

IN VITRO EVALUATION OF ANTI OXIDANT ACTIVITY OF *FICUS CARICA* LEAVE EXTRACT

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ABSTRACT

Antioxidants are compounds that can inhibit or prevent the oxidation of the easily oxidized substrate. One of the plants as a potential source of bioactive compounds and antioxidant activity (*Ficus carica*). This study aimed to determine the proximate compositions, bioactive compounds and antioxidant activity from large-leafed mangrove fruit which extracted by methanol. The phytochemical screening was carried on the both extracts of leaves of *Ficus carica*, revealed the presence of some active ingredients such as Alkaloid, Glycoside, Steroids Gums, Flavonoids, Saponins, Reducing sugar, Tannins. The aqueous and alcoholic leaves extract were also evaluated for their antioxidant activity using FRAP assay, Metal chelating assay, DPPH radical scavenging assay, superoxide-radical scavenging assay and Hydrogen peroxide scavenging assay. The result of the present study showed that the ethanolic leaves extract of *Ficus carica* has shown the greatest anti-oxidant activity than aqueous extracts. The high scavenging property of may be due to hydroxyl groups existing in the phenolic compounds. Further work is needful to isolate the exact compound which is responsible for antioxidant activity and biophysical characterization can be done in the future.

Keywords: *Ficus carica*, Antioxidants activity, FRAP assay.

1. INTRODUCTION

Depressed patients commonly complain about feeling sad, lack of interest in their day to day work, inability to find pleasure in activities that would normally please others, and feelings of discontent. It is also associated with feeling of guilt or reduced self-worth, disturbed sleep, changes in appetite, and low energy. In severe forms of depression, patients are known to cause self harm or even suicide. Although drugs available for the therapy of depression, they have their limitations, such as delayed therapeutic response and low responders to these drugs, which poses a problem with patient compliance. Hence, there is a requirement for alternative treatment of depressive disorders with the use of medicinal plants.

The plant *Mimosa pudica* is a weed that grows in humid areas, open fields and by roadsides. It grows as a shrub, under 100 cm in height, and is easily identifiable by its characteristic 15 – 20 pairs of leaflets that folds when disturbed, and is hence known as “Lajwanti” in Hindi, and “Touch me not” in English. It is believed to have originated from the Middle Americas and is now found in other in all tropical countries of the Asian subcontinent and South East Asia. It has been used as a folk lore medicine since many years because of its various medicinal properties, and because it is an easily cultivable plant and is abundantly available.

2. MATERIAL AND METHODS

2.1. Plant Material Collection

The leaves of *Ficus carica* were collected and was identified and authenticated from Department. The plant material was cleaned, reduced to small fragments, air dried under shade at room temperature and coarsely powdered in a mixer. The powdered material was stored or taken up for extraction process.

2.2. Preparation of plant extracts

Fresh leaves of *Ficus carica* were collected and washed under tap water. The leaves extract used was prepared by taking 20gms of finely cut leaves into 250ml beaker containing 200ml of water. The contents were mixed well and then the mixture was boiled up to 80-100°C for 4-5hrs. Further the extract was filtered with whatmann filter paper. The filtrate was boiled until the concentrated residue is formed. The concentrated product was sealed in sample covers and stored under room temperature and used for further experiment to check the activities.

Fresh leaves of *Ficus carica* were collected and washed under tap water. The leaves extract used was prepared by taking 20gms of finely cut leaves into 250ml beaker containing 200ml of alcohol. The contents were mixed well and then the mixture was boiled up to 50-60°C for 4-5hrs. Further the extract was filtered with whatmann filter paper. The filtrate was boiled until the concentrated residue is formed. The concentrated product was sealed in sample covers and stored under room temperature and used for further experiment to check the activities.

2.3. In vitro methods of anti-oxidant activity

2.3.1 Ferric Reducing-Antioxidant Power (FRAP) Assay

The FRAP assay was done according to the method described by Benzie and Strain (1999) with some modification. This method is based on reduction of TPTZ-Fe³⁺ complex to TPTZ-Fe²⁺ form in the presence of antioxidants. The stock solutions included acetate buffer (300 mM, pH 3.6), 2, 4, 6-tripyridyl s-triazine (TPTZ) solution (10 mM in 40 mM HCl) and ferric chloride (FeCl₃.6H₂O) solution (20 mM). The fresh working FRAP solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ solution and 2.5 ml FeCl₃ solution. Extracts were made up to 2.0 ml with distilled water and 1.0 ml of FRAP solution was added. An intense blue color developed was measured at 593 nm, after an incubation period of 20 min. The absorbance was related to absorbance changes of a ferrous sulphate solution (0 – 100 NM) tested in parallel. All results were based on three separate experiments and antioxidant capacity was expressed as NM FeSO₄/ g of dry extract. Quercetin and Butylated Hydroxy Toluene (BHT) were used as positive control.

2.3.2 Metal chelating activity

The chelating capacity of *Ficus carica* extracts on Fe²⁺ ions was determined according to the method of Dinis et al (1994), wherein Fe²⁺ chelating potential of extracts was monitored by measuring ferrous iron – ferrozine complex at 562 nm. Briefly, extracts (0.05 – 1.0 mg/ml), quercetin, BHT and EDTA (10 – 250 Ng/ml) were made up to 4.7 ml with distilled water and then mixture was allowed to react with 0.1 ml of Ferrous chloride (2.0 mM) and 0.2 ml of ferrozine (5.0 mM) for 20 min. Absorbance of mixture was measured at 562 nm against a blank, which contained distilled water, instead of extracts/EDTA/standard antioxidants. The ability of extracts to chelate ferrous ion was calculated using the following equation: Chelating effect (%) = [Ab control 562 – Ab sample 562/ Ab control 562] x 100.

Experiments were done in triplicate.

2.3.3 DPPH radical-scavenging activity

DPPH radical-scavenging activity of *Ficus carica* extracts was determined as previously described (Burits and Bucar, 2000). The capacity of extracts to scavenge lipid soluble 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, which results in bleaching of purple color exhibited by stable DPPH radical, is monitored at an absorbance of 517 nm. Briefly, 1.0 ml of extracts (0.05 – 1.0 mg/ml) and Quercetin/BHT (10 – 250 Ng/ml) in ethanol were added to 4 ml of 0.004% methanolic solution of DPPH. After incubation for 30 min at room temperature in the dark, absorbance was read against a blank at 517 nm. Tests were carried out in triplicate. The ability of extracts and Quercetin/BHT to scavenge DPPH radical was

Calculated using the following equation:

$$\text{Radical scavenging activity (\%)} = [A_0 - A_1 / A_0] \times 100.$$

Where A₀ was absorbance of negative control (containing all reagents except test compounds) at 517 nm and A₁ was absorbance of the extracts or Quercetin/BHT at 517 nm. DPPH scavenging activity of extracts and standard was expressed as IC₅₀, which was interpolated from a graph constructed, using percent inhibition (Y-axis) against concentration (X-axis) of extracts and standards.

2.3.4 Superoxide radical-scavenging activity

The ability of *Ficus carica* extracts, Quercetin and BHT to quench generation of superoxide radicals was determined according to the method of Nishikimi et al (1972) with a slight change. Superoxide radicals were generated in PMS-NADH system by oxidation of NADH and analyzed by NBT reduction. 0.1 ml of extracts (0.05 – 1.0 mg/ml) and quercetin/BHT (10 – 250 Ng/ml) were mixed with 2.9 ml of phosphate buffer (40 mM, pH 7.4), 1.0 ml of nitroblue tetrazolium (NBT) solution (150 NM in 40 mM phosphate buffer, pH 7.4) and 1.0 ml of NADH (468 NM in 40 mM phosphate buffer, pH 7.4). The reaction was initiated by addition of 1.0 ml of phenazine methosulphate (60 NM in 40 mM phosphate buffer, pH 7.4) to reaction mixture. After incubation at 25°C for 5 min, absorbance was measured at 560 nm. Negative control was subjected to the same procedures as extracts, except that only solvent was added for negative control. All measurements were made in triplicate. The ability of extracts and quercetin/BHT to scavenge superoxide radical was calculated using the following equation:

$$\text{Superoxide radical scavenging activity (\%)} = [A_0 - A_1 / A_0] \times 100.$$

Where A₀ was absorbance of negative control at 560 nm and A₁ was absorbance of the extracts or quercetin/BHT at 560 nm. IC₅₀ value, which represents concentration of extracts and standards that caused 50% inhibition, was determined by a linear regression analysis.

2.3.5 Hydrogen peroxide scavenging activity

The method of Sinha (1972) originally designed for the estimation of antioxidant enzyme, catalase, was adopted to evaluate hydrogen peroxide scavenging effect of *Ficus carica* extracts. Extracts (0.05 – 1.0 mg/ml) and Quercetin/BHT (10 – 250 Ng/ml) were incubated with 0.6 ml of H₂O₂ (40 mM in a phosphate buffer, 0.1 M pH 7.4) in the dark for 10 min. A negative control was set up in parallel with entire reagent except extract or standard. After incubation, remaining H₂O₂ was allowed to react with 1.0 ml of dichromate in acetic acid (5% potassium dichromate and glacial acetic acid in the ratio of 1:3) in a boiling water bath for 10 min. Absorbance of green color developed was determined at 620 nm. All experiments were performed in triplicate. The percentage scavenging of H₂O₂ by *Ficus carica* extracts and standards were calculated using the following equation:

$$\text{H}_2\text{O}_2 \text{ scavenging activity (\%)} = [A_0 - A_1 / A_0] \times 100.$$

Where A₀ was absorbance of negative control and A₁ was absorbance of the extracts or standards. H₂O₂ scavenging activity of extracts and standards was expressed as IC₅₀, which was interpolated from a graph constructed using percent inhibition (Y-axis) against concentration (X-axis) of extracts and standards.

3. RESULTS AND DISCUSSION

3.1. Ferric reducing ability of *Ficus carica*

Table 1: Ferric Reducing Ability - FRAP (expressed as mM FeSO₄/g dry weight) of leaves of *Ficus carica*

Group	Drugs	IC ₅₀ value µg/ml
I	Quercetin	13.59±0.098
II	Butylated Hydroxy Toluene (BHT)	3.10 ±0.025
III	AQFC	1.88±0.037
IV	ALFC	2.34±0.065

3.2. Metal chelating activity of *Ficus carica*

Table2: Metal chelating activity of leaves of *Ficus carica*

Group	Drugs	IC ₅₀ value µg/ml
I	EDTA	6.98
II	Quercetin	143
III	Butylated Hydroxy Toluene(BHT)	89
IV	AQFC	25.18
V	ALFC	31.61

3.3. DPPH radical scavenging activity of *Ficus carica*

Group	Drugs	IC ₅₀ value µg/ml
I	Quercetin	4.2 ±0.024
II	Butylated Hydroxy Toluene (BHT)	2.16 ±0.075
III	AQFC	1.22 ±0.052
IV	ALFC	2.2 ±0.000

3.4. Superoxide radical scavenging activity of *Ficus carica*

Group	Drugs	IC ₅₀ value µg/ml
I	Quercetin	0.013 ±0.001
II	Butylated Hydroxy Toluene (BHT)	0.010 ±0.009
III	AQFC	0.34 ±0.008
IV	ALFC	0.312 ±0.001

3.5. Hydrogen peroxide scavenging activity of *Ficus carica*

Group	Drugs	IC ₅₀ value µg/ml
I	Quercetin	0.040±0.003
II	Butylated Hydroxy Toluene (BHT)	0.063±0.009
III	AQFC	0.072±0.015
IV	ALFC	0.597±0.042

4. CONCLUSION

In this study, all antioxidant methods (FRAP assay, Metal Chelating assay, DPPH radical-scavenging assay, Superoxide radical scavenging assay and Hydrogen peroxide scavenging assay) showed that the both aqueous and alcoholic extracts of *Ficus carica* contain more antioxidant activities. Moreover, this study demonstrated the important source of phenol compounds, which are a good source of antioxidant activity. The phenol component has a high inhibitory effect that prevents lipid peroxidation. However, the solvent type has an important role in detecting phenol compounds and antioxidant factors. Thus, we concluded that *Ficus carica* act via its free radical scavenging to prevent lipidperoxidation. Therefore, natural antioxidants and phenol compounds in *Ficus carica* have the capability to be used medically and in food systems to preserve food quality.

5. REFERENCES

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