Hi-Media Laboratories, Mumbai , India. All other Chemicals

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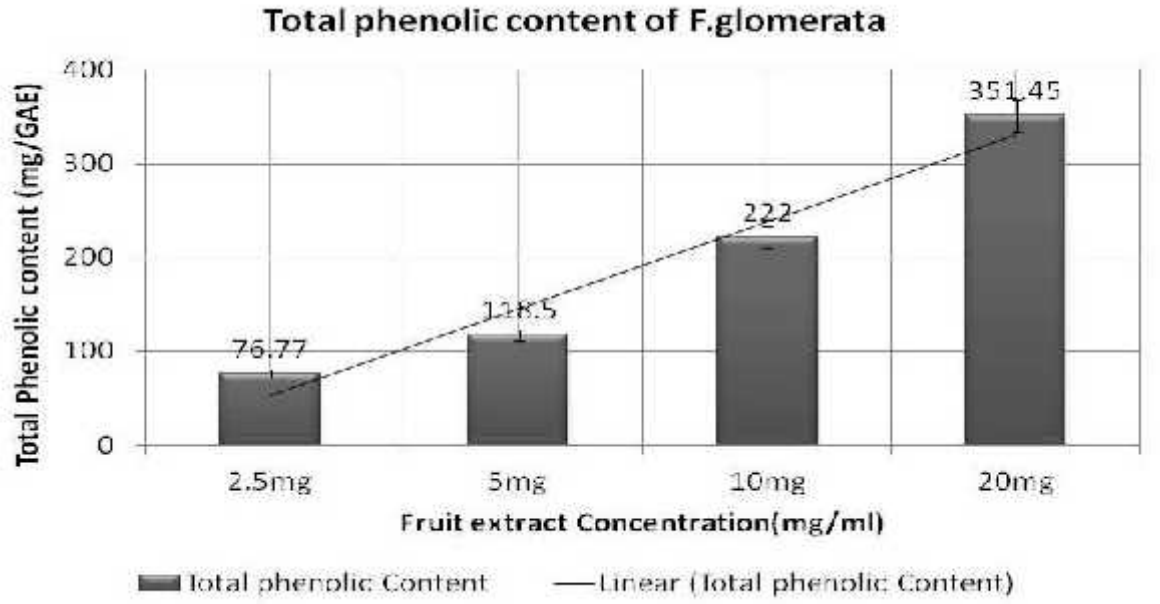

Figure 1: Total Phenolic content of F. glomerata fruit extract at different concentrations

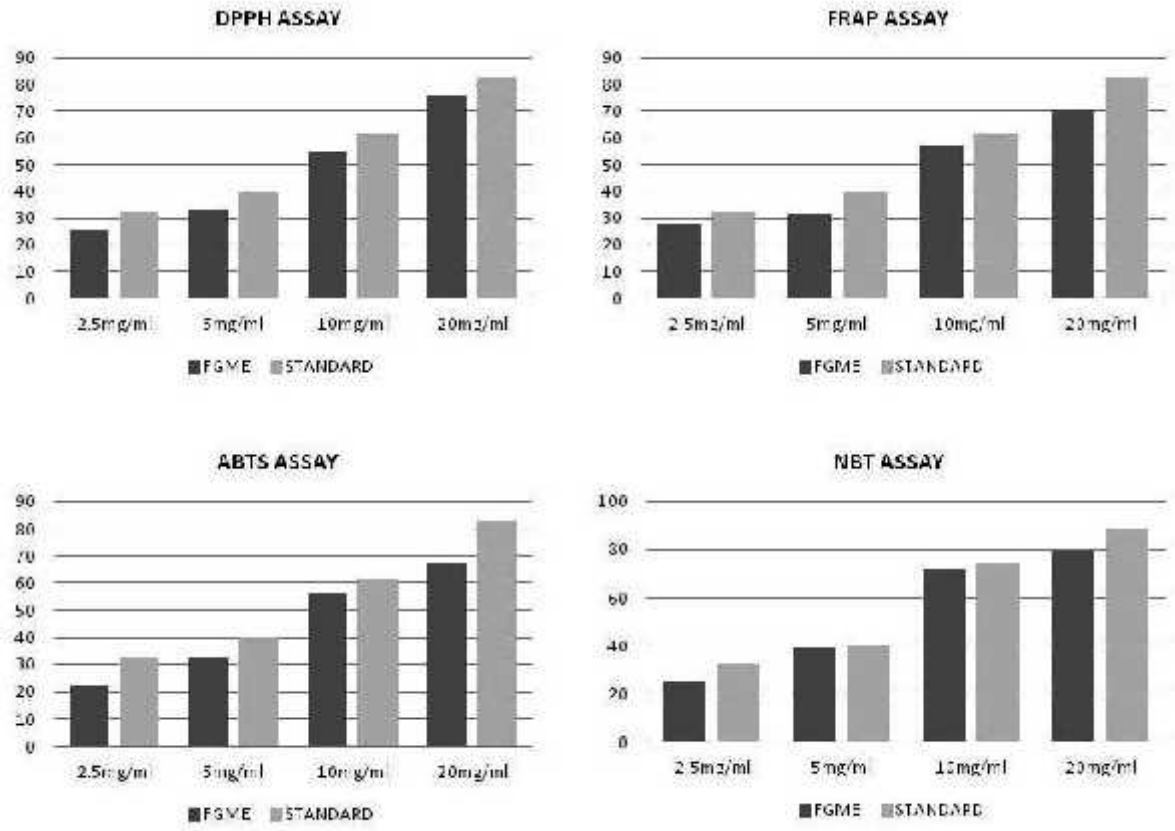

Figure 2: Free radical scavenging activities of F. glomerata fruit extracts

and Solvents used were of high purity and of analytical grade marketed by Sigma Aldrich, Mumbai, India.

Estimation of total Phenolic Content: The total phenolic content of the extracts was determined by the Folin-Ciocalteau method described by A Bleniski[11]. 1gm/10 ml of sample was filtered with whatman no.1 paper. 0.5 ml of the sample was added to 2.5 ml of 0.2 N Folin-Ciocalteau reagent and placed for 5 minutes. 2 ml of 75 g/l of Na2CO3 were then added and the total volume made up to 25 ml using distilled water. The above solution was then kept for incubation at room temperature for 2 hours. Absorbance was measured at 760 nm using 1 cm cuvette in UV-VIS spectrophotometer. Gallic acid (0 - 800 mg/L) was used to produce standard calibration

Table 1: Antioxidant activity (IC_{50} Value) of *F. glomerata* extract

Test	IC50 value (mg/ml)
DPPH	7.23
ABTS	9.89
FRAP	16.49
NBTS	35.08
MCA	11.38

curve. The total phenolic content was expressed in mg of Gallic acid equivalents (GAE) / g of extract.

Invitro Antioxidant Studies: DPPH Radical Scavenging Assay: The free radical scavenging activity of *Ficus glomerata* methanolic extracts was measured *in vitro* by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay[12]. 0.3mM solution of DPPH in 80% methanol was prepared and 1ml of this solution was added to 3 ml of the extract dissolved in methanol at different concentrations (2.5-20 mg/ml). The mixture was shaken and allowed to stand at room temperature for 30 min under dark conditions and the absorbance was measured 517 nm using a spectrophotometer. The percentage of scavenging activity at different concentrations was determined and the IC_{50} values of the extracts were compared with that of ascorbic acid, which was used as the standard. DPPH radical-scavenging activity was calculated according to the following equation:

% Inhibition = ((A0 – A1) / A0 × 100)

Where A0 was the absorbance of the control (without extract) and A1 was the absorbance in the presence of the extract.

ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging activity: ABTS radical scavenging activity was estimated[13]. The stock solutions included 7 mM ABTS solution and 2.4 mM potassium persulphate solution. The working solution was prepared by mixing the two stock solutions in equal quantities and allowing them to react for overnight at room temperature in the dark. The solution was diluted by mixing 1 ml of ABTS solution with 60 ml ethanol. Different concentrations (2.5–20mg) of the extracts (1 ml) were allowed to react with 1 ml of the ABTS solution and the absorbance was measured at 734 nm after 7 min using a UV-Visible Spectrophotometer.

FRAP (Ferric reducing ability of plasma) Assay: Reducing power of the plant extracts was determined[14]. Briefly, different concentrations of extracts (2.5mg/ml–20mg/ml) were added 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricynide (1%). The reaction mixture was allowed to incubate at 50°C for 20 min. Then 2.5 ml of trichloroaceticacid acid (10%) was added to the reaction mixture, which was then centrifuged at 9500 rpm for 10 min. The upper layer of solution (2.5 ml) was recovered and mixed with 2.5 ml distilled water and 2.5 ml FeCl3 (0.1%). The absorbance was recorded at 700 nm in a spectrophotometer. An increase in the absorbance of reaction mixture indicated the increased reducing power.

NBT (Nitro blue tetrazolium) superoxide radical scavenging assay: The scavenging activity of the plant extracts towards superoxide anion radicals was measured[15,16]. The superoxide anion was generated in 3ml of Tris-HCL buffer (100 mM, pH 7.4) containing 750µl of NADH (936µM) solution and 300µl of different concentrations (10–100µgml−1) of extracts. L-ascorbic acid was used as positive control. The reaction was initiated by adding 750µl of PMS (120µM) to the mixture. After 5 min of incubation at the room temperature, the absorbance was measured at 560 nm. The percent NBT decolourization of the sample was calculated.

Total antioxidant activity: Total antioxidant activity was measured[17,18]. In this method, different concentration (2.5mg–20 mg/ml) of extract in acetic acid was combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction mixture were incubated at 95^0C for 90 min and cooled to room temperature. Then the absorbance was measured at 695 nm using single beam UV-visible spectrophotometer against blank. The antioxidant activity was expressed as the number of equivalents of ascorbic acid.

Correlation Study: Relationship between phenolic content, radical scavenging and total antioxidant was analyzed using correlation studies.

RESULTS

Total Phenolic content: Total phenolic content of Ficus glomerata fruit extract is reported in Fig.1. The amount of phenolics varied from 76.77mg/GAE to 351.45 mg/GAE in different concentration of extracts (2.5mg-20mg).

Results of the free radical scavenging activities of FGME are depicted in Fig.2. The different concentrations of extract exhibited the antioxidant potentiality in all the assays. Percentage of inhibition of free radical scavenging activity ranges from DPPH (25-76%), ABTS (22-67%), FRAP (29-70%), NBTS (27-77%), MCA (25-79%) and it is lesser than standard antioxidant Ascorbic acid.IC50 values of plant extract with respect to DPPH,ABTS,FRAP,NBTS and is shown in Table.1.

Total antioxidant activity (TAA): Total antioxidant capacity of different concentrations of F. *glomerata* fruit extract has shown in Fig .4. TAA of plant extract ranging from 15% to 78% while standard drug Ascorbic acids total antioxidant activity is ranging from 27% to 97%. The correlation between total phenolics and total antioxidants is determined by square regression co-efficient and found significant (r^2=0.88) and which is shown in Fig.5

Correlation exhibited positive and strong correlation of TPC with DPPH (r = 0.98), ABTS (r = 0.99) and TAC (r = 0.95). Correlation analysis also shows positive and strong correlation of DPPH with ABTS (r = 0.99) and TAC (r = 0.98); ABTS with TAC (r = 0.97). The findings suggest strong involvement of phenolics in the antioxidant activity of FGME. The high degree of correlation in the simple spectrophotometric assay showed that it would be a useful technique for rapid evaluation of antioxidant activity in this plant**.**

DISCUSSION

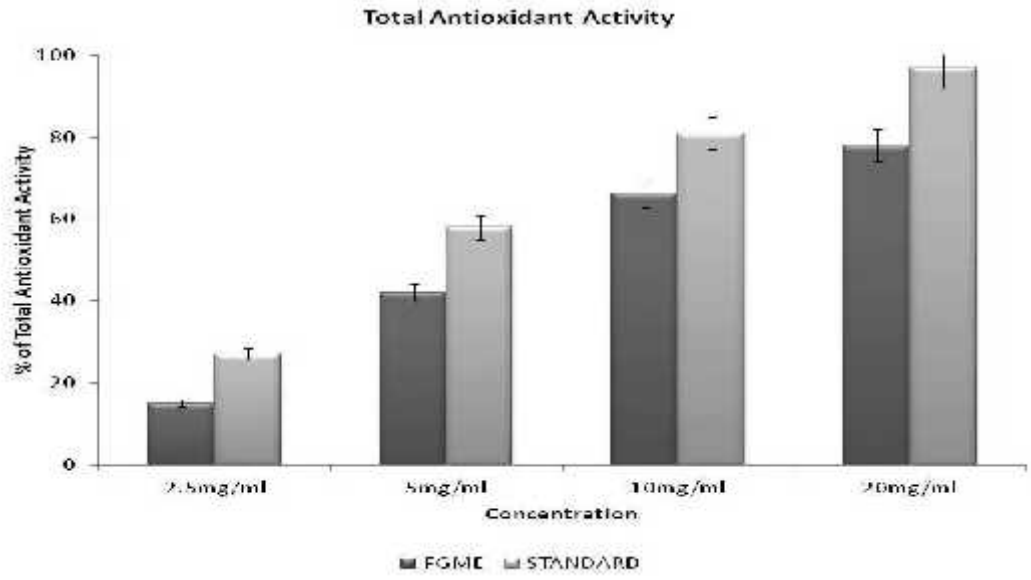

Figure 3: Total Antioxidant activity of F. glomerata extract and Standard

Table 2: Correlation between TPC, DPPH and TA of *F. glomerata* fruits.

	TPC	DPPH	ABTS	TAC
TPC	1			
DPPH	0.98	1		
ABTS	0.99	0.99	1	
TAC	0.95	0.98	0.97	1

The antioxidant activity of plant origin components can be due to the presence of phenolic compounds[19]. Phenolics are aromatic secondary plant metabolites extensively distributed throughout the plant kingdom and associated with colour, sensory qualities, nutritional and antioxidant properties of food[20]. The results of the present experiment indicates that the TP contents of different concentrations of methanol extracts was ranging from, 76.77 mg/GAE to 351.45 mg/GAE. Similar studies have reported[21,22,23] the existence of positive linear relationship between antioxidant activity and TP content in many spices and herbs. The antioxidant activity of phenolic compounds is essentially due to redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, hydroxyl radical quenchers and heavy metal chelators[24]. Further research is underway to determine which component plays the major role. The total antioxidant capacity of *F. glomerata* was measured and compared with that of BHT. The results of above experiment showed that the extract had significant concentration dependent antioxidant activities when compared with that of BHT. Reactive oxygen species (ROS) encompass a spectrum of diverse chemical species including superoxide anions, hydrogen peroxide, hydroxyl radicals, nitric oxide and others. In vivo studies showed that oxidants play a variety of role in both animals and plants[25]. ROS are involved in cellular signaling, cell growth regulation, specific cellular physiology, and energy production. However, the oxidation of lipids, DNA, protein, and carbohydrate by toxic ROS and cause DNA mutation also damage target cells or tissues which results in cellular senescence and death[26]. Recent, studies implies that the knowledge and application of antioxidants in reducing oxidative stress at *in vivo* condition has promoted many investigators to search for potent natural antioxidants from plant sources[27]. Various techniques have been used to determine the antioxidant activity *in vitro* in order to allow rapid screening of substances.

In the present study, it is reported that the methanol extracts of *F. glomerata* fruits have the ability to display antioxidant properties in a dose dependent manner, which is evaluated by various *in vitro* assays. Radical scavenging activity of *Ficus glomerata* resolved in the study using various test systems shows that the extract and its active substances could be promising for the further studies. Reveals that fruits and vegetables have high values of important nutrients and phytochemicals which exhibit antioxidant functions[28]. In the above-mentioned test systems in comparison can provide the important information for development of plant extracts and assessment of their antioxidant properties. In present study *in vitro* antioxidant tests reveals that extracts had poor activity against the formation of hydroxyl radicals a harmful radical which is formed during *in vivo* studies, but these are more potent in decreasing the concentration of nitrite after the spontaneous decomposition of sodium nitroprusside it indicates that the *F. glomerata* extracts may be able to scavenge nitric oxide. The scavenging effect on DPPH also provides a strong antioxidant effect for the extracts, and this activity may be attributed to their capacity of trapping free radicals by donating a hydrogen atom.

CONCLUSION

In this study *in vitro* antioxidant activities of *Ficus glomerata* methanolic extract have been evaluated. Methanolic extract at different concentration has expressed a considerable amount of phenolic content and exhibited significant free radical scavenging antioxidant activities. This activity of *Ficus glomerata* may be due to presence of high amount of phenolic content in fruits. From the above observations we can draw the conclusion that the *Ficus glomerata* has not only the more phenolic components in it but the extract also has a good free radical and reactive oxygen species scavenging activities.

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