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**ORIGINAL RESEARCH ARTICLE**

# Antioxidant Activity of Four Different Solvent Extracts of the Bark of *Ficus arnottiana* Miq. (M)

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## ABSTRACT

**Objectives:**

Many diseases are associated with oxidative stress caused by free radicals. The Present research was carried out to evaluate in vitro antioxidant activity potential by five different methods of various extracts of bark of *Ficus arnottiana* Miq.

**Methods:**

Antioxidant activity was determined by using five different *in vitro* assay including total phenolic content (TPC), Total reducing power, DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging, Total flavonoid content and Hydroxyl ion Scavenging assay.

**Results:**

The decreasing order of antioxidant activities is acetone extract (FAAE)>Methanol extract (FAME)>petroleum ether extract (FAPEE)>chloroform extract (FACE) in all the methods which is in conformity with TPC. The results clearly demonstrate that acetone extract has highest TPC and displayed strongest activity, and can be used to prevent oxidative stress related diseases.

**Conclusion:**

The processing of perishable bark of *Ficus arnottiana* Miq.by selective extraction with acetone can give better yield of antioxidants and the extract can be stored as food supplement with longer shelf life. Further investigation of individual isolated compounds, their *in vivo* antioxidant activities and in different antioxidant mechanisms is needed.

**Key words**: Antioxidant activity DPPH, Total phenolic content, *Ficus arnottiana* Miq.

**Abbreviations**

DPPH-1, 1-Diphenyl-2-picrylhydrazyl; FAME- Methanol extract of the bark of *Ficus arnottiana* Miq.
FAAE- Acetone extract of the bark of *Ficus arnottiana* Miq.; FACE- Chloroform extract of the bark of *Ficus arnottiana* Miq.; FAPEE- Petroleum ether extract of the bark of *Ficus arnottiana* Miq.
BHT- Butylated hydroxytoluene ; TPC- Total phenolic content.

## INTRODUCTION

Consumption of dietary antioxidants of vegetables and fruits origin plays a positive role in the enhancement of health status in human being [1]. Particularly, regulated production of reactive oxygen species (ROS) maintains the redox homeostasis that is essential for the physiological health of organisms [2]. However, during these metabolic processes, excessive production of ROS escapes from the protective shield of antioxidant mechanisms, causing oxidative damage to cellular components such as DNA, proteins, and lipids. Moreover, the oxidative stress caused from imbalance between the generation and the neutralization of ROS by antioxidant mechanism is responsible for many human diseases, including aging, cancer, sexual dysfunction and neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, and Huntington's disease [3-6].

*Ficus arnottiana (F. arnottiana)* Miq. is a glabrous tree belonging to family Moraceae also known as Paras pipal. It is distributed throughout India; mostly in rocky hills 1 350 m elevations. The leaves of the plant are used for controlling

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fertility. Bark of the plant is used as astringent, aphrodisiac, demulcent, depurative, emollient. It is also useful in inflammation, diarrhea, diabetes, burning sensation, leprosy, scabies, wounds and skin diseases. The fruits of the plant contain - sitosterol, gluanol acetate, glucose, friedelin [7].

Though the plant and its extracts have been used in the folk medicine extensively, but no scientific evidence for such activities is available in established scientific journals of repute. The present study aims to study the antioxidant potential of the bark of this plant. Four different solvents were used to prepare the bark extracts in order to investigate the best solvent for antioxidant activity .

## MATERIALS AND METHODS

### Instruments

Shimadzu UV–VIS Spectrophotometer (1700) was used for all spectrophotometric studies. Rotavapor was used for vacuum drying and Centrifuge was used for centrifugation. Cyclomixer was used for rapid mixing.

### Chemicals and reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH), quercetin and gallic acid were obtained from Hi-Media. And others chemicals were obtained from SD fine chemicals, Mumbai. All the other chemical and reagents used in this study are analytical grade.

### Plant material

The bark of *Ficus arnottiana* Miq. were collected fresh from Balawala, Dehradun, Uttarakhand, India in the month of Nov. 2011. The plant was identified, authenticated and certified by botanist Dr. R. M. Painuli, Department of Botany H. N. B. Garhwal (A Central University) Srinagar Garhwal, Uttarakhand India.

### Preparation of the Plant extracts

The bark was extracted successively with petroleum ether, chloroform, acetone and Methanol. All the extracts thus obtained and kept in desicators for future use. The extracts were cooled at room temperature, filtered and evaporated to dryness under reduced pressure in a rotatory evaporator.

### DPPH radical scavenging activity

DPPH radical scavenging activity was evaluated according to the method described by Nagai *et al*. The assaymixture contained 0.3 ml of 1.0 mM DPPH radical solution, 2.4 ml of 99 % ethanol, and 0.3 ml of test sample solution of different concentrations. The solution was rapidly mixed and scavenging capacity was measured spectrophotometrically by monitoring the decrease in absorbance at 517 nm. Ascorbic acid was used as positive control while reaction mixture (DPPH radical solution) minus extract solution was taken as control. The percent (%) radical scavenging was calculated by the following equation [8].

$$\% \text{ radical scavenging} = \frac{Ac - As}{Ac} \times 100$$

Where Ac = Absorbance of control at 517 nm ; As= Absorbance of sample

### Total Reducing power assay

Total reducing power was determined as described by Zhu *et al*. Plant extracts or compounds (varying concentrations) in 1 ml of distilled water were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1 % potassium ferricyanide [$K_3Fe(CN)_6$]; the mixture was then incubated at 50°C for 30 min. Afterward, 2.5 ml of trichloroacetic acid (10 %) was added to the mixture, which was then centrifuged at 3,000 rpm for 10 min. Finally 2.5 ml of the upper layer solution was mixed with 2.5 ml of distilled water and 0.5 ml $FeCl_3$ (0.1 %) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power [9].

### Determination of total phenolic content

The method of Saucier and Waterhouse was used with slight modification, and the results are expressed as gallic acid equivalents (GAE). In each analysis, 20 µl of sample solution was mixed with 1.58 ml of water and 100 µl of Folin-Ciocalteau (FC) reagent. After 2 min, 300 µL of a 20 % sodium carbonate solution was added. The solutions were left at room temperature for 2 h. Then the absorbance of the developed blue color was determined at 765 nm. The amount of light absorbed is proportional to the amount of oxidizable material present, that is, phenolic compounds. Gallic acid was used as a standard for the calibration curve. The total phenolic content is reported as gallic acid equivalents (µg) using the following linear equation based on the calibration curve:

$$A = 0{:}0011x + 0{:}0025 \; ; \quad R^2 = 0{:}9995$$

Where A is the absorbance and x is the gallic acid equivalents (µg) [10].

### Total flavonoid content

The total flavonoid content in the extracts was determined using Aluminum chloride colorimetric Method. Quercetin was used to make the calibration curve. Quercetin was dissolved in 80% ethanol and then diluted to 4, 8, 12, 16 and 20

mg/100 ml. The diluted standard solutions (0.5 ml containing 500 µg) from extracts were separately mixed with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. After incubation at room temperature for 30 minutes, the absorbance of the reaction mixture was measured at 415 nm with a double beam UV spectrophotometer. The amount of 10% aluminum chloride was substituted by the same amount of distilled water in blank. The total flavonoid content was expressed as mg Quercetin equivalent/g of sample [11].

**$OH^-$ Scavenging assay:**

$OH^-$ Scavenging ability was measured according to a Literature procedure (Wang et al., 2008) with few modifications. OH radicals were generated from $FeSO_4$ and $H_2O_2$, and detected by their ability to hydroxylate salicylate. The reaction mixture (3 ml) contained 1 ml $FeSO_4$ (1.5 mM), 0.7 ml $H_2O_2$(6mM), 0.3 ml sodium salicylate (20 mM) and varying concentrations of extracts. After incubation for 1 hour at 37 °C, the absorbance of the hydroxylated salicylate complex was measured at 562 nm. Butylated hydroxytoluene (BHT) was used as a positive control.

The percentage scavenging effect was calculated as:

**Scavenging Rate= $1-(A_1-A_2)/A_0 \times 100\%$**

Where $A_0$is the absorbance of the control (without extract) and $A_1$ is the absorbance in the presence of the extract, $A_2$ is absorbance of Standard [12].

**Data analysis**

All assays were carried out in triplicate and the results were expressed as Mean ± SD.

## RESULTS

**Total Phenolic content**

Total Phenolic content in the acetone extract was found to be 53.42 µg/ml. Gallic acid equivalent of Phenol/g of sample respectively.

**Total Flavonoid Content:**

Total Flavonoid Content of acetone extract was found to be 13.6 42 µg/ml. Quercetin equivalent/g of sample respectively. Acetone extract was rich in flavonoids.

**Total reducing Power**

The results of this study show that the reducing power of all the extracts was less than that of BHT. There was significant change in the reducing power of the various extracts with increase in their concentration. The reducing power of the FAAE was most active than all extracts of the plant bark. Table 1 Show the reducing activity of various extracts of *Ficus arnottiana* Miq. Bark.

The extracts exhibited the activity in a dose dependent manner. In an overall reducing power analysis the test can be arranged as BHT > FAAE > FAME > FAPEE > FACE.

**Table 1: Total reducing power of various extracts of *Ficus arnottiana* Miq. Bark**

| Concentration (mg/ml) | BHT | FAPEE | FACE | FAAE | FAME |
|---|---|---|---|---|---|
| 0.025 | 0.214±0.003 | 0.172±0.0017 | 0.162±0.0007 | 0.192±0.0005 | 0.183±0.0005 |
| 0.05 | 0.253±0.002 | 0.192±0.0010 | 0.184±0.0011 | 0.213±0.0006 | 0.201±0.0008 |
| 0.1 | 0.432±0.002 | 0.267±0.0006 | 0.254±0.0007 | 0.404±0.0008 | 0.354±0.0009 |
| 0.2 | 0.602±0.002 | 0.396±0.0008 | 0.353±0.0099 | 0.592±0.0008 | 0.512±0.0008 |
| 0.3 | 0.986±0.002 | 0.547±0.0007 | 0.457±0.0011 | 0.952±0.0012 | 0.822±0.0016 |

**$OH^-$ Scavenging activity**

The results of hydroxyl ion scavenging activity show that the scavenging power of all the extracts was less than that of BHT. There was significant change in the scavenging activity of the various extracts with increase in their concentration. The hydroxyl ion scavenging activity of FAAE was most active than all extracts of the plant bark. Table 2: Show the scavenging activity of various extracts of *Ficus arnottiana* Miq. Bark. The extracts exhibited the activity in a dose dependent manner. In an overall reducing power analysis the test can be arranged as BHT > FAAE > FAME > FAPEE > FACE.

**Table 2: The $OH^-$ scavenging activity of various extracts of *Ficus arnottiana* Miq. Bark. (DPPH scavenging activity)**

| Concentration (mg/ml) | BHT | FAPEE | FACE | FAAE | FAME |
|---|---|---|---|---|---|
| 0.05 | 30.255±0.005 | 23.131±0.002 | 21.882±0.001 | 28.502±0.0026 | 26.881±0.0017 |
| 0.1 | 42.762±0.002 | 35.303±0.002 | 33.882±0.002 | 40.416±0.0031 | 38.632±0.0014 |
| 0.2 | 58.621±0.002 | 46.812±0.002 | 40.633±0.0019 | 54.511±0.0017 | 50.252±0.0021 |
| 0.3 | 75.823±0.002 | 51.381±0.001 | 48.129±0.0017 | 68.102±0.0018 | 59.631±0.0016 |

The results of DPPH Scavenging activity show that the Scavenging power of all the extracts was less than that of ascorbic acid. There was significant change in the reducing power of the various extracts with increase in their concentration. The DPPH Scavenging power of the FAAE was most active than all extracts of *Ficus arnottiana* Miq. Bark. Table 3: Show the DPPH scavenging activity of various extracts of *Ficus arnottiana* Miq. Bark. In an overall Scavenging power analysis the test can be

arranged as Ascorbic acid > FAAE > FAME > FAPEE > FACE.

**Table 3: The DPPH scavenging activity of various extracts of *Ficus arnottiana* Miq. Bark.**

| Concentration (mg/ml) | FAPEE | FACE | FAAE | FAME | Ascorbic Acid |
|---|---|---|---|---|---|
| 0.025 | 11.04±0.008 | 4.79±0.006 | 45.83±0.006 | 18.95±0.006 | 73.83±0.006 |
| 0.05 | 36.87±0.007 | 33.54±0.005 | 58.12±0.006 | 44.58±0.006 | 78.95±0.006 |
| 0.1 | 58.75±0.012 | 58.12±0.008 | 77.50±0.006 | 67.08±0.006 | 83.12±0.006 |
| 0.2 | 81.04±0.015 | 77.50±0.013 | 84.37±0.006 | 82.91±0.006 | 87.08±0.006 |
| 0.3 | 83.75±0.009 | 79.58±0.006 | 92.91±0.006 | 88.54±0.006 | 94.58±0.006 |

## DISCUSSION

In our study, the decreasing order of antioxidant activity among the *Ficus arrnottiana* Miq.bark extracts assayed through all the five methods was found to be FAAE>FAME>FAPEE>FACE. The results revealed that the acetone extract exhibited highest antioxidant activity followed by methanol, petroleum ether and chloroform extract. The antioxidant effect of *Ficus arrnottiana* Miq. Bark could be exhibited due to the presence of tannins and flavonoids.

## CONCLUSION

The bark extracts of *Ficus arrnottiana* Miq. exhibited good but different levels of antioxidant activity in all the models studied. The FAAE had potent antioxidant activity as compare toother extracts. Further investigation of individual isolated compounds, their *in vivo* antioxidant activities and in different antioxidant mechanisms is needed.

## CONFLICT OF INTEREST STATEMENT:

We declare that we have no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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