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# Assessment of antioxidant activity of *Ficus asperifolia* Miq aqueous extract - In vitro studies

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## Abstract

Antioxidants play an important role in inhibiting and scavenging free radicals, thus providing protection to human against degenerative diseases. The aim of this study was to assess the in vitro antioxidant activity of *Ficus asperifolia* aqueous extract. In this study, the free radical scavenging potential of a aqueous extract of the leaves of *Ficus asperifolia* was assessed by measuring its capability for scavenging 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, hydroxyl radical (OH), nitric oxide radicals (NO), iron chelation as well as its reductive ability. Total phenolic and flavonoid contents were determined by spectrophotometric methods. The aqueous extract of *Ficus asperifolia* had a DPPH scavenging activity of 78.65 ±1.15% at 5 mg/ml and a reductive potential of 44.05 ± 0.05 mm.AAE/g at 5 mg/ml. The plant extract yielded 69.20 ± 0.00 mg garlic acid equivalents phenolic content and 39.90 ± 0.00 mg rutin equivalents flavonoid content. The observed antioxidant potentials and phenolic content of the extract suggest that an aqueous extract of *Ficus asperifolia* leaves is a potential source of natural antioxidants and may be responsible for its popular and wide traditional use.

**Keywords:** *Ficus asperifolia*, Antioxidant activity, Phenolic content, Flavonoid content.

## Introduction

*Ficus asperifolia* Miq belonging to the Moraceae family is a small or average size tree, terrestrial or epiphyte which can reach 20 m in height. It is found in Nigeria, Senegal, Uganda, Tanzania, Natal (South Africa), Madagascar and Cameroon. *Ficus asperifolia* is abundant in the savannah regions, especially along river banks and marshy areas at an altitude of up to 1100 m. The leaves are enormous and displayed spirally, the limb is largely oval or has a form of ellipse and the roots are most often fibrous.[1] In many traditional medicines of Africa, the leaf extract of *F. asperifolia* is used as an anthelmintic and a purgative.[2] In living cells, reactive oxygen species (ROS) are continuously produced in numerous processes such as mitochondrial respiration, metabolism of Xenobiotics by cytochromes P450, inflammation, and phagocytosis. Exposure to UV and gamma radiation also generates ROS. ROS damage cellular macromolecules (lipids, proteins, nucleic acids) leading to oxidative stress. It has been demonstrated that oxidative stress is involved in many diseases such as cardiovascular diseases, rheumatoid arthritis, neurodegenerative diseases, alcoholic and non-alcoholic steatohepatitis, diabetes mellitus, and cancer. As the incidence of these diseases is constantly increasing the research in the field of natural and synthetic antioxidants is still of high interest.[3, 4] Some of these medicinal plants used in ethno medicine for the treatment and management of many of these diseases have been investigated for their

antioxidative properties.[5, 6] Many of the metabolites from these medicinal plants especially flavonoids exhibited potent antioxidant activity in vitro and in vivo.[7-9] Thus, the objective of this study is to justify the traditional claims we have assessed and the antioxidant effect of *Ficus asperifolia* aqueous extract.

## Materials and Methods

Fresh sample leaves of *Ficus asperifolia* were collected and authenticated at the Department of Plant Sciences, Ekiti State University, Nigeria. The fresh leaves were air dried and finely powdered with an electric grinder.

### Extraction

The aqueous extract of the powered *Ficus asperifolia* Miq was air dried in the laboratory at ambient temperature (30 ±2°C) for 10 days, pulverized using a laboratory mechanical grinder (Christy and Norris limited, machine type 8) and the fine powders obtained stored until further use. 50g of the powdered sample was extracted with distilled water of 500ml for 48hrs. The mixture was decanted and filtered using sterile whatman paper No1. The filterate measured up to 425ml and evaporated to dryness using a freeze dryer to obtain 12 % yield. The crude extract was later subjected to bioassay analyses. From the stock solution, concentration 10mg/ml was obtained by serial dilution.

### Chemicals and reagents

Chemicals and reagents used such as 1,10-phenanthroline, gallic acid, Folin–Ciocalteau's reagent were procured from Sigma–Aldrich, Inc., (St. Louis, MO), trichloroacetic acid (TCA) was sourced from Sigma–Aldrich, Chemie GmbH (Steinheim, Germany), dinitrophenyl hydrazine (DNPH) from ACROS Organics (New Jersey, USA), hydrogen peroxide, methanol, acetic acid and $FeCl_3$were sourced from BDH Chemicals Ltd., (Poole, England), $CuSO_4$•$5H_2O$, $H_2SO_4$, sodium carbonate, $AlCl_3$, potassium acetate, Tris–HCl buffer, sodium dodecyl sulphate, $FeSO_4$, potassium ferricyanide and ferric chloride were of analytical grade while the water was glass distilled.

### Determination of Total phenolic content

Total soluble phenols in the extracts were determined with Folin Ciocalteau reagent using Gallic acid as a standard phenolic compound.[10] 0.1 ml of extract solution taken in a test tube and 1ml of FC reagent was added and 3- 5 min later, 2.5 ml of 20% sodium carbonate was added and the mixture was allowed to stand for 30min with intermittent shaking. The absorbance of the blue colour that developed was read at 765 nm. The concentration of total phenols was expressed as mg/gm of dry extract. The concentration of total phenolic compounds in the extract was determined by using the formula: T=CV/M Where, T= Total phenolic content mg/gm of plant extract in GAE, C= Concentration of Gallic acid from the calibration curve, V= volume of the extract in ml, M= wt of the pure plant methanol extract.

### Determination of total flavonoids

The total flavonoid content of the extract was determined using a colourimeter assay developed.[11] 0.2ml of the extract was added to 0.3ml of 5% NaNO3 at zero time. After 5min, 0.6ml of 10% AlCl3 was added and after 6min, 2ml of NaOH was added to the mixture followed by the addition of 2.1ml of distilled water. Absorbance was read at 510nm against the reagent blank and flavonoid content was expressed as mg rutin equivalent.

### Determination Fe2+ Chelation

The ability of the extract to chelate $Fe^{2+}$ was determined using a modified method.[12, 13] Briefly, 150mM $FeSO_4$ will be added to a reaction mixture containing 168ml of 0.1M Tris-HCl pH 7.4, 218ml saline and extract and the volume is made up 1ml with distilled water. The reaction mixture will be incubated for 5min, before the additional of 13ml of 1, 10-phenantroline the absorbance will be read at 510nm.

### Determination of ferric reducing property (FRAP)

The reducing property of the extract was determined by Pulido R *et al*.[14] 0.25ml of the extract was mixed with 0.25ml of 200mM of Sodium phosphate buffer pH 6.6 and 0.25ml of 1% KFC. The mixture was incubated at 50$^{o}$C for 20min, thereafter 0.25ml of 10% TCA was also added and centrifuge at 2000rpm for 10min, 1ml of the supernatant was mixed with 1ml of distilled water and 0.2m of $FeCl_3$ and the absorbance was measure at 700nm. The ferric reducing antioxidant property was subsequently calculated using ascorbic acid as standard.

### Determination of DPPH radical scavenging ability

The free radical scavenging ability of the extract against DPPH (1, 1- diphenyl-2-picryhydrazyl) using ferric reducing/antioxidant power assay method.[15] 1ml of the extract was mixed with 1ml of the 0.4mM methanolic solution of the DPPH the mixture was left in the dark for 30min before measuring the absorbance at 516nm.

### Determination of Nitric Oxide radical scavenging ability

Sodium Nitropruside in aqueous solution at physiological pH spontaneously generates NO,which interacts with oxygen to produce nitrite ions that can be estimated by use of Greiss reagent. Scavengers of NO compete with oxygen, leading to reduce production of NO. Briefly 5mM sodium nitroprusside in phosphate- saline was mixed with the extract, before incubation at 25$^{o}$C for 150min. Thereafter the reaction mixture was added to Greiss reagent. Before measuring the absorbance at 546nm, relative to the absorbance of standard solution of potassium nitrate treated in the same way with Greiss reagent.[16]

### Determination of Hydroxyl radical scavenging activity

The ability of *Ficus asperifolia* extract to prevent $Fe^{2+/}H_2O_2$ induced decomposition of deoxyribose will be carried out using the method of Halliwell B.[17] Briefly, freshly prepared extract (0-100µl) will be added to a reaction mixture containing 120µl, 20mM deoxyribose, 400µl, 0.1M phosphate buffer pH 7.4, 40µl, 20mM hydrogen peroxide and 40µl, 500µM $FeSO_4$, and the volume will be made to 800µl with distilled water. The reaction mixture will be incubated at 37Oc for 30min and the reaction will be stopped by the addition of 0.5ml of 2.8% TCA, this will be followed by the addition of 0.4ml of 0.6% TBA solution. The tubes will subsequently be incubated in boiling water for 20min. The absorbance will be measured at 532nm in spectrophotometer

### Statistical analysis

For all assays, samples were analysed in triplicate and the results were expressed as mean ± SD. Student t test was used for statistical evaluation; $p < 0.05$ was considered statistically significant.

## Results

The total phenol content and total flavonoid content of leaves of *F. asperifolia* is presented in Table 1. Total phenolic compounds are reported as gallic acid equivalents. The total phenolic contents of the plant were 69.20±0.00 mg gallic acid equivalent/g of sample. The total flavonoid contents of the plant were 39.90±0.00 mg rutin equivalent/g of sample.

**Table 1:** Total Phenolic and Total flavonoids of leaves of *Ficus asperifolia* Miq

| Phenolic content (mg GAE/g) | Flavonoid content (mg RUT/g) |
|---|---|
| 69.20 ± 0.00 | 39.90 ± 0.00 |

Values represent means ± standard deviation of duplicate readings.
GAE - Gallic acid equivalent and RUT - Rutin equivalent

The plant extracts chelate $Fe^{2+}$ at the concentration of the extracts tested (5mg/ml) with *Ficus asperifolia* leaf extract Table 2. Furthermore, the reducing power of the extractable phytochemicals from *Ficus asperifolia* (leaves) expressed as ascorbic acid equivalent (AAE) is presented in Table 2.

**Table 2:** $Fe^{2+}$ chelating ability and Ferric reducing antioxidant properties (FRAP) of aqueous extract of *Ficus asperifolia* Miq

| Fe2+ chelating ability (%) | FRAP (mg.AAE/g) |
|---|---|
| 59.27 ± 0.33 | 44.05 ± 0.05 |

Values represent mean ± standard deviation, number of samples n = 2.

The DPPH radical scavenging ability of the aqueous extract from *Ficus asperifolia* leaves as represented in Table 3 revealed that the extracts scavenged DPPH radicals at the concentration tested. However, the extract competes with oxygen to react with nitric oxide and thus inhibits generation of the anions. Table 3 illustrates the percentage inhibition of nitric oxide generation by the extract of *Ficus asperifolia*. Furthermore, the effect of the aqueous extract of *Ficus asperifolia* on hydroxyl radicals generated by Fe3+ ions was measured by determining the degree of deoxyribose degradation, an indicator of thiobarbituric acid–malonaldehyde (TBA-

MDA) adduct formation. As shown in Table 3 the extract inhibited hydroxyl radical induced deoxyribose degradation with a inhibition of 29.25 ± 0.04% observed at a concentration of 5 mg/ml of extract.

**Table 3:** DPPH radical, Nitric Oxide (NO) and Hydroxyl (OH) radical activity of aqueous extract of *Ficus asperifolia* Miq

| DPPH (%) | NO (%) | OH (%) |
|---|---|---|
| 78.65 ±1.15 | 47.03 ± 0.00 | 29.25 ± 0.04 |

Values represent mean ± standard deviation, number of samples n = 2

## Discussion

Many plants are rich sources of phytochemical, and intakes of these plant chemicals have protective potential against degenerative diseases.[18] Phenolic compounds can protect the human body from free radicals, whose formation is associated with the normal metabolism of aerobic cells. They are strong antioxidants capable of removing free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce a- tocopherol radicals and inhibit oxidases.[19] It has been reported that green leafy vegetables, soft fruits and medicinal plants exhibited higher levels of flavonoids.[20] Phenols and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources, and they have been shown to possess significant antioxidant activities.[21] The presence of derivatives of flavonoids has been found in many herbs; moreover, numerous studies have conclusively shown that the majority of the antioxidant activity maybe from compounds such as flavonoids, isoflavones, flavones, anthocyanins, catechin and isocatechin rather than from vitamins C, E and β-carotene.[19, 22] Flavonoids have antioxidant activity and could therefore lower cellular oxidative stress.[22] Polyphenols are considered to be strong antioxidants due to the redox properties of their hydroxyl groups.[23] The results from the present investigation shows that *Ficus asperifolia* is rich in phytochemicals. Specific biologically important compounds have been identified in extracts from the plant by previous workers.[24-26] The present work also reveals that the extract from the leaves of *Ficus asperifolia* possesses good antioxidant potential presumably because of its phytochemical constituents.[27, 28] The DPPH scavenging activities of *Ficus asperifolia* showed a good correlation with its reductive potentials. These facts justify the medicinal use of the plant for the treatment of various maladies.[29, 30] However, further work is necessary to ascertain the clinical safety of extracts from the plant determine appropriate concentration for therapy so as to safeguard the health of the teeming mass of traditional uses.

## Conclusion

This study shows that *Ficus asperifolia* aqueous extract is an effective scavenger of DPPH, nitric oxide, superoxide and hydroxyl radicals. The antioxidant activity of the extract can mainly be attributed to the polar phenolic compounds which are known to develop significant antioxidant effects.

Regarding the results of in vitro antioxidant assays, *Ficus asperifolia* seems to be a candidate for in vivo assessment of a protective activity against disorders associated with oxidative stress.

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