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RESEARCH ARTICLE

An *In vitro* Analysis of *Ficus carica*'s Antioxidant Potential

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ABSTRACT:
Since antiquity, plants naturally produce free radical scavengers and they act as potential source of antioxidants. Researchers show huge interest towards the traditional uses of medicinal plants against ailment related to oxidative stress. The present analysis measures the antioxidant capacity of *Ficus carica* fruits (Sample A - Pulp with seeds and Sample B - Pulp). Therefore, ethanol extracts of *Ficus carica* fruits were assessed for determining their anti-oxidative potentials using both free radical scavenging and reducing ability assays which is compared with standard ascorbic acid (control). Results strongly suggest that ethanol extract of fig possess a good antioxidant potential, moreover Sample A - fig pulp with seed possess a better antioxidant activity when compared to pulp alone. This finding is useful for further advancements in the fields of food supplements, food additives and drug synthesis in future.

KEYWORDS: Figs, Antioxidant assay, *Ficus carica*, Free radical scavengers, DPPH.

INTRODUCTION:
The human body has a complex system of natural enzymatic and non-enzymatic antioxidant defences which counteract the harmful effects of free radicals and other oxidants[1]. Free radicals are responsible for causing a large number of diseases including cancer, cardiovascular disease, neural disorders, Alzheimer's disease, mild cognitive impairment, Parkinson's disease, alcohol induced liver disease, Ulcerative colitis, aging and atherosclerosis[2,3]. Protection against free radicals can be enhanced by ample intake of dietary antioxidants. Substantial evidence indicates that foods containing antioxidant nutrients may be of major importance in disease prevention. There is, however a growing interest among scientists that a combination of antioxidants, rather than single entities, may be more effective over the long term[4]. Antioxidants may be of great benefit in improving the quality of life by preventing or postponing the onset of degenerative diseases. In addition, they have a potential for substantial savings in the cost of health care delivery.

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Various extraction methods are used to investigate the antioxidant property of samples[5].

Nowadays, most food and pharmaceutical products contain synthetic antioxidants. These compounds are added to food in order to prolong product shelf life, mainly by preventing the oxidation of unsaturated double bonds of fatty acids. In pharmaceutical products to antioxidants are added to enhance the stability of therapeutic agents that are susceptible to chemical degradation by oxidation[6]. The nutraceutical trend towards doubling the impact of natural antioxidants that stabilize food and maximize health impact presents distinct challenges in evaluating antioxidant activity of purified individual compounds, mixed extracts, and endogenous food matrices and optimizing applications[7]. The two most common synthetic antioxidants used today are butylated hydroxyanisole (BHA) and butylated hydroxyltoluene (BHT). The synthetic antioxidants used in the industry could have carcinogenic effects on human cells, thus an intense search for new, natural and efficient antioxidants. Plants and plant-derived substances, collectively termed "phytonutrients," or "phytochemicals," are becoming increasingly known for their antioxidant activity.

Figs *(Ficus carica)* originated in the Middle East areas such as Syria, Asia Minor, and Iran, then, it was spread to the Mediterranean basin countries[8]. It belongs to the family of Moraceae, and is one of the unique edible fruits with high commercial value[9]. The fruits of *F. carica* are an abundant source of vitamins, carbohydrates, minerals, sugars, phenolic compounds and organic acids[10]. The *Ficus carica* being a medicinal plant it have a significant anti hyperglycemic effect[11], anti- arthritic property[12] helps to maintain blood glucose and reduces the risk of arthritis, anti- depressant[13] and anti- bacterial activity[14]. It also possesses a potent anthelmintic effect which helps in reducing parasitic problems in children[15]. Thus, the present study was undertaken to analysis the antioxidant potential of fig pulp with seed and fig pulp separately.

MATERIALS AND METHODS:

a) Collection of Figs:

The samples (Figs) for the study were collected from Local Organic Store in Chennai, Tamil Nadu. The Figs were washed and sliced using a sterile-knife.

b) Preparation of Fruit Extract:

About 10g of the figs (sample A - pulp with seeds and Sample B - only pulp without seeds) was soaked separately in 100ml of ethanol for 72 hours. After 72 hours, the supernatant liquid was filtered using a whatman filter paper and then stored at 4^0C for the further analysis.

Doses such as 20, 40, 60 and 80µg/ml were chosen for *In vitro* antioxidant activity. L-Ascorbic acid used as reference standard.

c) *In vitro* Antioxidant Activity:

The antioxidant potential of the ethanol extract of the 2 samples of fig were estimated using three assays comprising of two radical scavenging assay and one reducing power assay (Figure 1).

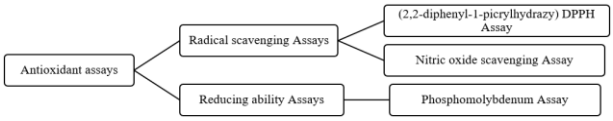

Figure 1: Antioxidant assay of ethanol fig extract

Free radical scavenging assay:

a) (2,2-diphenyl-1-picrylhydrazy) DPPH radical-scavenging activity:

The method of Shimada *et.al.*, (1992)[16] and Blois (1958)[17] was used to determine the DPPH radical-scavenging activity in the samples.

Reagents:

1. DPPH
2. Methanol

Procedure:

To the ethanol extract of fig (Sample A and B) at different concentrations (20 to 80µg/ml), then the 2ml DPPH methanol solution was added. Once added the mixture is shaken strongly and allowed to settle at room temperature in dark for 30 minutes. The absorbance was measured in spectrophotometer in 517nm. Higher free-radical scavenging activity is identified if there is lower absorbance of the reaction mixtures.

$$\text{Radical scavenging activity (\%)} = \frac{(A_C - A_S)}{A_C} \times 100$$

Where $A_{C=}$ control is the absorbance and A_S = Extract is the absorbance of reaction mixture (in the presence of Extract).

b) Nitric oxide scavenging activity assay:

Nitric oxide radical scavenging activity was determined according to the method reported by Garrat (1964)[18].

Reagents:

1. Sodium nitroprusside : 10 mM
2. Phosphate buffer saline : pH 7.4
3. Sulfanilic acid reagent : 33%
4. Naphthylethylenediamine dihydrochloride: 0.1%

Procedure:

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be determined by the use of the Griess Illosvoy reaction. 2ml of 10mM sodium nitroprusside in 0.5ml phosphate buffer saline (pH 7.4) was mixed with 0.5ml of extract at various concentrations and the mixture incubated at 25ºC for 150 min. From the incubated mixture 0.5ml was taken out and added into 1.0ml sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. Finally, 1.0ml naphthylethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min. The absorbance at 540nm was measured with a spectrophotometer. The nitric oxide scavenging activity was calculated according to the following equation:

$$\% \text{ Inhibition} = ((A_c - A_s) / A_c \times 100)$$

Where A_c was the absorbance of the control (blank, without extract) and A_s was the absorbance in the presence of the extract.

Reducing ability assay:

a) Phosphomolybdenum reduction assay:

The antioxidant activity of the extracts was evaluated by the phosphomolybdenum method according to the procedure of Prieto *et al*., (1999)[19].

Reagents:

1. Sulfuric acid : 0.6M
2. Sodium phosphate : 28mM
3. Ammonium molybdate : 4mM

Procedure:

The analyse is based on the decrease of Mo(VI)–Mo(V) by the extract and succeeding configuration of a green phosphate/Mo(V) complex at acid pH. 0.3ml extract was combined with 3ml of reagent solution (0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95°C for 90 min. Then the absorbance of the solution was measured at 695nm using a spectrophotometer against blank after cooling to room temperature. Methanol (0.3ml) in the place of extract is used as the blank. The antioxidant activity is expressed as the number of equivalents of ascorbic acid. The scavenging activity was calculated according to the following equation:

$$\% \text{ of Reduction} = \frac{(A_s - A_c)}{A_s} \times 100$$

Where A_c was the absorbance of the control (blank, without extract) and A_s was the absorbance in the presence of the extract.

Statistical analysis:

To study the variance of antioxidant activity of *F. carica* extracts at different concentration, data were processed by one-way ANOVA was analysed statistically using IBM SPSS Statistics version 25 software. Statistically significant differences were determined by Tukey's Honest Significant Difference (HSD) post hoc test. $p < 0.01$ were considered statistically significant. All data were analysed in triplicate and expressed as means±standard deviation (SD). The experimental results obtained were expressed as a mean±standard deviation of the mean of three replicates. One way analysis of variance (ANOVA) was computed to compare the free radical scavenging activity and reducing ability of the extracts at different concentrations.

RESULTS AND DISCUSSION:

DPPH radical scavenging activity:

DPPH radical scavenging activity of figs (sample A and B) and standard as ascorbic acid were investigated. The DPPH radical was widely used to evaluate the free-radical scavenging capacity of antioxidants[20]. Recently, the use of the DPPH• reaction has been widely diffused among food technologists and researchers, for the evaluation of free radical scavenging activity on extracts from plant, food material or on single compounds. In the DPPH assay, the antioxidant was able to reduce the stable radical DPPH to the yellow colored 1, 1-diphenyl-1, 2-picryl hydrazine. The molecule of 2, 2-diphenyl-1-picryl hydrazine is characterised as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole. The proton transfer reaction of the DPPH• free radical by a scavenger (A-H) causes a decrease in absorbance at 517nm, which can be followed by a common spectrophotometer set in the visible region. The effect of antioxidants on DPPH• is thought to be due to their hydrogen donating ability[21]. The DPPH radical scavenging activity of fig extract is shown in table 2.

Table.2: DPPH radical scavenging activity of fig extract

Concentration µg/mL	Sample A (% of inhibition) Mean±SD#	p value	Sample B (% of inhibition) Mean±SD#	p value	Standard as Ascorbic acid (% of inhibition) Mean±SD#	p value
20	24.50±0.50[a]	0.001**	24.64±0.16[a]	0.001**	25.16±0.37[a]	0.001**
40	52.00±0.18[b]		46.38±0.77[b]		61.11±0.64[b]	
60	73.27±0.71[c]		69.49±0.41[c]		88.34±0.74[c]	
80	86.43±0.86[d]		77.86±0.92[d]		99.22±0.65[d]	

**Different letters (a-d) for each column symbolise Significant differences at $p < 0.01$ by means of Tukey's HSD test.
#Values are the mean of triplicates

The ethanol extract of Sample A (with seeds) and B (without seeds) exhibited a significant dose dependent inhibition of DPPH activity. The potential of L-ascorbic acid to scavenge DPPH radical is directly proportional to the concentration. The DPPH radical scavenging activity of extracts is near to the scavenging activity of standard ascorbic acid. The maximum DPPH radical scavenging activity of Sample A was 86.43±0.86% and Sample B was 77.86±0.92% at 80µg/mL concentration. The IC_{50} (Inhibitory Concentration$_{50}$) of DPPH radical scavenging activity of fig extracts was Sample A 38.46µg/mL and Sample B 43.12µg/mL concentration respectively which was compared to the IC_{50} of standard ascorbic acid of 32.72µg/mL concentration. It can be inferred that the Sample A had a better free radical scavenging activity than the Sample B. This is probably because the antioxidant compounds in seeds and pulp have a synergistic antioxidant potential in Sample A was better when compared to Sample B.

Nitric oxide scavenging activity:

Nitric oxide (NO•) released from sodium nitroprusside (SNP) has a strong NO^+ character which can alter the structure and function of many cellular components. The

extract of sample A and B exhibited good NO• scavenging activity leading to the reduction of the nitrite concentration in the assay medium. The fig *sample A and B* in SNP solution significantly inhibited the accumulation of nitrite, a stable oxidation product of NO• liberated from SNP in the reaction medium with time compared to the standard ascorbic acid. The toxicity of NO• increases when it reacts with superoxide to form the peroxynitrite anion (•ONOO-), which is a potential strong oxidant that can decompose to produce •OH and NO_2[22]. The nitric oxide scavenging activity of sample A and B and ascorbic acid is presented in (Table 3).

The maximum Nitric oxide radical scavenging activity of Sample A was 88.37±1.01% and Sample B was 84.35±1.20% at 80 µg/mL concentration. The IC_{50} (Inhibitory Concentration$_{50}$) of Nitric oxide radical scavenging activity of fig (Sample A and B) extract was Sample A 35.35 µg/mL and Sample B 37.74 µg/mL concentration respectively which was compared to the IC_{50} of standard ascorbic acid of 34.08 µg/mL concentration. It can be inferred that the Sample A had a better free radical scavenging activity than the Sample B. This is probably because the antioxidant compounds in Sample A are better when compared to Sample B.

Phosphomolybdenum reduction assay:
The phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/ Mo (V) complex with a maximal absorption at 695 nm. The assay is successfully used to quantify vitamin E and, being simple and independent of other antioxidant measurements commonly employed, it was decided to extend its application to fig (Sample A and B) extract[19]. Phosphomolybdenum reduction by Sample A, B and ascorbic acid is presented in (Table 4).

The Phosphomolybdenum reduction activity reveals that the antioxidant activity of the extract is in the increasing trend with the increasing concentration of the extract. The maximum phosphomolybdenum reduction activity of Sample A was 83.64±0.77% and the Sample B was 79.26±0.72% at 80µg/mL concentration. The RC_{50} of Sample A fig was 41.18µg/mL concentration and Sample B fig was 45.53µg/mL concentration, compared to the 39.03µg/mL concentration of the standard ascorbic acid. It is obvious that Sample A had a better Phosphomolybdenum reducing power when compared to Sample B of fig.

From the results it is evident that the free radical scavenging activity and reducing ability of both the Samples of fig extract increased with increasing concentration. There was a significant difference ($p<0.01$) by means of Honest Significant Difference (HSD) post hoc test.

Table 3: Nitric oxide scavenging activity

Concentration µg/mL	Sample A (% of inhibition) Mean±SD[#]	p value	Sample B (% of inhibition) Mean±SD[#]	p value	Standard as Ascorbic acid (% of inhibition) Mean±SD[#]	p value
20	24.53±0.65 [a]	0.001**	19.22±0.25 [a]	0.001**	26.90±0.65 [a]	0.001**
40	56.58±0.59 [b]		52.99±1.72 [b]		58.68±1.29 [b]	
60	79.39±0.69 [c]		76.02±0.57 [c]		84.14±0.85 [c]	
80	88.37±1.01 [d]		84.35±1.20 [d]		95.81±0.57 [d]	

** Different letters (a-d) for each column symbolise Significant differences at p <0.01 by means of Tukey's HSD test.
[#]Values are the mean of triplicates

Table 4: Phosphomolybdenum reduction activity

Concentration µg/mL	Sample A (% of reduction) Mean±SD[#]	p value	Sample B (% of reduction) Mean±SD[#]	p value	Standard as Ascorbic acid (% of inhibition) Mean±SD[#]	p value
20	21.24±0.72 [a]	0.001**	20.12±0.79 [a]	0.001**	21.85± 0.43 [a]	0.001**
40	48.56±0.88 [b]		43.93±0.50 [b]		51.23± 0.12 [b]	
60	71.48±1.21 [c]		68.95±0.57 [c]		72.99± 0.53 [c]	
80	83.64±0.77 [d]		79.26±0.72 [d]		86.18± 0.87 [d]	

** Different letters (a-d) for each column symbolise Significant differences at p <0.01 by means of Tukey's HSD test.
[#]Values are the mean of triplicates

CONCLUSION:

When the concentration of fig (Sample A and Sample B) extract increases the radical scavenging activity and reducing ability of the extract increases gradually. Thus, the result of the free radical scavenging assays and reducing ability assays strongly suggests that the two samples of fig ethanol extracts possess good antioxidant property, in which sample A (pulp and seed) possess greater antioxidant potential than Sample B (pulp) when compared to standard ascorbic acid. This might be due the additional effect of seeds which could have enhanced the antioxidant value of Sample A. Thus, fig being rich source of antioxidants helps as free radical scavenger and the it can be effectively used as an

alternative for synthetic antioxidant since the antioxidant potential of figs is nearly equal to the IC_{50} and RC_{50} values of standard ascorbic acid, which could positively impact our health.

CONFLICT OF INTEREST:

Nil .

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