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Phytochemical and antioxidant activity of *Ficus glomerata* ethanolic leaf extract

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Abstract
The present study is to design new attempt on *ficus glomerata leaves* one of important medicinal sources. *Ficus glomerata* is a species of plant in the moraceace family popularly known as the cluster fig tree present in all over India has been recommended for the treatment of diarrhea diabetes, hypertension, Cancer, gastric ulcer, wound healing etc. The leaves of *Ficus glomerata* was collected, washed with saline water, shade dried room temperature for 20 days. The dried plant material was pulverized in to fine powder using a grinder (mixer) About 9gm of leaf powder was extracted in soxhlet apparatous 50° with ethanol. Crude extract thus obtained is subjected to column chromatography for purification of principle compound. The individual fractions were subjected to qualitative chemical investigation for the identification of the type of active principles. Hexane and ethanolic extrct (1:5) showed the presence of carbohydrate, saponin, phytosterol, protein, amino acid, tannin, phenolic compounds and flavonoids. The quantification of total alkaloid, total flavanoid, total tannin and total phenol has been identified from the leaves of plant crude extract and column purified samples by spectrophotometer. The present study deals with the standardization of the plant leaves on the basis of various phytochemical and antioxidant parameters. The column purified extract enhanced free radical scavenging activity of stable radical 2,2-diphenyl-1-picryl-hydrazyl (DPPH), SO &ABTS in *In-vitro* assay methods. The results of the study indicate that the ethanolic extract of *ficus glomerata* possess significant antioxidant activity, evidentially proves to be a powerful pharmacological agent.

Keywords: *Ficus glomerata* pytochemical antioxidant activity

Introduction
The plant kingdom is a virtual gold mine of potential drug and other active molecules waiting to be discovered. It has been estimated that only 10-15% of the 7,50,000 existing species of higher plants have been surveyed for biologically active compounds. Approximately one-third of the top-selling drugs in the world are natural products or their derivatives often with ethno-pharmacological background (Dnyaneshwar, 2010). Plants contain several phytochemical, which possess strong antioxidant activities. Phytochemicals are a rich source of antioxidants such as polypenols and flavanoids, which can delay or inhibit the oxidation of biomolecules by regulation of oxidative chain reactions (Aray v & yadav s 2011) [5]. The (ROS) Reactive oxygen species are free radicals wich include superoxide, Hydroxl raical, Hydrogen peroxide. Reactive oxygen species have been implicated more than 100 diseases, including malaria, acquired immune deficiency syndrome, heart disease, Diabetes mellitus, Obesity & stroke. The antioxidant from natural resources has received much attention and efforts have been put in to identify compound. The antioxidants may cause and cure cancer and other diseases by the protecting the cells from damage caused by 'free radicals'- the highly reactive oxygen compounds (American cancer society, 2006) [2]. *Ficus glomerata* is a species of plant in the moraceace family, popularly known as the cluster fig tree or country fig goolar [Gular] this is native to Australia, South East Asia and the Indian sub-continent (Jagtap Supriya *et al*., 2012) [12]**.** The genus *ficus glomerata* have been showed immense medicinal value (Anita *et al*., 2011) [4]. *Ficus glomerata*, medicinally it has been various pharmacological activities astringent antidiabetic, refrigerant, antiasthmatic, anti-inflammatory, hepatoprotective antioxidant, anti-ulcer, antipyretic, antidiuritics, anti hyperglycimic, anti-diarrheal**,** (Jagtapsupriya *et al*., and 2011). The leaves are good wash for wounds and ulcers. They are useful in dysentery and diarrhea. The infusion of bark and leaves is also employed as mouth was to spongy gums and internally in dysentery, menorrhagia, effective remedy in glandular swelling, chronic wounds, cervical adenitis and haemoptysis. The extract of bark, leaves and fruits are used as antitumor, anticancer and as an antimicrobial agent. (Kambali *et al*., 2014) [13]. Nutritional studies include quantitative estimation of crude protein, lipids, ascorbicacid, phenols, anthocyanin, lycopene, carotenoids, chlorophyll, carbohydrate, starch, reducing and

non-reducing sugars, crude fat and crude fiber in fresh as well as dry tissues, minerals. (Bhogaonkar *et al*., 2014) [6]. Antioxidants-based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer (Devasagayam *et al*., 2004) [8]. Therefore**,** in recent years, considerable attention has been directed towards the identification of plants with antioxidant ability.

Fig 1: *Ficus glomerata*

Materials and Methods

Collection of plant materials

The fresh leaves of *Ficus glomerata* were collected in the month of Auguest (2016) from pulugandiur (Vill), krishnagiri (Dt).

Processing of plant Extract

The leaves of *Ficus glomerata* were collected from natural habitat and Washed thoroughly with saline water and shade dried. The dried leaves is uniformly using mechanical grinder to make fine powder. Then the coarse powder 9g was extracted with 150ml ethanol in a hotsoxhlet apparatus for 48 hours. The ethanolic extracts were then distilled evaporated and dried. The crude extracts thus obtained are subjected to column chromatography.

Column chromatography for isolation & purification

Column chromatography is commonly used purification technique isolation of pure, phamocologically active constituents from plants remain a long and tedious process. It is necessary to have methods available for efficient separation from plant extracts, to isolate the purify compound. The column chromatography (length: 450mm; Bore: 30mm) was performed using 60-120 mesh silica gel to elute out individual components from the crude plant extract. The column was rinsed with hexane and completely dried before use. The column was filled 3/4th with mixture of solvents ratio (1:2:0.5) (Distilled water: Acetic acid: Chloroform) and 20g of silica gel was packed 2/3rd of the column length with simultaneous draining of the solvent to aid proper packing. The packing was performed after activating the silica gel gently poured on the top of the column with constant tapping to avoid air bubbles and cracks after mixing with solvents. Add 2mm layer of sea sand into the top of the column after settled the gel. The column was run with solvent of (Hexane & Ethanol 1:5) after loading with the crude plant extract (2-3g) mixed with activated silica gel. The fractions collected were dried for further analysis (fig-2).

Fig 2: Column chromatography

Qualitative Phytochemical Analysis (Harborne JB., 1998) [10]

Chemical tests for the screening and identification of bioactive chemical constituents in the crude extract and column purified sample were carried out by standard procedure in the different solvents are Hexane, Ethanol, methanol, chloroform, Ethylacetate and water.

Quantitative Phytochemical Analysis

Determination of total alkaloid

The plant extract (1mg) was dissolved in dimethyl sulphoxide (DMSO), added 1ml of 2N HCl and filtered. This solution was transferred to separating funnel, 5ml of bromocresol green solution and 5ml of phosphate buffer were added. The mixture was shaken with 4ml chloroform by vigorous shaking and collected in a 10ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (20, 40, 60, 80 and 100μg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg /g of extract. By using standard atropine calibration curve, measured the concentration of alkaloid content in atropine equivalents using unit's mg/g of Gallic acid (GAE) (Fazel Shamsa *et al*., 2008) [9].

Determination of total phenolic content

Total phenolics in extracts were determined by Folin-Ciocalteu method (Ranalli *et al*., 2006) [18]. Briefly, 0.5 mL (0.1%, w/v) of each sample was mixed with 2.5 mL of a 10 fold diluted Folin-Ciocalteu reagent followed by 2 mL of 7.5% sodium carbonate. The tubes were covered with parafilm (American National Can, Chicago) and allowed to stand for 30 min at room temperature before the absorbance was recorded at 760 nm (U-1800, Spectrophotometer, Hitachi, Japan). Different concentrations of gallic acid (0.1 to 0.60 mg/mL) were prepared in methanol for preparation of standard curve. All determinations were analyzed in triplicate and results expressed in mg gallic acid equivalents (GAE)/g dried extract. (Ranalli *et al*. (2006) [18].

Determination of total flavonoid content

The total flavonoid content of plant extracts were estimated according to method described by (Zhishen *et al*. (1999) [20]. 1.0 mL (0.1%, w/v) of sample was mixed with 4 mL of distilled water and subsequently with 0.3 mL of $NaNO2$ solution (10%, w/v). After allowing the mixture to stand for 5 min, 0.3 mL $AlCl3$ solution (10%, w/v) was added followed

by 2.0 mL of (1%, w/v) NaOH solution. The mixture was thoroughly mixed immediately and absorbance was determined against blank at 510 nm. Standard curve of quercetin (Sigma Aldrich, USA) was prepared in a concentration ranging from 0 to 12 mg/mL and the results were expressed as quercetin equivalents (mg quercetin equivalents/g dried extract).

Determination of total tannin content

Tannin content in sample was determined using insoluble polyvinyl- polypirrolidone (PVPP) which binds tannins (Makkar *et al*., 1993) [14]. Briefly, 1 mL of extract (0.1%, w/v) dissolved in methanol in which the total phenolics were determined, was mixed with 100 mg PVPP, vortexed, kept for 15 min at 4°C and then centrifuged for 10 min at 3000 rpm. In the clear supernatant tannin phenolics were determined. Tannin content was calculated as a phenolic content read at 450nm.

Antioxidant activity

Ethanolic crude extract and column purified sample of *Ficus glomerata* were analyzed using free radical scavenging assay such as DPPH, ABTS & SO for evaluating antioxidant attributes.

DPPH (1, 1-Diphenly-2-picryl-hydrazil.) Free Radical Scavenging Activity: (Shimada *et al*., 1992) [19]

Various concentrations of plant extracts (20-100 µg/ml) were mixed with 1.0ml of methanolic solution containing DPPH, resulting in the final concentration of DPPH being 0.2mM. The mixture was shaken vigorously, left for 30min, at room temperature and the absorbance was measured at 517 nm. The reaction was initiated by the addition of 1.0ml of diluted DPPH to 10µl of methanol as control. Ascorbic acid, Trolox, BHA (20-100 µg/ml) was used as positive controls. The DPPH radical scavenging activity was calculated as follows: Scavenging activity = $[(A_0-A_1/A_0)] \times 100$, where $A_0$was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract. EC50 value (µg extract/ml) was the effective concentration at which DPPH radicals were scavenged by 50% and were obtained by interpolation from linear regression analysis.

ABTS radical scavenging activity (Re *et al*., 1990)

The plant extracts (20-100 µg/ml) were mixed with 1.0ml of ABTS solution resulting in the final concentration of ABTS being 7mM. The mixture was shaken vigorously; the reaction was initiated by the addition of 1.0ml of diluted ABTS+ to 10µl of methanol as control. The absorbance was read at 734nm after 6min and the percentage inhibition was calculated. Ascorbic acid, trolox, BHA (20-100 µg/ml) was used as positive controls. The inhibition was calculated according to the equation Scavenging activity = $[(A_0-A_1/A_0)] \times 100$, where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract.EC50 value (µg extract/ml) was the effective concentration at which ABTS radicals were scavenged by 50% and were obtained by interpolation from linear regression.

Super oxide radical scavenging activity (Ock-Sook *et al*. 2011)

4.5ml of 0.05 M Tris HCL buffer pH 8.2 was added in to test tube, followed by adding 1ml of the extract and 0.5ml of 2.5mM pyrogallol solution. The reaction mixture was incubated at 25 ºC for 5 min and then the reaction was stopped by to addition of 1ml of 8.0 m HCL and the absorbance was measured at 320nm The inhibition was calculated according to the equation Scavenging activity = $[(A_0-A_1/A_0)] \times 100$, where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract. EC50 value (µg extract/ml) was the effective concentration at which SO radicals were scavenged by 50% and were obtained by interpolation from linear regression.

Results and Discussion

All the results of phytochemical analysis are showed in the Table1 and table 2.The results of Antioxidant studies using DPPH, ABTS &SO assay are shown in figure 3, 4 & 5 respectively.

Table 2 showed the preliminary phytochemical screening of ethanolic crude extract of *Ficus glomerata* leaves revealed the presence of Alkaloids, flavonoids, tannins, carbohydrates, amino acids, steroids, phenols and proteins. The present study also carried out in the column purified extracts was found to be rich in alkaloids (3+), in the solvents used ethanol and methanol in other solvents presents, other compounds like tannins, flavanoids & phenols, etc. were expressed in lower grade (2+) when compared to alkaloids. These phytoconstituents are the key factors for pharmacological activity of a medicinal plant. The results of quantitative screening of phytochemical presented in Table 1. The ethanolic extract of *ficus glomerata* crude sample and column purified sample were evaluated for the presence of total alkaloids. The total alkaloids content was found to be high in crude extract 13.00mg/g of sample followed by column purified extract was found to be 10.20mg/g. The results of total flavanoids content of crude extract and column purified extract 10.02mg/g, 4.21mg/g respectively. Flavanoids have been showed to be highly effective scavengers of most oxidizing molecules include single oxygen and various free radicals. (Bravo l, 1998) [7]. The result of total tannin content of crude extract & column purified extract was found to be 4.31mg/g & 2.01mg/g respectively. The total phenol content of crude extract & column extract 6.50mg/g & 4.20mg/g respectively.

The DPPH radical scavenging (%) activity of ethanolic leaves extract of *ficus glomerata,* compared to standard ascorbic acid shown in fig-3 DPPH radical scavenging activity of *ficus glomerata*, crude extract and column purified sample, IC_{50} value was found to be 65. 68µg/ml &51.11µg/ml respectively. The mean IC_{50} value of ascorbic acid was found to be 44.8µg/ml. The plant extract exhibit potent ABTS radical cation scavenging activity in concentration dependent manner was showed in fig 4.The ABTS radical scavenging activity of *ficus glomerata*, crude extract and column purified sample IC_{50} value was found to be 61. 43µg/ml &44.8µg/ml respectively. The mean IC_{50} value of ascorbic acid was found to be 41.118µg/ml. The SO radical scavenging activity of *ficus glomerata*, crude extract and column purified sample IC_{50} value was found to be 44.17µg/ml 42.27µg/ml respectively. The mean IC_{50} value of ascorbic acid was found to be 38.96µg/ml (fig-5). The antioxidant activity is expressed as the number of gram equivalent of ascorbic acid. Ascorbic acid equivalents were calculated using standard graph of ascorbic acid in µg/mg of extract. (Aderogba *et al*., 2005) [3] This maximum antioxidant activity of plant extract showed to the

presence of maximum concentration of alkaloid flavanoids and phenols. Hence the scavenging activity DPPH, ABTS &SO radicals by the *ficus glomerata* plant extract was found to be potential; to treat as various free radical related diseases.

Table 1: Shows the quantity of phytochemical compounds in crude extract and column etract of ethanolic leaf extract of *Ficus glomerata*

Phytochemicals	Crude extract in mg	Column purified extract in mg
Total alkaloids	13.20	10.5
Total flavanoids	10.02	4.21
Total tannins	4.31	2.01
Total phenols	6.50	4.20

Table 2: Shows the presence of phytochemicals in column purified extract of different solvents

Phytochemical Tests	Inference					
	Hexane	Chloroform	Ethyl acetate	Ethanol	Methanol	water
Alkaloids	++	++	+	+++	+++	++
Flavanoids	-	+	-	++	++	+
Tannins	+	+	-	+	+	-
Phenols	+	-	+	+	+	-
Proteins	-	-	-	+	+	-
Cabohydrates	+	+	++	++	++	++
steroids	-	-	+	++	++	+

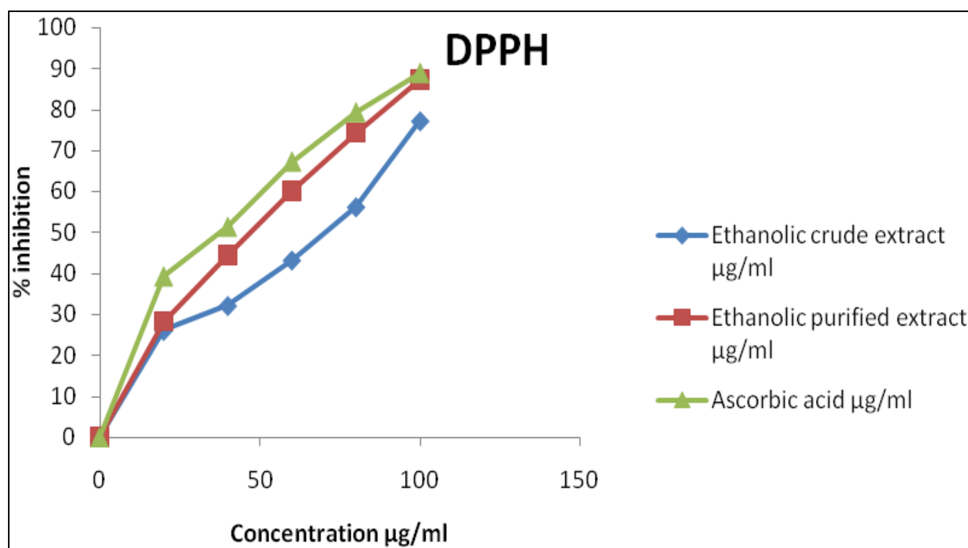

Fig 3: presents Inhibition @ 50% concentration (IC50) of ethanolic crude extract and column purified extract of *ficus glomerata* leaf against DPPH Scavenging assay

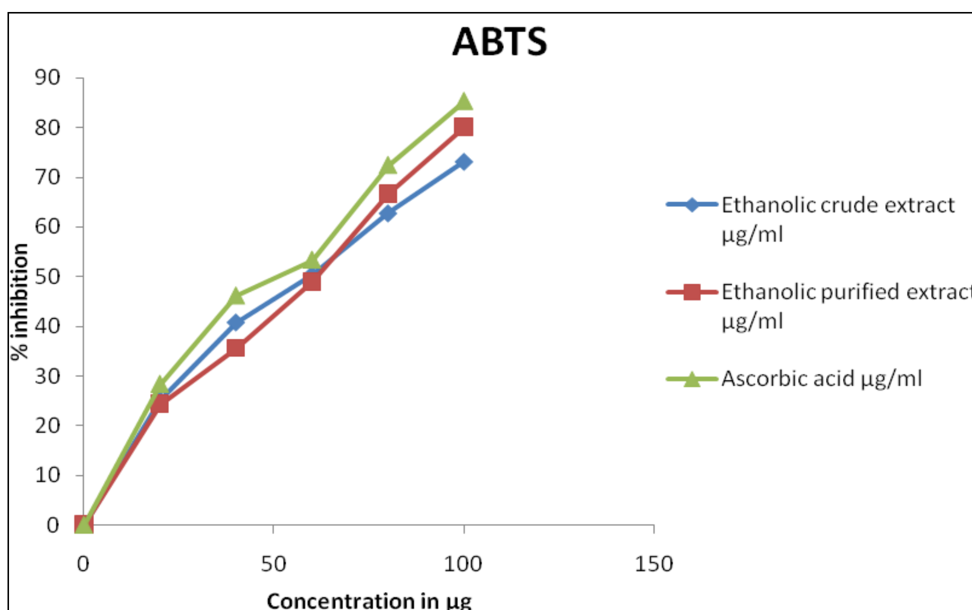

Fig 4: presents Inhibition @ 50% concentration (IC50) of ethanolic crude extract and column purified extract of *ficus glomerata* leaf against ABTS Scavenging assay

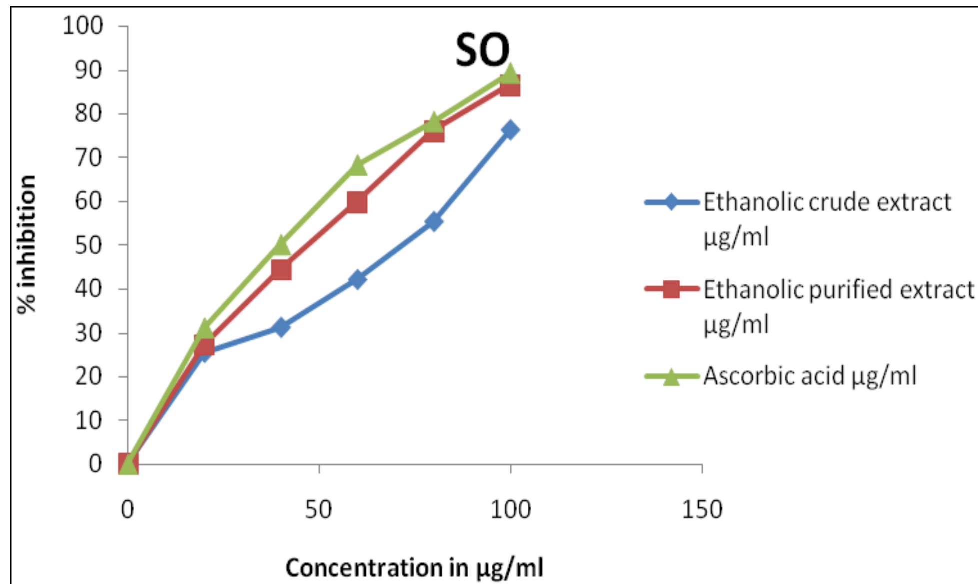

Fig 5: presents Inhibition @ 50% concentration (IC50) of ethanolic crude extract and column purified extract of *ficus glomerata* leaf against Super oxide Scavenging assay

Conclusion

From the above study reveals that the presence of phyto-constituents like alkaloids, phenol, flavanoids, tannins, found to play an active role on free radical scavenging potential, that infers the promising antioxidant activity of ethanolic leaf extract of column purified sample from *ficus glomerata*.

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