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Determination of Antioxidant Activity, Total Phenolic Content and Total Flavonoid Content of *Ficus Carica* Stem Bark

***Dr. A. SwaroopaRani* [a*], *Dr.A.Kiran Kumar* [b]**

[1.] *Associate Professor of Bio-Technology, JNTUA-OTPRI, Beside Collector Office, Anantapuramu, Andhra Pradesh-515001, India.*

[2.] *Assistant professor, Deartmnt of Chemistry, University College of Technology, Osmania University, Hyderabad-500007, Telangana, India.*

ABSTRACT

Phytochemicals like carotenoids, tocopherols, ascorbates and phenols present in the plants are strong antioxidants and have an important role in the health care system. There is growing interest in correlating the phytochemical constituents of a plant with its pharmacological activity. Therefore, the present study investigates the content of total phenolics, flavonoids and the antioxidant activity of stem bark extracts of Ficus carica by using in vitro antioxidant models.

Keyword: *Ficus carica stem bark,* antioxidant activity, total phenolic content, total flavonoid content

1 INTRODUCTION

Antioxidant phytochemicals are present in almost all fruits, vegetables, and medicinal plants and play an important role in the prevention and treatment of chronic diseases instigated by oxidative stress. Some of the phytoconstituents of *Ficus carica* are used in the production of sunscreen and coloring agents [1]. Thousands of phytochemicals have been identified so far, yet there are a lot more to be studied. Phytochemicals are basically present in plants for protecting them against diseases. When consumed by humans, they protect us as well like mild constipation [2]. Polyphenols and carotenoids are the two main types of antioxidant phytochemicals, which contribute the most to the antioxidant properties [3]. Natural polyphenols are the most abundant antioxidants in human diets, and their radical scavenging activities are well known [4]. The present study was conducted on *Ficus carica* stem bark crude extracts to evaluate their phenolic and flavonoid content and to make a base for further study of their radical scavenging capacity, a most suitable solvent for maximum phenolic and flavonoid content is determined in this study. Antioxidants protect cells against the damaging effects of reactive oxygen species otherwise called, free radicals such as singlet oxygen, super oxide, peroxyl radicals, hydroxyl radicals and peroxynite which results in oxidative stress leading to cellular damage [5].

2 MATERIALS AND METHODS

SIMPLE INVITRO ASSAY METHODS

2.1 ANTIOXIDANT ACTIVITY ASSAYS

Spectrometric techniques of antioxidant assay rely on the reaction of a radical, radical cation or complex with an antioxidant molecule capable to donate a hydrogen atom.

2.1.1 DPPH- *(Diphenyil – picrylhydrazyl) radical scavenging meth*

The molecule, 2, 2 – Diphenyil – picrylhydrazyl (DPPH•) is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecule do not dimerise, as would be the case with most other free radicals. The delocalization gives rise to the deep violet colour, characterized by an absorption band in methanol solution centered at about 517 nm. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives reduced form with the loss of the violet colour. Therefore, the absorbance diminution depends linearly on the antioxidant concentration. The pale colour may appear at the end due to still presence of pecryl residue [6].

2.1.2 PFRAP (potassium ferricyanide reducing power) method

Reducing power activity by Oyaizu method **[7]** Antioxidants in plants may disrupt the Fe^{3+} to Fe^{2+} transformation by competing with $O_2^{·-}$ and thereby, causing a decrease in the formation of hydroxyl radicals. The antioxidant present in the sample reduced the oxidant probe and the respective product interacted with some colouring agents to form a coloured complex, in this method. The antioxidants reduced the Fe^{3+} to Fe^{2+}. This ion then conjugated with the ferricyanide ion to form a Prussian blue coloured product, which id spectrophotometrically measured at 700 nm. The change in optical density is directly related to total reducing power of the electron donating antioxidants available in the reaction mixture.

2.1.3 Total antioxidant capacity by Phosphomolybdenum

Total antioxidant capacity of the samples was evaluated by the green phosphomolybdenum complex formation according to the method [8]. In this method is based on the reduction of phosphomolybdic acid to phosphomolybdenum blue complex by sodium sulfide. The obtained phosphomolybdenum blue complex is oxidized by the addition of nitrite and this causes a reduction in intensity of the blue colour.

2.2 TOTAL PHENOLIC CONTENT

DETERMINATION OF TOTAL PHENOL BY FOLIN-REAGENT METHOD

Total phenol content was determined by Folin-Ciocalteu reagent method [9] with modification. From each 1mg of crude extracts was dissolved in 1ml of solvents. A total of 10% Folin-Ciocalteu reagent was prepared by adding 5ml of Folin-Ciocalteu reagent in 45ml water. Then, 10% Na_2CO_3 was prepared by dissolving 5 g of Na_2CO_3 in 50ml water. Each crude sample was taken in a test tube and 0.5ml of 10% Folin-Ciocalteu reagent was added and vortex them. Finally, 0.5 ml of 10% Na_2CO_3 was added to the solution and mixed well. Again all the test tube was kept in the dark for 1hr. The absorbance was measured for all solution by using UV-spectrophotometer at constant wavelength 760 nm. Total content of phenolic in the plant extracts were expressed as gallic acid equivalents (mg of GAE/g sample) and were calculated by the formula:

$$T= (C \times V) / M$$

Where, T=total content of phenolic compounds (mg of GAE/g sample) C=the concentration of gallic acid established from the calibration curve (mg/ml) V= volume of extract (ml) M= weight of plant extract (gram).

2.3 TOTAL FLAVONOID CONTENT

DETERMINATION OF TOTAL FLAVONOID CONTENT BY ALUMINIUM CHLORIDE COLORIMETRIC METHOD

In this method [10] quercetin was used as standard flavonoid to make the calibration curve. 10 mg of quercetin was dissolved in10ml methanol. A calibration curve was made by measuring the absorbance of the dilutions at 415 nm (λmax of quercetin) with a ShimadzuUV-180 spectrophotometer. 10% Aluminium chloride and 1M potassium acetate solutions were prepared. Total content of flavonoid in the plant extracts were expressed as quercetin equivalents (mg of QE/g sample) and were calculated by the formula:

$$T = (C \times V) / M$$

Where, T = total content of flavonoid compounds (mg of QE/g sample) C = concentration of quercetin established from the calibration curve (mg/ml) V = volume of extract (ml) M = weight of plant extract (gram).

Preparation of Test Solutions: Different concentrations of Plant extract samples were taken (i.e., 10, 20, 30, 40, 50, 60, 70, 80, 90). 0.1ml aluminium chloride, 0.1 ml potassium acetate solution and 2.8 ml distilled water were added and mixed well after 30 min of incubation in dark their absorbance was measured at 415nm.

All tests such as antioxidant activity, total phenolic content and flavonoid content were performed in triplicate for each independent sample to be analysed. All data were expressed as mean ± standard deviation. Linear regression analysis was used to calculate IC50 value.

3.RESULTS & DISCUSSION

The total phenolic content of *Ficus carica* was determined according to the method reported by using folin-ciocalteu reagent (FCR) as oxidizing agent and gallic (GA) acid as standard. Phenolic compounds are a class of antioxidant agents which act as free radical terminators. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donars and singlet oxygen quenchers. Table shows the contents of total phenolics and flavonoids. Total phenolic contents were measured by Folin Ciocalteu reagent and gallic acid as standard (Standard curve equation: $Y = 0.0392x - 0.3906$, $R2= 0.999$). Total phenolic content of methanolic plant extract as shown higher phenolic content 0.1147 ±0.06755 than ethyl acetate 0.08988 ±0.09708, acetone 0.0593 ±0.05683 and ethanol 0.0364 ±0.02401 plant extract.

Total flavonoidal contents were measured by aluminium chloride method and Quercetine as standard (Standard curve equation: $Y = 0.022x - 0.2376$, $R2= 0.996$). Total flavonoid content of acetone 0.1572 ±0.1160 extract as shown higher flavonoid content than methanol 0.038 ±0.03199, ethanol 0.0238 ±0.01848 and ethyl acetate 0.00466 ±0.003905.

Table 1: TPC &TFC values of extract were represented by mean±SD (TPC-Total phenolic content, TFC-Total flavonoid content).

Plant Extract / Methods	Solvent extract				Standard	
	Methanol	**Ethanol**	**Acetone**	**Ethyl acetate**	**Gallic acid**	**Quercetine**
TPC	0.1147± 0.06755	0.0364± 0.02401	0.0593± 0.05683	0.08988± 0.09708	1.3726 ± 0.7358	-
TFC	0.038± 0.03199	0.0238± 0.01848	0.1572± 0.1160	0.00466± 0.003905	-	0.861± 0.6027

Antioxidant Activity assays

The antioxidant activity of DPPH scavenging assay, reducing power assay and total phosphomolybdenum assay of different solvent extracts of plant results were shown in the table 2 & table 3 below with their mean ±sd values and Ic_{50} values respectively with standard extract.

DDPH scavenging activity of acetone extract has high antioxidant activity than other extracts when compared to the standard extract i.e., The standard ascorbic acid has shown 398.23 ±0.2 to which methanol plant extract is higher i.e., 165.95±0.12 thus, it indicates methanol extract has higher antioxidant activity than the other solvent plant extracts.

Reducing power activity of ethanolic and methanolic extract has high antioxidant activity than the ethyl acetate and acetone extract when compared to the standard extract i.e., methanolic extract as 13.86886 ±9.53981 and ethanolic extract as 13.2885 ±10.67648 which is higher than the standard BHT 11.79 ±6.57435 which are expressed by mean ±standard deviation of triplicates

Total phosphor molybdenum assay of acetone extract has shown higher antioxidant activity than the other solvent plant extract when compared to the standard extract. The standard ascorbic acid has shown 369.0512 ±202.09 to which acetone plant extract is higher thus, it indicates acetone extract has higher antioxidant activity than the other solvent plant extracts.

Table 2: Antioxidant activities were expressed as mean±SD values for DPPH, Reducing power and Phosphomolybdenum assay (BHT- Butylated hydroxytoluene, AA- ascorbic acid).

Plant extract	Solvent extract				Standard	
Assays	Methanol	Ethanol	Acetone	Ethyl acetate	BHT	AA
DPPH	165.9± 0.12	76.253± 0.18	98.593± 0.58	45.983± 0.37	-	398.2± 0.2
Reducing Power assay	13.86886 ± 9.53981	13.2885 ± 10.67648	3.57086 ± 3.47912	7.30851 ± 4.3295	11.79 ± 6.57435	-
Phospho Molybde-num assay	109.59 ±94.17236	165.6503 ±133.5957	410.402 ±335.9248	30.5891 ±30.10263	-	369.0512 ±202.09

As IC_{50} value increases the antioxidant activity decreases and vice versa. Thus, the below table shows that DPPH activity of methanol and acetone plant extract has shown higher activity i.e., 28.75 and 42.469 respectively.

Table 3: IC_{50} values for DPPH, reducing power and phosphomolybdenum activity assay (BHT- Butylated hydroxytoluene, AA- ascorbic acid).

Plant extract	IC_{50}				
Assays	Methanol	Ethanol	acetone	ethyl acetate	Standard
DPPH	28.75	53.526	42.469	69.231	25.62(AA)
Reducing power	122.46	114.9236	353.835	256.371	165.56(BHT)
Phospho-molybdenum assay	35.587	28.4595	21.0996	76.1225	7.1778(AA)

Reducing power activity of ethanol and methanol plant extract as shown higher activity i.e., 114.92 and 122.46 respectively while acetone and ethyl acetate as shown higher IC_{50} than the standard BHT 165.56 which implies lower antioxidant activity. Total phosphomolybdenum assay of acetone plant extract 21.099 as shown lower IC_{50} thus, higher antioxidant activity.

4. CONCLUSION

The present study includes preliminary qualitative phytochemical constituents and quantitative phenolic and flavonoid content of extract antioxidant activity. Phytochemicals can be used for the formulation of compound drugs. These plants have a great medicinal value as it has been reported to have versatile phytochemical constituents including flavonoid, phenols, tannins, proteins, alkaloids, steroids, glycosides, quinones, coumerins, resins, terpenoids etc. plant extract of different solvents exhibited Phenolic content for methanol extract and flavonoid content for acetone extract has shown maximum results compared to the other solvent extracts. Antioxidant activity of methanol and acetone extract has were considerably more effective radical scavengers then the other solvent extracts of *Ficus carica* stem bark. Further, it was observed that there was a strong correlation of higher antioxidant activities with that of total phenolic and flavonoid content. Therefore, the results encourage the use of *Ficus carica* stem bark extracts for medicinal health, functional food and nutraceuticals applications, due to their antioxidant properties.

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