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PHYTOCHEMICAL CONTENT, RADICAL SCAVENGING ACTIVITY, AND THE SAFETY PROFILE OF THE STEM BARK OF *FICUS SUR* (FORSSK), MORACEAE

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ABSTRACT

Background: *Ficus sur,* a small tree belongs to the Moraceae family. In Nigeria, it is locally called 'Uwar yara' and 'Ópoto' in Hausa and Yoruba languages respectively. Its leaves, bark, and root are used traditionally to treat diarrhoea, anaemia, threatened abortion, infertility, pains, etc.

Aim: This study focused on the phytochemical contents, the free radical scavenging activity, and the safety profile of the stem bark of *Ficus sur* with the view to validate its ethnomedicinal uses.

Methods: The stem bark was extracted by maceration using methanol. Qualitative and quantitative phytochemical constituents were evaluated using standard methods. The 2,2 -Diphenyl-1-picryhydrazyl (DPPH) was used to assess the radical scavenging activity of the methanol extract using Vitamin C as the standard. An acute toxicity test was carried out using OECD 425 method. Parts of this study were conducted in the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Kaduna State University, Kaduna-Kaduna state while the other parts were conducted at ABU- Zaria animal house and the histology department between May–August, 2023.

Results: The percentage yield of the stem bark was 12.01%. Phenols, tannins, saponins, flavonoids, triterpenoids, anthocyanins, and alkaloids were present. Total phenolic and flavonoid contents of the stem bark extract were 113.603 mg GA/g, and 62.001mg QE/g. The IC50 value of the stem bark was 0.44mg/ml. The observed behavioural pattern and histology results suggested the LD_{50} of methanol stem bark extract was greater than 5000 mg/kg body weight.

Conclusion: The study revealed that *Ficus sur* stem bark methanolic extract is rich in polyphenols, has an antioxidant capacity, and its toxicity profile was found to be safe for human consumption. Further research is needed to establish the aforementioned biological activities and isolate the compound(s) responsible for these activities.

Keywords: *Ficus sur*, Extraction, Phytochemicals, Antioxidant, toxicity.

INTRODUCTION

Ficus sur belongs to the Moraceae family of flowering plants, it is commonly called Cape fig, petit sycamore, or broom cluster fig and is primarily found in tropical and subtropical regions, [1]. Generally, Ficus species produced latex in all its aerial parts [2]. One interesting phenological and pathological event with *F. sur* irrespective of water scarcity or abundance is the shedding of its leaves. *F. sur* is found mainly along river banks, stream heads, or moist habitats [1,3]. In Nigeria, its local names among the major tribes are Uwarýara or Beru in Hausa, Akokoro in Igbos, Opoto in Yoruba [3]. Morphologically, the tree can grow up to 35

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m in height, it has a leafy twig and the periderm does not flake off when dried. The leaves, stem bark, and root are used to treat various ailments including diarrhea, inflammation, anemia, threatened abortion, etc. [3,4]. In Nigeria *F. sur* stem bark is used to treat female infertility [4], and the leaves are used to treat male infertility [5]. The presence of phytochemicals such as phenols, tannins, flavonoids, triterpenoids, and anthraquinones, is largely responsible for these activities [6; 7].

Phytochemicals are non-nutritional bioactive components that are primarily responsible for generating antioxidants to scavenge toxic radicals [6]. In humans, many diseases are associated with free radicals [7]. Free radicals, reactive oxygen species (ROS), and reactive nitrogen species (RNS) are derived from both endogenous sources and exogenous sources. Free radicals induce oxidative stress in humans and have been reported to be involved in several diseased conditions such as cancers, diabetes, cardiovascular diseases, chronic inflammation, etc [8,9]. Phytochemicals, with antioxidant activity, have potential protective effects on these disease conditions [10,11]. Certain medicinal plants used in ethnomedicines have been reported to have some toxic effects in humans [12,13,14]. Hence, it becomes imperative to ascertain the safety of all medicinal plants used as ethnomedicines. This study aims to determine the phytochemical content, the antioxidant capacity, and the safety of the plant *F. sur* with the view to providing supporting materials for its traditional uses.

METHODOLOGY

Collection, Authentication and Preparation

The plant's leaves and fruits (fig 1) were collected in the wild in Samaru-Zaria, Kaduna state, and were identified and authenticated as *F. sur* (Moraceae) at the Department of Botany, ABU-herbarium section, and the voucher number, ABU02368 was given. A sufficient quantity of the stem bark was collected and dried at room temperature. These were pulverized and kept in a closed container for further uses.

Fig. 1 *Ficus sur* Stem Bark

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Extraction

The powdered plant material (150g) was macerated in methanol (800ml each) and allowed to stand for three days with frequent shaking until the soluble matter dissolved. The mixture was then strained, marc pressed and the combined liquid was clarified by decantation after standing [15]. The menstruum was evaporated from the filtrate using a hot water bath and the solid crude extract was collected in an airtight container for future use.

Qualitatative Phytochemical Screening

The extract was subjected to qualitative phytochemical screening to test for the presence of phenols, tannins, flavonoids, alkaloids, anthocyanins, saponins, and glycosides using standard methods as described by some authors [16].

Quantitative Phytochemical Study

The total phenolic content and total flavonoid content of the stem bark of *F. sur* were determined using the spectroscopic method.

Determination of Total Phenolic content

The concentration of total Phenolic content was determined using the method described by some authors [5; 17]. Briefly, the methanolic concentration of extract 1mg/ml was prepared. From this concentration, 0.5 ml was mixed with 2.5 ml of 10 % Folin-Ciocalteu's reagent and 2.5 ml of 7.5% of $NaHCO_3$, and the preparation was made up to 10 ml with 4.5 ml of distilled water. Blank was concomitantly prepared containing 0.5 ml methanol, 2.5 ml 10% Folin -ciocalteu's, 7.5% of $NaHCO_3$, and 4.5 ml of distilled water. Gallic acid was used as standard and it was prepared in concentration range of (20, 40, 60, 80, and, 100) µg/ml. To the different concentrations, 2.5ml of 10 % Folin-Ciocalteu's reagent and 2.5ml of 7.5% of $NaHCO_3$ and the preparation was made up to 10 ml with 4.5 ml of distilled water. These were incubated for 45 minutes and the absorbance was taken at 765 nm. Based on the measured absorbance, the concentration of phenolics (mg/ml) was read on the calibration line and the content of total phenolics was expressed in terms of gallic acid equivalent (mg of GA/g of extract).

Determination of Total Flavonoid Content

The total flavonoid content in the examined stem bark extract was determined as described [5;17] with some modifications. The methanol solution of the extracts in the concentration of 1 mg/ml was prepared and 1 ml of the solution was mixed with 1.25 ml of $NaNO_3$ was added and then the mixture was placed in the dark for 6 minutes then 1.25 ml of 10% $AlCl_3$ was added and again incubated in dark for 6 minutes. Then, 2 ml NaOH was added and the volume was made to 10 ml with distilled. The set of standard solutions of quercetin was prepared with concentrations (0.02, 0.04, 0.06, 0.08, and 0.10 mg/ml). The absorbance of the standard and the extract solutions was measured against the reagent blank at 510 nm with a UV/Visible spectrophotometer. The total flavonoid content was determined from the calibration curve and expressed as milligrams of quercetin equivalent (QE) per gram of extracts.

DPPH-Free Radical Scavenging Activity of Plant Extract

Using 2,2 -Diphenyl-1-picrylhydrazyl (DPPH), the ability of the extract to scavenge free radicals was assessed using methods described [18]. Briefly, 1 mg/ml stock

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solution of each extract was prepared in methanol. Dilutions were made to obtain concentrations of 500, 250, 125,62.5, 31.25 µg/ml. Vitamin C concentration of 1 mg/ml was also prepared and dilution was also made as above. This was used as the standard. DPPH solution (1mg/ml) was prepared and 1 ml of the prepared DPPH was added to each dilution of extracts and Vitamin C. The control was 10 ml methanol. These were incubated in the dark for 30 minutes at room temperature and the absorbances were taken at 517nm.

Percentage Radical Scavenging Activity were calculated using equation:

$$\% \text{ RSA} = \frac{\text{ABS. control} - \text{ABS. sample}}{\text{ABS. control}} \times 100$$

IC_{50} values were calculated from the % RSA versus concentration plot, using a non-linear regression algorithm [19].

Oral Acute Toxicity Testing (OECD 425 Method)

Following the OECD 425 method, rats were selected randomly, marked to allow individual recognition, and kept in their cages for at least 5 days before dosing to allow for acclimatization. An acute toxicity study was performed with a Limit test at 5000 mg/kg p.o. as a single dose. The initial weight of all the animals was recorded from day 1. The dose was calculated and administered first to one animal in each group based on their body weight. Normal saline was used as a control. The animals were closely observed for signs of toxicity first 30 min, then for 4 h. Food was provided after 1–2 h of dosing. Then, since no sign of toxicity was recorded, 3 more animals each from the groups were treated with the same dose of the extract. All the groups were closely observed for 6 h and then at regular intervals for 14 weeks. The animals were weighed on days 7 and 14. At the end of the experiment (day 14), the animals were sacrificed by cervical dislocation, and vital organs (liver and kidney) were excised, weighed, and preserved in 10% formalin for histopathological evaluation [19].

RESULTS

Extraction

The extraction of the powdered stem bark produced 18.02g methanol extract, with the corresponding 12.01 percentage yield. The solubility of *F. sur* stem bark powder in methanol and the subsequent percentage yield indicated that the extract may contain polar compounds. This is shown in Table 1

Table 1: Percentage Yield of the Stem Extract

s/n	Plant material	Initial weight (g)	Final weight(g)	Percentage yield (%)
1	*F. sur* stem bark	150	18.02	12.01

Qualitative Phytochemical Screening

The therapeutic properties of any medicinal plant are due to the presence of phytochemicals. Qualitative phytochemical screening revealed the presence of phenols, tannins, saponins, flavonoids, triterpenoids, anthocyanins, and alkaloids. Cardiac glycosides and anthraquinones were absent. This is shown in Table 2

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Table 2: Qualitative Phytochemical Screening of the stem bark of *F. sur* extract

Phytochemicals	Reagent/Test	Inference
Phenol	$FeCl_3$	+
Flavonoids	NaOH	+
	Shinoda	+
Cardiac glycoside	Keller- killani test	-
Alkaloids	Wagner's reagent	+
	Meyers reagent	+
Triterpenoids	Salkowski	+
Anthraquinones	Liebermann Burchard test	-
Saponins	Foam test	+
Anthocyanins	NaOH test then, H_2SO_4	+
Tannin	$FeCl_3$	+

Keys: (+ = present; - = absent)

Quantitative Phytochemical

Figure 2 shows the estimated total phenolics and total flavonoid contents of the stem bark extract based on the calibration curve equation. Polyphenols are known to possess great antioxidant properties and are effective against most pathogenic organisms.

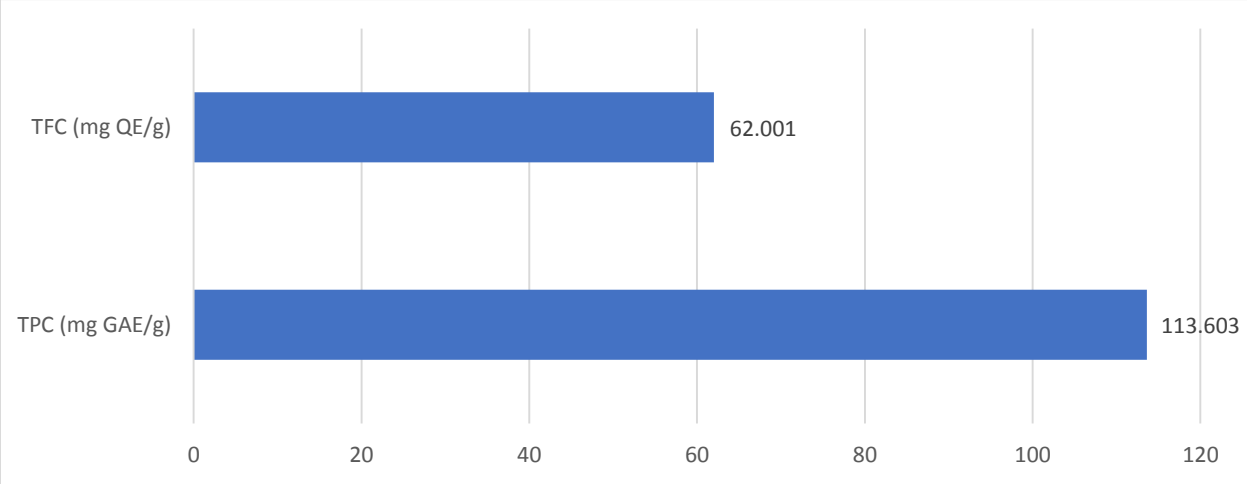

Figure 2: Quantitative Phytochemicals of FSB Extract

***Keys**: FSB = Ficus sur bark, TPC= Total phenolic content, TFC= total flavonoids content.*

DPPH Radical Scavenging Activity

F. sur methanol extract demonstrated free radical scavenging ability at varying increasing concentrations as shown in Table 3. The higher the concentration, the lower the absorbance value. The lower absorbance values are due to the lower amount of hydrogen [18]. This was observed in the discoloration of the prepared samples from purple to a yellow hue as the number of electrons collected increased. Table 3 shows the absorbance values and %RSA at each concentration.

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Table 3: The absorbance value and Percentage Radical Scavenging Activity (RSA) of standard (Vitamin C) and *F. sur* stem bark extract.

Conc. (µg /ml)	Absorbance@517nm		(%RSA)	
	Vitamin C	*F. sur stem bark*	Vitamin C	*F. sur stem bark*
31.5	0.183	0.215	44.54545	34.84848
62.5	0.16	0.202	51.51515	38.78788
125	0.147	0.191	55.45455	42.12121
250	0.114	0.175	65.45455	46.9697
500	0.085	0.152	74.24242	53.93939
Control = 0.33			*IC_{50} = 0.05*	*IC_{50} = 0.44*

The IC_{50} Values of stem bark of *F. Sur* (Extract) and Vitamin C (Standard).

IC50 represents the concentration at which an extract exerts half of its maximal free radical scavenging effect [19]. The antioxidant capacity is indirectly proportional to the IC_{50} value of an extract. A lower IC_{50} value indicates a higher antioxidant capacity. The IC_{50} value is then estimated using the fitted line from the plot of percentage radical scavenging activity (%RSA) and the concentrations of extract as shown in Figure 3.

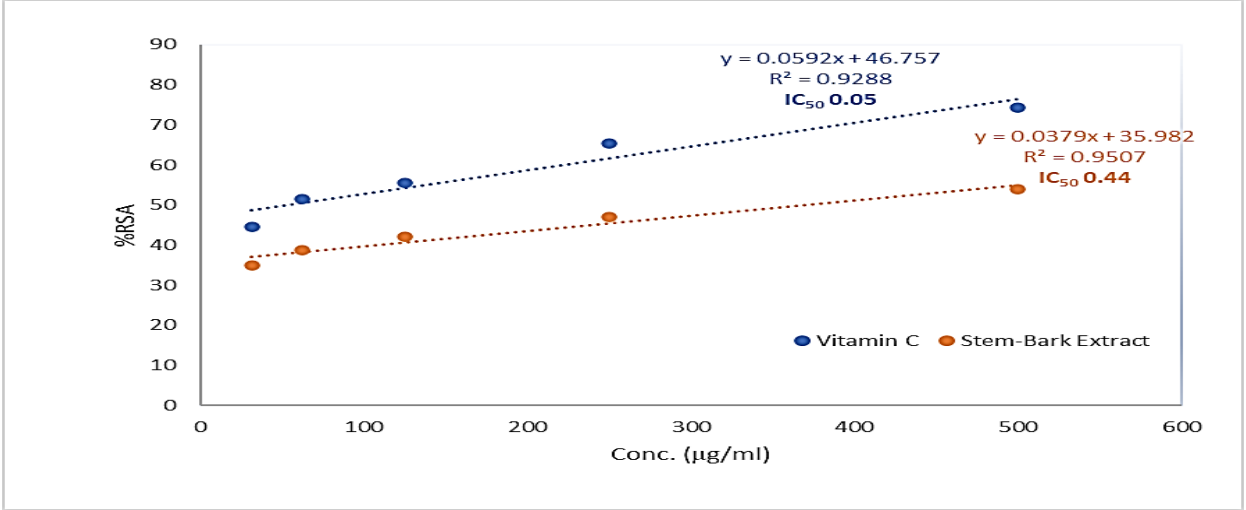

Fig 3: the IC_{50} values estimated from a plot of Percentage radical scavenging activity (RSA) against various concentrations of vitamin C, and *F. sur* stem bark

Oral Acute Toxicity Study

Following the OECD 425 method, *F. sur* stem bark extract caused no mortality in rats at a dose of 5000 mg/kg body weight. The results are shown in Fig. 4 and Fig. 5 below

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Behavioural Pattern and Body Weight

The study found all parameters normal, with no signs of convulsion or itching, and no significant increase in body weight of the animals.

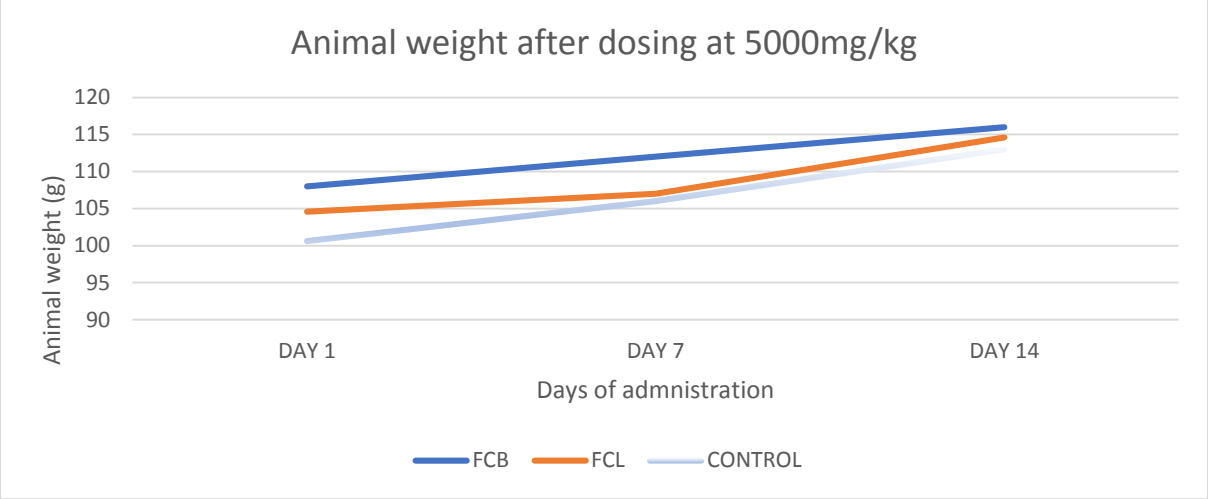

Fig 4: Effects of *F. sur* stem bark extract on body weight of rats in acute toxicity study

Histopathological Studies

The histopathological report of the vital organs viz. liver and kidney suggested no significant change in any treated group as compared to the control. Signs of slight inflammatory hyperplasia of cells were observed in the kidneys (extract and normal saline) and the liver (normal saline).

F. sur bark extract Normal saline

Fig. 5. Effect of Extracts on the Histology of the Vital Organs of *F. sur* Stem Bark Extract

DISCUSSION

Plants have been used as medicines since the beginning of time [20;21]. Extraction is the first step in phytochemical analysis. Extraction is the separation of medicinally active plant components from inactive or inert components through the use of selective solvents in standard extraction procedures [21;22]. The obtained extract may be suitable for use as a medicinal agent [22]. In Table 1, the extraction of 150 g of powdered stem bark with methanol yielded 12.01 percent crude extract. Because of its high polarity, methanol has been shown in most studies to be a better extractive solvent, producing higher extraction yields than other solvents. [23;24]. According to the law of similarity and inter-miscibility, *F. sur* stem bark, may contain

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high polar compounds due to its solubility in methanol and high yield.

Plants' medicinal properties are due to their phytochemical constituents [24;25]. Table 2 shows the presence of phenols, tannins, flavonoids, triterpenoids, anthocyanins, saponins, and alkaloids in methanol extracts *of F. sur* stem bark. The presence of the aforementioned phytochemicals is consistent with another report of phytochemical analysis of *F. sur* extracts. [5;25;26]. Cardiac glycosides and anthraquinones were found to be absent in our study. Although glycosides are present in many Ficus species, anthraquinones are absent in many Ficus species [27]. Compounds such as flavonoids, saponins, phenols, and tannins have been shown to have therapeutic properties against most disease-causing organisms [5;6;28]. The total phenolic and total flavonoid contents of *Ficus sur* methanol stem bark extract were 113.603 mgGAE/g and 62.00 mgQE/g, respectively, as shown in Table 2. This result is consistent with previous reports on *F. sur* stem bark methanol extract [5;25]. The antimicrobial, abortifacient, anti-sickling, and anti-inflammatory properties of *F. sur* stem bark could be attributed to its high total phenol and total flavonoid contents.

Many diseases in humans have been linked to free radicals, and oxidative stress has been linked to the onset and progression of many human diseases [10; 11; 28;29]. Antioxidants work by donating electrons or hydrogens to free radical-damaged cells, converting the free radicals into waste products for elimination by the body [18;19;29]. The methanol extract of *Ficus sur* stem bark demonstrated free radical scavenging activity, as shown in Tables 4, and Figure 3 above. The radical scavenging activity (% RSA) of *F. sur* stem bark extract at 500ug/ml concentration was 53.93%. This finding backs up previous findings in which *F. sur* methanolic stem bark extract demonstrated significant in-vitro antioxidant potential using the DPPH, ABTS, and CUPRAC assays [5]. The IC_{50} value of the methanol extract (0.44mg/ml) was found to be higher than that of the standard Vitamin C (0.05 mg/ml) in this study. The higher the IC_{50} value, the lower the antioxidant capacity [19].

Clinical signs and symptoms, as well as other toxicity indicators, can be used to predict toxic outcomes [30]. There were no obvious signs of toxicity, no significant change in body weight, and no animals died at the extract dose of 5000 mg/kg body weight of rats. When the animals were sacrificed at the end of the study, however, histological examination of the kidney and liver revealed minor signs of toxicity. In organs isolated from rats treated with the extract and the control, minor hyperplasia of inflammatory cells and Kupfer cells was observed (fig.5). Because hyperplasia was also observed in the control group, we were unable to attribute the observed condition to the extract. Other causes of hyperplasia may exist, and in most cases, mild hyperplasia of cells is not harmful and is not associated with toxicity [12;30].

CONCLUSION

The study established that the methanol stem bark extract of *F. sur* is rich in phytochemicals such as polyphenols which could be responsible for its radical scavenging activity and the various ethnomedicine uses of the plant. Acute toxicity testing indicated that *F. sur* methanol stem bark extract could be safe for human consumption. However, a detailed chronic

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toxicity study is recommended to ensure its safety in long-term use.

Competing Interests

Authors have declared that no conflicting interest exists.

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Authors Contribution

Oduma S.E: Designed the study, managed the literature search, provided fund, and performed the laboratory analysis and write-ups. Abdullahi I: Assisted in the laboratory analysis.

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