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Research Article

IN VITRO ANTIOXIDANT CAPACITY, ESTIMATION OF TOTAL PHENOLIC AND FLAVONOID CONTENT OF *FICUS AURICULATA LOUR.*

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ABSTRACT

The study was carried out to determine the antioxidant activity of *Ficus auriculata* Lour and to estimate their phenolic and flavonoid content. DPPH (1,1-diphenyl-2-picryl hydrazyl) radical scavenging capacity and reducing power assay (RPA) were done for evaluating *In vitro* antioxidant activity. Total phenolic content (TPC) was estimated by Folin-Ciocalteau's method and total flavonoid content by Aluminium chloride colorimetric method. It is observed that DPPH EC_{50} was 251.33mg/ml and Reducing Power Assay (RPA) was 53.40 ± 0.01. Total phenolic and flavonoid content were 21.404 ± 0.134 & 50.83 ± 1.32 mg GAE/mg dry weight respectively. This study validated the medicinal potential of the leaf of *Ficus auriculata* and the positive relationship between total phenolic, flavonoid content and antioxidant activities.

Keywords: Antioxidant activity, DPPH (1, 1-diphenyl-2-picryl hydrazyl), *Ficus auriculata* Lour., RPA (reducing power assay), TF (Total flavonoid), TP (Total phenolic).

INTRODUCTION

Halliwell and Gutteridge propose to define an antioxidant as "any substance that, when present at low concentration compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate"[1]. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols[2].

Recently there has been an upsurge of interest in the therapeutic potential of medicinal plants as antioxidants in reducing such free radical-induced tissue injury[3]. Besides, well known and traditionally used natural antioxidants from teas, wines, fruits, vegetables and spices, some natural antioxidants (e.g. rosemary and sage) are already exploited commercially either as antioxidant additives or as nutritional supplements[4]. Because of potential health benefits of natural antioxidants[5,6], they are expected to be an alternative to synthetic ones. Therefore, there is an increasing interest for researchers in seeking for new resources of natural antioxidants[7].

The increasing practical use of medicinal Plant preparations possessing antioxidant properties for the regulation of free radical processes in the human organism poses the task of quantitative characterization of the antioxidant activity of these complex preparations. Many of the biologically active substances found in plants, including phenolic compounds (flavonoids, phenolic acids), sugars, vitamins, saponins, ethereal oils, polyunsaturated fatty acids, phospholipids, enzymes, amino acids, etc., are known to possess antioxidant properties[8]. It has been mentioned the antioxidant activity of plants might be due to their phenolic compounds[9].

Phenolics are an important class of secondary plant metabolites possessing an impressive array of pharmacological activity. One of the more prominent properties of the phenolics is their excellent radical scavenging ability. Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action[10]. Some evidence suggests that the biological actions of these compounds are related to their antioxidant activity[11]. An easy, rapid and sensitive method for the antioxidant screening of plant extracts is free radical scavenging assay using 1,1-diphenyl-2-picryl hydrazyl (DPPH) stable radical spectrophotometrically. In the presence of an antioxidant, DPPH radical obtains one more electron and the absorbance decreases[12].

Ficus auriculata (Roxburgh Fig), this is an evergreen to semi-deciduous, spreading large shrub or small tree reaching 25 feet tall and as wide. This plant is native to India, China, Bhutan, Malaysia, Myanmar, Nepal, Pakistan, Thailand and Viet Nam. *Ficus auriculata* is commonly known as Heiba in Manipuri language.Fruits is edible and can be made into jams and curries. Leaves are lopped for fodder. The species *Ficus auriculata* is used as a tree fodder in the Himalayan foothills of India and Nepal[13].

The Leave and fruit parts of *Ficus auriculata* is reported to have Antibacterial, Anti-inflammatory and Antioxidant Activities[14]. Preliminary phytochemical screening of the plant leaf extract revealed the presence of alkaloids, glycosides, flavonoids, terpenoids, tannins and reducing sugar but saponin was found absent in the leaf of *Ficus auriculata.* The leaf of *Ficus auriculata* is reported to have antibacterial properties[15].

Several tribal in North eastern India especially in Manipur use the leaf of *Ficus auriculata*, traditionally for the treatment of diabetes. In the present studies attempt has been made to establish a correlation between the total antioxidant activity and the phenolic & flavonoid content of the methanolic leaf extract of *Ficus auriculata.*

MATERIALS AND METHODS

Standards and reagents

Folin-Ciocalteau reagent and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were used as reagents. All chemicals and solvents were of analytical grade.

Sample preparation

The leaf of *Ficus auriculata* was collected randomly from the Phayeng village of Imphal West district, Manipur, India. The collected plant material was botanically authenticated by Botanical Survey of India, Shillong. The leaves are dried and powdered. The powdered sample (100g) *Ficus auriculata* was weighed and subjected to soxhlation with methanol for 72 hrs. The solvents was distilled off at lower temperature under reduced pressure in the rotary evaporator and concentrated to dryness (crude extract). The dried extract was weighed and then stored in a freezer. The crude extract was used for the experiments.

Scavenging activity on DPPH radical

The radical scavenging activities of the plant extract against 2,2-Diphenyl-1-picryl hydrazyl (DPPH) radical was determined by a slightly modified method using UV spectrophotometry (electronic GEAESYD-20 thermo spectronic) at 517 nm[16]. Briefly, I ml of the crude extract solution at variable concentrations (0.001-0.003mg/ml) was placed in a test tube, and 3 ml of methanol was added followed by 0.5 ml of 1 mM DPPH in methanol, kept for 35 mins at room temperature until to produce a stable colour and subsiquently absorbance was measured at 517 nm. L- ascorbic acid was used as the positive control. A blank solution was prepared containing the same amount of methanol and DPPH. The radical scavenging activity was calculated using the following formula:

DPPH radical scavenging activity (%) = {[Ab-Aa]/Ab} x 100 .....(1)

Where Ab is the absorption of the blank and Aa is the absorption of the extract sample.

Different concentrations of the extract were selected in such a way that percentage of inhibition of colour of DPPH solution is above and below 50%. EC_{50} value was obtained from the linear regression equation. Correlation coefficient ($R^2 \geq 0.90$) was taken as highly correlated.

Reducing power assay

Reducing ability was performed by using potassium ferricyanide- ferric chloride system [17]. One milliliter of extract solution (0.2, 0.4, 0.06, 0.8, and 1.0 mg/ml) was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml, 1%). The content was mixed and heated at 50 °C for 20 min and cooled; then trichloroacetic acid (2.5 ml, 10%) was added, and the mixture was centrifuged at 3,000 rpm for 10 min. The supernatant (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml, 0.1%), and the absorbance was measured at 700 nm against a blank. Increasing absorbance of the reaction mixture indicates increasing reducing power. All the tests were performed triplicate.

Determination of total phenolic content (TP)

The total phenolic content was determined using Folin–Ciocalteu reagents[18] with analytical grade gallic acid as the standard. 1 ml of extract or standard solution (25 mg/ml) was added to deionized water (60 ml) and Folin–Ciocalteu phenol reagents (5.0 ml). After 5 minutes, 20% sodium carbonate (15.0 ml) was added to the mixture. After being kept in darkness for 2 hrs, the absorbance was measured at 760 nm using a spectrophotometer (GEAESYS-20, Thermospectronic). The same solution was used without the extraction solution as blank.

Amounts of TP were calculated based on gallic acid standard.

$$\text{Total Phenols \%} = \frac{\text{Asample X Wstandard X 5}}{\text{Astandard X Wsample X 50}} \text{ X 100\% .....(2)}$$

The results were expressed as gallic acid equivalents (GAE) mg/mg of dry extract.

Determination of total flavonoid (TF) content

TF content was determined by Aluminium chloride colorimetric method [19]. Extract solution (1 ml, 0.1 mg/ml) was placed in a 10-ml volumetric flask, and then 5 ml of distilled water was added followed by $NaNO_2$ solution (0.3 ml, 5%). After 5 min, $AlCl_3$ solution (0.6 ml, 10%) was added. After another 5 min, NaOH solution (2 ml, 1 M) was added and volume was made up with distilled water. The solution was mixed thoroughly and absorbance was measured at 510 nm. TF amounts were expressed as milligrams of gallic acid equivalent per milligram of dry weight. All the tests were performed in triplicate.

RESULT AND DISCUSSION

Radical scavenging activity by DPPH method

DPPH is a relatively stable free radical. The assay is based on the measurement of the scavenging ability of antioxidants towards the stable radical DPPH. DPPH method allows estimation of hydrogen radical donating ability of the extract[20]. This model represents the situation in metabolic system where an antioxidant will stabilise a free radical by reacting with the hydrogen radical. The results are expressed in EC_{50} (Effective concentration to reduce the initial concentration of DPPH to 50%). Lesser the value of EC_{50} value for an extract is considered to be associated with higher ability to donate hydrogen radical i.e antioxidant activity. In the present study EC_{50} values were found to be 251.33mg/ml and 4.86 mg/ml for *Ficus auriculata* and Ascorbic acid respectively.

Reducing power assay

The reducing capacity of the extract is another significant indicator of antioxidant activity. In the reducing power assay, the presence of antioxidants in the sample would result in the reduction of Fe_3^+ to Fe_2^+ by donating an electron. The amount of Fe_2^+ complex can then be monitored by measuring the formation of Perl's blue at 700 nm. Increasing absorbance indicates an increase in reductive ability[21]. The results show that there was increase in reducing power of the plant extract as the extract concentration increases. The result is expressed as absorbance X 100. The Reducing Power Assay of *Ficus auriculata* was found to be 53.40.

Estimaton of total phenolic content

Phenolic compounds are having wide bioactivity including antioxidant properties/activity. The antioxidant activity of phenolic compound is due to hydroxyl functional group, however other factors eg., presence of electron withdrawing or releasing group in the aromatic ring having hydroxyl moiety wil increase or decrese the activity. The phenols contain hydroxyls that are responsible for the radical scavenging effect mainly due to redox properties[22]. In the current study total phenolic content was found to be 21.404 mg GAE/mg dw for *Ficus auriculata*.

Estimation of total flavonoid content

Flavonoids have been shown to exhibit their actions through effects on membrane permeability, and by inhibition of membrane-bound enzymes such as the ATPase and phospholipase A2[23]. Flavonoids serve as health promoting compound as a results of its presence as anion radicals[24].

Total flavonoid contents were found to be 50.83 mg GAE/mg dw for *Ficus auriculata*. The compounds such as flavonoids, which hold hydroxyl groups, are responsible for the radical scavenging activity in the plants[25,26]. It has been acknowledged that flavonoids show significant antioxidant action on human health and fitness. It is known that flavonoids act through scavenging or chelating process[27,28].

Table 1: Showing data of *in vitro* antioxidant activity of the plant extracts in different bioassays

Plant	DPPH EC_{50} (mg/ml)	RPA #	TP* (mg GAE/mg dw)	TF* (mg GAE/mg dw)
Ficus auriculata	251.33	53.40 ± 0.01	21.404 ± 0.134	50.83 ± 1.32

EC_{50} in mg/ml. The significance difference was analysed by one- way Anova followed by Tukey's post hoc test. $P<0.05$ was considered significant.

*Result are expressed as mean ± S.D (n=3) from 3 independent observation, gallic acid as standard.

#Absorbance given by 1mg/ml solution of extract X 100.

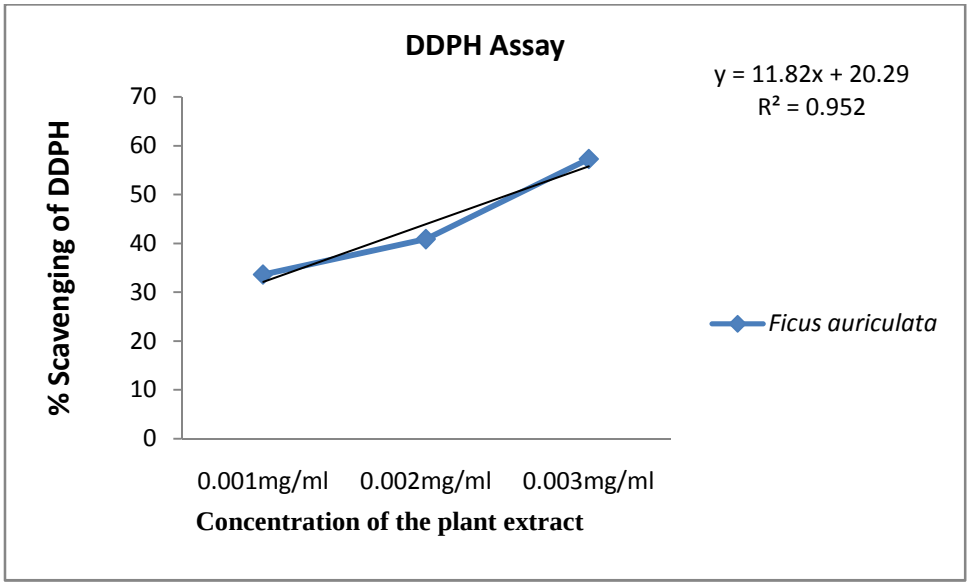

Fig. 1: Linear regression of %DPPH Scavenging Vs Concentration

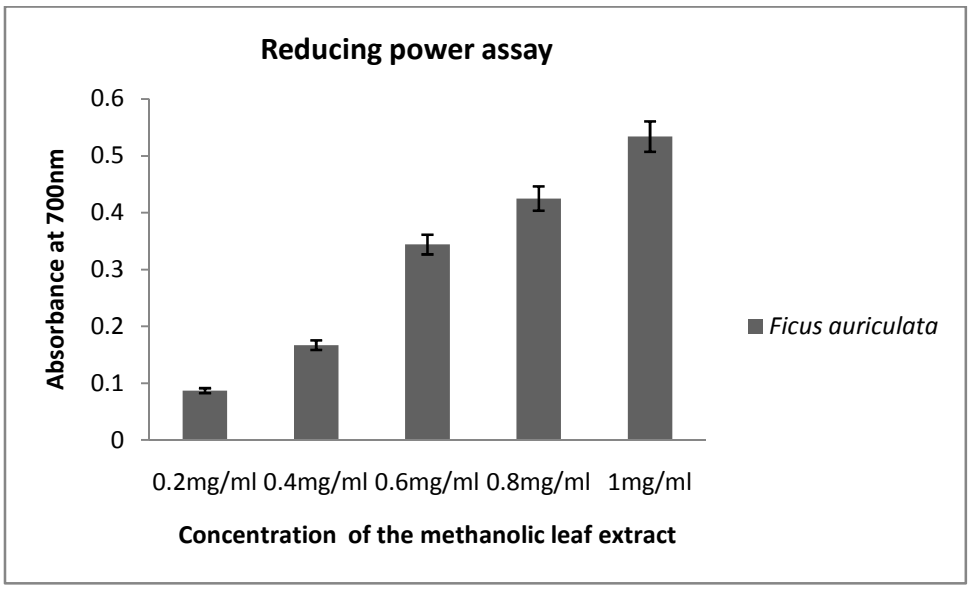

Fig. 2: Antioxidant activity of the plant extract estimated by Reducing Power Assay.

CONCLUSION

This study suggests that *Ficus auriculata* leaf extract has antioxidant activity which might be helpful in preventing or slowing the progress of various oxidative stress induce diseases such as diabetes, which would be beneficial to the human health. We have also established the relationship of total phenolic, flavonoid contents and the free radical scavenging activity. The methanolic leaf extract of the plant in this study exhibited its extent of antioxidant activity. This may be related to the high amount of flavonoid and phenolic compounds present in the plant extract. Further studies are needed to clarify the *in vivo* potential of this plant in the management of human diseases resulting from oxidative stress and this is a subject of investigation in our group.

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