



## Preliminary Phytochemistry and Antioxidant Activities of the Ethanol Extract of *Ficus abutilifolia* Leaves

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### Abstract

Medicinal plants harbor bioactive compounds utilized for addressing various health challenges. This current research focuses on the study of *Ficus abutilifolia*, collected from Askira Uba, Borno State, Nigeria. Ethanol was employed to extract compounds from the plant leaves. The extracts underwent qualitative phytochemical screening using established procedures. The screening identified the presence of Alkaloids, Saponins, Tannins, Anthraquinones, Steroids, Glycosides, Flavonoids, reducing sugar, amino acids, and terpenoids, while Phytosterol was notably absent. The antioxidant activity of the crude extracts was assessed using two complementary test systems: 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydrogen peroxide free-radical scavenging assays, with Ascorbic acid serving as the standard. The free radical scavenging activity of the leaf crude extracts at different concentrations (2.5, 5, 7.5, and 10  $\mu\text{L}/\text{mL}$ ) demonstrated a concentration-dependent increase. In the DPPH method, the crude extract at a concentration of 10  $\mu\text{g}/\text{mL}$  exhibited 58.2 % antioxidant activity. Similarly, in the hydrogen peroxide radical scavenging assay, it was observed that at a concentration of 10  $\mu\text{g}/\text{mL}$ , the crude extract demonstrated an inhibition of 72.7 %. In conclusion, the ethanol extract from the plant exhibited potent inhibition of free radicals, possibly attributed to the presence of secondary metabolites.

**Keywords:** Phytochemical, DPPH, Antioxidant, Extract, *Ficus abutilifolia*

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## 1 Introduction

The pursuit of natural antioxidants, particularly those of plant origin, has experienced a notable upsurge in recent years. Consequently, various plant fruits, vegetables, seeds, barks, and leaves are subjects of investigation for the detection of these bioactive compounds [1]. Primarily, antioxidants have the greatest defensive role in protecting the cell against oxidative damage. Generally, antioxidants have a vital role in keeping optimal cellular functions and systemic health and well-being [2]. The plant kingdom, being a primary reservoir for materials crucial to the food, pharmaceutical, and cosmetics industries, has given rise to numerous supplements, nutricosmetics, and cosmetics crafted from botanical ingredients, many of which have a rich history of use in traditional or folk medicine. Throughout history, medicinal plants and herbal teas have played a significant role in everyday human life, providing traditional remedies with curative and therapeutic attributes. These plants represent abundant sources of phytochemicals, secondary metabolites responsible for the beneficial properties of herbs [3], [4]. Plant antioxidants, being a natural reservoir of bioactive compounds, play crucial roles in plant acclimation to environmental challenges and confer health benefits to humans [5].

*Ficus*, a genus comprising approximately 850-900 species, with some sources indicating over 1000 species, stands as the largest genus in the Moraceae family [6]. *Ficus abutilifolia*, a small to medium-sized tree seldom exceeding 5 m in height, is commonly found along streams and widely distributed across the African continent [7], [8]. Chemically, *Ficus abutilifolia* contains mainly glycosides such as saponins, flavonoids, anthraquinones, alkaloids, and tannins [9]. Ajayi *et al.* [10] reported the presence of tannins, phlobatannins, flavonoids,

cardiac glycosides, saponins, alkaloids, and steroids in the leaf extract of *Ficus exasperata* of the Moraceae family. Similarly, according to Achi *et al.* [11], phytochemical studies on the aqueous extract of *Ficus capensis* leaves showed high levels of flavonoids, terpenoids, tannins, and alkaloids, while glycosides, saponins, and steroids were present in trace amounts. Muanda *et al.* [12] reported the presence of phenolic compounds in *Ficus capensis*, metabolites responsible for its activity against pathogenic bacteria. Thagriki *et al.* [13] found Alkaloids, Tannins, Saponins, Flavonoids, Glycosides, Proteins, and Phenols in the leaf extract of *Ficus sycomorus*, while Balogun *et al.* [14] reported the presence of reducing sugar, alkaloids, saponins, pyrocatecholic tannins, and free amino acids/amines in the aqueous ethanolic stem bark extract of *Ficus trichopoda*.

The antiradical and antibacterial activity of *Ficus sur* and *Ficus sycomorus* extract could provide scientific support for their traditional use in treating sickle cell disease [15]. Traditionally, the plant has been used to treat various diseases in Nigeria, such as typhoid fever, dysentery, food poisoning, and sexually transmitted infections [9]. Similarly, the leaves have been employed to promote fertility, the latex to remove skin warts, and the bark as a strengthening tonic. Anticonvulsant activity has also been reported against maximum electroshock for the ethanol extract of the root-bark of the plant [8].

While various research studies have explored different species of the genus *Ficus*, scientific findings on the pharmacological activities of *Ficus abutilifolia*, a dominant plant found in Askira Uba, Borno State, Nigeria, remain limited. The objective of this research is to compare the therapeutic properties of this plant with established therapeutic data of other species of the genus *Ficus* from the literature.

## 2 Materials and Methods

### 2.1 Sample Collection

*Ficus abutilifolia*, a member of the Moraceae family (L.), was sourced from the bush area of Askira Uba, Borno State, Nigeria.

### 2.2 Preparation of Sample

The leaves of *Ficus abutilifolia* (L.) plants were meticulously washed and air-dried in Chemistry Laboratory 2, Science Complex of the Faculty of Science, Adamawa State University, Mubi, under shade at room temperature. The dried leaves were subsequently weighed, ground to a coarse powder using a sterile mortar and pestle, and stored in an airtight container for successive analyses [16].

### 2.3 Plant Preparation and Extraction

A 100 g sample of *Ficus abutilifolia* (L.) leaves powder was weighed and subjected to ethanol extraction in an airtight container for 24 hours. The resultant mixture underwent filtration using Whatman No. 1 filter paper under gravity. The filtrate was then dried at 60 °C on a water bath, yielding *Ficus abutilifolia* (L.) leaves ethanolic extract residue [17].

### 2.4 Phytochemical Screening

Phytochemical screening was conducted using standardized procedures to detect the presence of various natural product groups in the ethanolic extracts of *Ficus abutilifolia* (L.).

#### 2.4.1 Test for Essential Oils

Two drops of FeCl<sub>3</sub> were introduced to 90 % alcohol containing a small quantity of the extracts. The appearance of a greenish coloration indicated the presence of essential oils [18].

#### 2.4.2 Test for Alkaloids

Mayer's test: Two drops of Mayer's reagent were added to a few milliliters of the plant sample extract. The appearance of a white creamy precipitate indicated the presence of alkaloids [19].

#### 2.4.3 Test for Phenolic Compounds and Tannins

Ferric Chloride test: The extract (50 mg) was dissolved in 5 mL of distilled water. A few

drops of neutral 5 % ferric chloride solution were added. A dark green color indicated the presence of phenolic compounds and alkaloids [19].

#### 2.4.4 Detection of Saponins

Foam test: A small amount of the extract was shaken with a small quantity of water. The persistence of foam for ten minutes indicated the presence of saponins [20].

#### 2.4.5 Test for Flavonoids

Dilute ammonia (5 mL) was added to a portion of an aqueous filtrate of the extract. Concentrated sulfuric acid (1 mL) was added. A yellow coloration that disappeared on standing indicated the presence of flavonoids [21].

#### 2.4.6 Detection of Cardiac Glycosides

Killer-killani test: 0.5 g of the extract dissolved in 5 mL water was added to 2 mL of glacial acetic acid solution containing one drop of ferric chloride solution. This was underlaid with 1 mL of concentrated H<sub>2</sub>SO<sub>4</sub>. A brown ring at the interface indicated the presence of deoxysugar characteristics of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just above the brown ring and gradually spread throughout this layer [20].

#### 2.4.7 Detection of Phyosterols

Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrate was treated with a few drops of Conc. Sulphuric acid, shaken, and allowed to stand. The appearance of golden yellow color indicated the presence of triterpenes [20].

#### 2.4.8 Test for Reducing Sugars (Fehling's Test)

The aqueous ethanol extract (0.5 g in 5 mL of water) was added to boiling Fehling's solution (A and B) in a test tube. The solution was observed for a color reaction [21].

#### 2.4.9 Detection of Proteins and Amino acids

Xanthoproteic Test: The extract was treated with a few drops of concentrated Nitric acid solution. The formation of yellow color indicated the presence of proteins [20].

#### 2.4.10 Test for Terpenoids (Salkowski Test)

To 0.5 g of each extract, 2 mL of chloroform was added. Concentrated H<sub>2</sub>SO<sub>4</sub> (3 mL) was

carefully added to form a layer. A reddish-brown coloration of the interface indicated the presence of terpenoids [21].

#### 2.4.11 Test for Anthraquinones

0.5 g of the extract was boiled with 10 mL of sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) and filtered while hot. The filtrate was shaken with 5 mL of chloroform. The chloroform layer was pipetted into another test tube, and 1 mL of dilute ammonia was added. The resulting solution was observed for color changes [21].

### 2.5 Determination of Antioxidant Activity of Extract

#### 2.5.1 Free radical scavenging activity

DPPH has been widely used for measurement of free radical scavenging ability of antioxidants. This method is based on the reduction of an alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant [22]. The DPPH assay was performed using a standard method with minor modification. The hydrogen atom or electron donating abilities of the compounds were measured from the bleaching of the purple colored methanol solution of DPPH. This spectrophotometric assay uses the stable free radical, DPPH as a reagent. One thousand microlitres of diverse concentrations (2.5 µL/mL) of the essential oil in ethanol were added to 4 mL of 0.004 % methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm and compared to the standard antioxidants, Ascorbic acid (vitamin C). The DPPH radical scavenging effect was calculated as inhibition of percentage (I %) using the following formula in equation 1 [23].

$$\% \text{ Inhibition} = \frac{\text{absorbance of Blank} - \text{absorbance of sample}}{\text{absorbance of Blank}} \times 100$$

(Equation 1)

Where, A is blank is the absorbance of the control reaction (containing all reagents except the test compound) and A (sample) is the absorbance of the test compound. The values of inhibition were calculated for various

concentrations of the extract. Tests were conducted in triplicate.

#### 2.5.2 Hydrogen Peroxide Scavenging Activity

The ability of the extract to scavenge hydrogen peroxide was determined according to the method with modification. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH = 7.4). Extracts (2.5-25 µg/mL) in methanol were added to a H<sub>2</sub>O<sub>2</sub> solution (0.6 mL, 40 mM). The absorbance value of the reaction mixture was recorded at 230 nm. Blank solution contained the phosphate buffer without H<sub>2</sub>O<sub>2</sub> [24]. The percentage of H<sub>2</sub>O<sub>2</sub> scavenging was calculated as equation 2.

$$\% \text{ Inhibition} = \frac{\text{Control Absorbance} - \text{Sample Absorbance}}{\text{Control Absorbance}} \times 100$$

(Equation 2)

Where A (control) is the absorbance of the control, and A (sample) is the absorbance in the presence of the sample or standards.

### 2.6 Statistical Analysis

All determinations were replicated three times and results were reported in mean.

## 3 Results and Discussion

### 3.1 Phytochemical Screening of Ethanol Extract of *Ficus abutilifolia* Leaves

The preliminary phytochemical screening conducted on the leaves of *Ficus abutilifolia* revealed the presence of various bioactive secondary metabolites, confirmed through chemical color reaction tests, as detailed in Table 1. The results from the phytochemical analysis of *Ficus abutilifolia* leaf extracts demonstrated the presence of essential oil, alkaloid, phenol/tannin, saponin, flavonoids, glycosides, reducing sugar, protein/amino acid, terpenoids, anthraquinones, while phytosterol was found to be absent.

Eshwarappa *et al.* [25] previously reported the presence of only two phytonutrients, flavonoids, and carbohydrates, in the methanol extract of *Ficus glomerata*, which contrasts with the findings of this research. The results of the present study align more closely with the work reported by Taiwo *et al.* [7].

Table 1. Result for qualitative Phytochemical Screening of Ethanolic Extract of *Ficus abutilifolia* Leaves

Phytonutrients	<i>Ficus abutilifolia</i>
Essential oil	+
Alkaloid	+
Phenol/Tannin	+
Saponin	+
Flavonoids	+
Glycosides	+
Phytosterol	-
Reducing sugar	+
Protein/amino acid	+
Terpenoids	+
Anthraquinones	+

Key: + = Present, - = Absent

### 3.2 DPPH and H<sub>2</sub>O<sub>2</sub> Radical Scavenging Activity for crude extracts of *Ficus abutilifolia*

From the results displayed in Tables 2 and 3, it is evident that the ethanolic extract of leaves from *Ficus abutilifolia* exhibits potent antioxidant ability. Minimum and maximum DPPH inhibition are observed at 44.2 % and 58.2 %, respectively at concentrations of 2.5 µg/mL and 10 µg/mL.

The crucial role of phenolic compounds as scavengers of free radicals is emphasized in several reports. Asha *et al.* [26] reported a fair correlation between antioxidants, free radical scavenging activity, and phenolic contents.

The extract of *Ficus religiosa*'s fruit and bark demonstrated antioxidant activity, assessed using the oil stability index and radical scavenging capacity against DPPH [27]. Alcoholic fruit extracts of *Ficus capensis* Thunb showed an antioxidant potency of 13.05 % using the DPPH assay [28].

Similarly, Rathee *et al.* reported that the methanolic extract of *Ficus religiosa* fruits reduces the DPPH radical to the corresponding hydrazine when it reacts with the hydrogen donors in the antioxidant principles [29]. In a related study, Etratkhah *et al.* reported that the aerial root of *Ficus benghalensis* exhibits an activity of IC<sub>50</sub> 54.40 µg/mL against DPPH, using Vitamin C as a standard [30].

Gupta and Sharma's research results revealed a significant decrease in the concentration of DPPH radicals due to the scavenging ability of both aqueous extracts and standards. A 250 µg/mL of *Ficus bengalensis* Linn aqueous extract and Ascorbic acid (std.)

exhibited 96.07 % and 90.41 % inhibition, respectively [31].

In a related research, Ojo and Akintayo revealed that the aqueous extract of *Ficus asperifolia* scavenged 78.65 % of DPPH radicals at a concentration of 5 mg/mL against 47.03 % and 29.25 % of nitric oxide (NO) and hydroxyl (OH) radical activities, respectively [32]. This indicates that *Ficus* of the Moraceae family is a good source of antioxidants.

In another study, Anago *et al.* reported the antioxidant potency of the ethanolic extract of *Ficus exasperate* Roxb against DPPH as 23 % at 10 µg/mL [33]. Similarly, Ahoua *et al.* [34], investigated the DPPH radical scavenging power of extracts of a group of *Ficus* species, with the following results: Methanol extract of *Ficus elasticoides* leaves (96.69 %), , Methanol extract of *Ficus lyrata* leaves (94.53 %), Methanol extract of *Ficus mucoso* leaves (94.33 %), , Methanol extract of *Ficus thonningii* leaves (89.86 %), , Methanol extract of *Ficus umbellata* fruits (74.74 %), Methanol extract of *Ficus umbellata* leaves (73.03 %), Methanol extract of *Ficus elasticoides* fruits (71.44 %), dichloromethane extract of *Ficus umbellata* leaves (28.18 %), and dichloromethane extract of *Ficus lyrata* leaves (26.09 %). According to Tharini *et al.* [35], the maximum DPPH' radical scavenging activity was 75.74 % at 60 µg/mL concentration. The fruit extract of *F. benghalensis* demonstrated a high capacity to deactivate free radicals by reducing the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical to the yellow-colored 1,1-diphenyl-2-picrylhydrazine, and the reducing capacity increased with the concentration of the extract.

In a related research involving the methanol extract of *Ficus elastica*, Preeti *et al.* reported significant dose-dependent antioxidant activity with an IC<sub>50</sub> value of 20.17 µg/mL [36]. Madeleine *et al.* [37] reported an IC<sub>50</sub> antioxidant activity of 0.06 mg/mL for the methanol extract of *Ficus abutilifolia* against DPPH assay. Similarly, Boukhalfa *et al.* [38] reported a DPPH assay inhibition of 346.2 µg/mL by *Ficus carica* leaf extract. In a similar research, Abotsi *et al.* [39] confirmed the 0.499 IC<sub>50</sub> (mg/mL) and antioxidant activity against DPPH assay. Arun *et al.* [40] revealed that *Ficus carica* extract showed 86.43 % and 77.86 %, respectively for the seed and seedless plant samples, against DPPH at the highest

concentration of 80 µg/mL. Likewise, Flayyh *et al.* revealed 6.4 µg/mL E<sub>50</sub> antioxidant activity against DPPH for *Ficus elastica* extract [41].

The inhibition of hydrogen peroxide scavengers by *Ficus abutilifolia* shows 52.1 % at 2.5 µg/mL and 72.7 % at 10 µg/mL. Gupta and Sharma reported that the extracts demonstrated hydrogen peroxide scavenging activity in a concentration-dependent manner with an IC<sub>50</sub> of 0.65 mg/mL, while the standard (Ascorbic acid) had an IC<sub>50</sub> of 0.507 mg/mL [42]. Similarly, The highest activity was found in *F. exasperata* (25 % and 19 %) at 1 000 µg/mL and 750 µg/mL, respectively and The highest activity was found in *Arocarpus altilis* (Breadfruit), a member of the Moraceae (31.6 %) at 1 000 µg/mL, at 750 µg/mL (31.4 %), at 500 µg/mL (30.8 %), at 300 µg/mL (28.6 %) and with lowest activity at 100 µg/mL (9.6 %) using hydrogen peroxide assay. This appreciable scavenging of hydrogen peroxide may be attributed to the presence of phenolic groups that could donate electrons to hydrogen peroxide, thereby neutralizing it into water [43]. In a related research, Yadav *et al.* observed that hydrogen peroxide scavenging activity of the ethanolic extract of *Ficus racemosa* leaves (51.28 %) was comparable with the standard Ascorbic acid (49.2 %)[44]. Similarly, Chloroform Fruit Extract of *Ficus sycomorus* showed an increase in percentahe inhibition in a dose dependent pattern with the value of of IC<sub>50</sub> 117.6 µg/mL while Ascorbic acid (standard) showed an activity of 4.81 µg/mL [45]. *Ficus dalhousie* Ethyl Acetate extract and *Ficus dalhousie* Hydro Alcoholic Extract showed IC<sub>50</sub> activities of 86.56 µg/mL and 51.17 µg/mL, respectively [46]. This result aligns with the one reported by Putra *et al.* [6], indicating that *Ficus deltoidea* fruits extract exhibits excellent antioxidant activity. In a similar research, Zunoliza *et al.* reports that alcoholic and aqueous extracts of different varieties of the *Ficus deltoidea* plant exhibited different radical scavenging activities in different models. Both types of extracts displayed high antioxidant activity in DPPH and superoxide anion scavenging models, comparable to quercetin, rutin, butylated hydroxyanisole (BHA), Ascorbic acid, and allopurinol [47]. In general, antioxidants break up the chains formed during the propagation process by providing a hydrogen atom or an electron to the free radical

and receiving the excess energy possessed by the activated molecule to reduce oxidation. Antioxidants may offer resistance against oxidative stress by scavenging free radicals, inhibiting lipid peroxidation, and through other mechanisms, thus preventing disease and promoting healing [48].

The overall results indicate that the antioxidant activities for the plant extracts are concentration dependent.

Table 2. Result for Percentage Inhibition of DPPH by the plants crude extracts and Ascorbic Acid

Concentration (µg/mL)	FA (%)	AA (%)
2.5	44.2	67.6
5	47.9	71.1
7.5	53.1	81.4
10	58.2	91.6

Blank solution: 1.3681

FA= *Ficus abutilifolia*, AA= Ascorbic Acid

Table 3. Result for Percentage Inhibition of Hydrogen Peroxide by the plants' crude extracts and Ascorbic Acid

Concentration (µg/mL)	FA (%)	AA (%)
2.5	52.1	75.4
5	63.4	79.8
7.5	70.3	87.3
10	72.7	90.9

Blank solution: 1.2640

FA= *Ficus abutilifolia*, AA= Ascorbic Acid

#### 4 Conclusions

The investigation into the medicinal potential of *Ficus abutilifolia*, sourced from Askira Uba, Borno State, Nigeria, has revealed promising bioactive compounds through ethanol extraction of its leaves. The qualitative phytochemical screening identified a diverse array of compounds, including alkaloids, saponins, tannins, anthraquinones, steroids, glycosides, flavonoids, reducing sugar, amino acids, and terpenoids, with the notable absence of phytosterol. The antioxidant evaluation of the crude extracts using DPPH and hydrogen peroxide free-radical scavenging assays demonstrated concentration-dependent efficacy, with the highest concentrations exhibiting significant free radical inhibition.

In summary, the research on *Ficus abutilifolia* highlights its pharmacological potential, particularly in the inhibition of free radicals. The findings contribute valuable

insights into the therapeutic properties of this plant, addressing the existing gap in scientific knowledge regarding the pharmacological activities of *Ficus abutilifolia* in comparison to other species within the genus. The concentration-dependent increase in antioxidant activity underscores the plant's potential as a valuable source of bioactive compounds with significant implications for health and wellness. Further studies are warranted to delve deeper into the specific mechanisms of action and potential applications of *Ficus abutilifolia* in addressing health challenges.

## 5 Declarations

### 5.1 Author Contribution

Authors AC, LL and NBC designed the study, Author AC performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors LL, AC and NBC managed the analyses of the study. Author AC managed the literature searches. All authors read and approved the final manuscript.

### 5.2 Conflict of interests

The authors declare that they have no conflict of interest.

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