



PHARMACOGNOSTIC, ANTIOXIDANT AND ACUTE TOXICITY STUDY OF *Ficus sycomorus* (Linn) (Moraceae)
ROOT AND STEM BARK

*¹Namadina, M. M., ¹Haruna, H. and ²Sunusi, U.

¹Department of Plant Biology, Bayero University, Kano, Nigeria.

²Department of Biochemistry, Faculty of Basic Medical Science, Bayero University, Kano

*Corresponding Author's Email: Hajiyaiyalle@gmail.com 07035939668

ABSTRACT

Most of biochemical reactions in the body generates Reactive Oxygen Species (ROS), which are involved in the pathogenesis of oxidative stress-related disorders like diabetes, nephrotoxicity, cancer, cardiovascular disorders, inflammation and neurological disorders when they attack biochemical molecules like proteins, lipids and nucleic acid. Antioxidants are used to protect the cells or tissues against potential attack by ROS. Most medicinal plants possess a rich source of antioxidants such as flavonoids, phenols, tannins, alkaloids among others. These phytochemicals are currently pursued as an alternative and complimentary drug. In this study, phytochemical components, antioxidant and acute toxicity study of the methanol extract of stem bark and root of *F. sycomorus* were carried out using standard methods. Findings from this study revealed the presence of some diagnostic microscopical features such as calcium oxalate, starch, gum/mucilage, lignin, Aleurone grain, suberized/Cuticular cell wall and inulin but calcium carbonate was absent in stem bark but present in the powdered root. Quantitative physical constants include moisture contents (6.40% and 7.82%), ash value (7.20% and 9.30 %) in stem bark and root respectively. Carbohydrates, alkaloid, flavonoids, saponins, tannins, glycoside, steroid, triterpenes and phenols were present in all the extracts. They were found to exhibit potent 1,1,-diphenyl 2-picryl hydrazyl (DPPH) free scavenging activity. The DPPH radical scavenging ability of the extracts showed the following trend Ascorbic acid < stem bark extract > root extract. The LD50 of the methanolic stem bark and root extracts were found to be greater than 5000 mg /kg and is considered safe for use. Nonetheless, further studies are encouraged to evaluate toxicity at much higher doses.

Keywords: DPPH radical scavenging, Antioxidants, phytochemicals, Quantitative physical constants, *F. sycomorus*

INTRODUCTION

F. sycomorus is a species member from Moraceae family commonly known as cape fig/broom-cluster in english found in grasslands, riverine forest, secondary scrub type and cape island, they are common in tropical Africa extending from Senegal to south Africa (Feleke and Brehane, 2005). They are mostly evergreen and widely distributed in tropical areas while some few species are found in semi-warm temperate areas. In Nigeria *Ficus sycomorus* is locally known by four different languages of Nigeria as uwaryara/dillu in Hausa, Opoto in Yoruba, Rima bichehi in Fulani, obada in Edo and Akokoro in Igbo (Njoku-oji *et al.*, 2016).

Ficus sycomorus are used traditionally in folkore from different part of the world for the treatment of different ailments depending on the region. Several investigators reported the uses of *Ficus sycomorus* in traditional African medicine in Sudan and in Nigeria it is used to treat leucoderma (root and leaves), swollen feet (root and leaves), leprosy (leaves and roots), epilepsy, wound dressing, tuberculosis, anaemia, sexually transmitted diseases, diarrhoea, chest infections, circumcision,

rickets, oedema, respiratory disorders, emollient, dysentery, infertility in men and gonorrhoea (Ishola *et al.*, 2013). *Ficus sycomorus* is locally called "Omora" in Kenya and "Mkuyu" in Tanzania and the stem bark has been used traditionally to treat malaria and fungal infections (Umeokoli *et al.*, 2013). The root and bark are used in South Africa to treat tuberculosis (Madikizela *et al.*, 2013). In south eastern Nigeria *Ficus sycomorus* is used traditionally in the management of haemolytic and sickle cell anaemia (Umeokoli *et al.*, 2013).

Oxidative stress has been implicated in pathogenesis and progression of several lifestyle diseases like atherosclerosis, diabetes mellitus, hypertension, ischemic complications, malignancies, cardiovascular diseases, eye disorders, lung, pancreatic and kidney disorders, cancer as well as, ageing and diseases associated with reproductive system (Rahman *et al.*, 2012).

Stress-related disorders have become epidemic in developing and under-developed countries. Conventional therapeutic strategies mostly attempt to relieve the clinical manifestations of

these disorders and their complications. However, studies have shown they tend to increase toxicity leading to damage of sensitive organs like liver and brain, they are also suspected to be mutagenic (Mohan *et al.*, 2013). Against this backdrop, the popularity of complementary drugs for oxidative stress-related disorders has increased and plant based antioxidants therapies are now widely practiced in most of the developing countries (Mohan *et al.*, 2013).

Since antioxidants hold a key in preventing oxidative stress-related disorders, many plant extracts and their secondary metabolites are being explored for their antioxidants effects (Gomathi *et al.*, 2013). The use of plant based antioxidants, plays an important role in preventing activation of the oxidation induced signaling pathways in our bodies (Joseph *et al.*, 2002). Therefore, pharmacognostic, antioxidants and acute toxicity study of stem bark and root extract of *F. sycomorus* are important step in increasing our understanding about their usage in treatment of various stress-related disorders.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

The stem bark and root of *Ficus sycomorus* were collected from local farm in June, 2019 at Madobi Local Government Area, Kano state with geographical coordinates of 11° 46' 38" North, 8° 17' 18" East. The plant was identified and authenticated in the Herbarium of the Plant Biology Department of Bayero University, Kano and was compared with a voucher specimen number.

Preparation of Plant extracts

The fresh root and stem bark were air dried at room temperature under a shade for a week. After drying they were milled into fine powder by use of an electric mill. The powdered plant materials were sieved using mesh pore of 0.5 mm and packed in closed, dry sealed bags and stored awaiting extraction. For extraction, 50 g of each powdered plant material was soaked in 500 ml of methanol and was allowed to stand for 3 days at room temperature (28 ±2°C), with agitations at intervals. The resultant extract was poured into a clean dry conical flask and then filtered using Whatman's No.1 filter papers. To obtain dried extra the filtrate was then concentrated under reduced pressure and vacuum using a rotary evaporator at a temperature of 40 °C. The concentrates were placed in airtight containers weighed and stored.

Chemo-microscopic Studies of the Powdered Stem bark and Root of *Ficus sycomorus*

Powdered sample (5g) of fresh root and stem bark was used for this study to detect the presence of cell wall materials and cell inclusions. Finely ground sample of plant was cleared in a test tube containing 70% chloral hydrate solution. It was boiled in a water bath for about thirty minutes to remove obscuring materials. The cleared sample was mounted with dilute glycerol onto a microscope slide. Using various detecting reagents the presence of cell wall materials and cell inclusions were detected in accordance to WHO (2011) guidelines.

Cell wall Materials

Test for Cellulose

A drop or two of iodised zinc chloride was added to the powdered sample and allowed to stand for a few minutes and observed under a microscope. It stained cellulose cell wall blue to blue- violet.

Test for Lignin

The powdered plant material was moistened on a slide with a small volume of phloroglucinol and allowed to stand for about two minutes or until almost dry. A drop of hydrochloric acid was added and viewed under a microscope. Pink stained or cherry red was observed for the presence of lignin.

Test for Suberized or Cuticular cell walls

A drop or two of Sudan red was added to the cleared powdered sample and allowed to stand for few minutes and observed under a microscope. Orange red or red colour was observed presence of suberin or cutin on the cell (WHO, 2011).

Test for Gum and Mucilage

To a small portion of the cleared powdered sample of the plant, a drop of ruthenium red was added. Appearance of pink coloration was considered positive for gums and mucilage (WHO, 2011).

Cell Inclusions/ Cell Contents

Test for Starch grains

To a small portion of the cleared powder sample of the plant, N/50 iodine was added. Appearance of blue-black or reddish-blue coloration on some grains would be considered positive for starch (WHO, 2011).

Test for Calcium oxalates and Calcium Carbonates

To a small portion of the cleared powdered sample of the plant, HCl was added, dissolution of crystals in the powdered drug without effervescence was considered positive for calcium oxalate while slow dissolution with effervescence was considered positive for calcium carbonate (WHO, 2011).

Inulin

A drop of 1-naphthol and that of sulphuric acid was added to the powdered sample and viewed under the microscope. Spherical aggregations of crystals of Inulin turned brownish red and dissolve (WHO, 2011).

Test for Tannins

To a small portion of the cleared powdered sample of the plant, 5% ferric chloride solution was added. Appearance of greenish black colour was considered as positive for tannins (WHO, 2011).

Determination of Physicochemical Constants of the Powdered Stem bark and Root of *Ficus sycomorus*

Some physicochemical parameters of the powdered sample were determined as described in the updated edition of quality control methods for medicinal plant materials (WHO, 2011).

Moisture Content

This is the quantity of moisture present in a plant material. Moisture content of the powdered sample will be determined by loss on drying method.

3.0g each of the powdered sample was accurately weighed and

placed in some clean, dried evaporating dishes of known weights. They were placed in an oven and heated at a temperature of 105°C for 1 hour, then cooled in a desiccator and re-weighed. Heating and weighing were repeated until a constant weight was obtained. The weight loss on drying was computed following the formula below:

$$\% \text{ Moisture content} = \frac{\text{Initial Weight of Powder} - \text{Final Weight of Powder}}{\text{Initial Weight of Powder}} \times 100$$

Total Ash Value

2g of powdered plant materials was accurately weighed and placed separately in a crucible of known weight. It was heated gently and the heat gradually increased until it is white indicating the absence of carbon. It was allowed to cool in a desiccator and weighed; this was repeated until a constant weight was obtained. The total ash value was determined as a percentage with the formula below

$$\% \text{ Ash Value} = \frac{\text{Weight of Residual Ash}}{\text{Original Weight of Powder}} \times 100$$

Acid-insoluble ash

This is the residue that remains after boiling the total ash with dilute hydrochloric acid.

This was determined for the powdered plant material. 25ml of dilute hydrochloric acid was added to the crucible containing ash. It was covered with a watch glass and gently boiled for 5mins. The watch glass was rinsed with 5ml of hot water and the liquid added to the crucible. The insoluble matter was collected on an ash less filter-paper and washed with hot water until the filtrate is neutral. The filter-paper containing the insoluble matter was transferred to the original crucible, dried in an oven and ignited to a constant weight. The residue was allowed to cool in a suitable desiccator for 30 minutes and then weighed without delay (Evans, 2002).

The acid-insoluble ash will then be calculated as a percentage for each of the two plants with the formula

$$\% \text{ Acid insoluble Ash} = \frac{\text{Weight of Residual Ash}}{\text{Original Weight of Powder}} \times 100$$

Water soluble ash

To the crucible containing the total ash, 25ml of water was added and boiled for 5 minutes. The insoluble matter was collected in a sintered glass crucible. It was then be washed with hot water and ignited in a crucible for 15 minutes at 105°C. The weight of the residue was subtracted from the weight of the total ash. The content of water soluble ash per air dried powdered sample was calculated and recorded (WHO, 2011).

$$\% \text{ Water Soluble Ash} = \frac{\text{Weight of Total Ash} - \text{Weight of Residual Ash}}{\text{Original Weight of Powder}} \times 100$$

Alcohol-Soluble Extractive Value

This is the amount of extraction in percentage of a plant sample with alcohol.

4g of each of the plant material was separately weighed in a conical flask. 100ml of ethanol was added and macerated for 24 hours, during which the mixture was frequently shaken within the first 6hours using a mechanical shaker. It was filtered and 25ml of the filtrate transferred into an evaporating dish of known

weight and evaporated to dryness on a water bath. It was dried to a constant weight, the percentage of alcohol-soluble extractive value was then determined for the plant as

$$\text{Alcohol-Soluble Extractive Value (\%)} = \frac{\text{Weight of Extract in 25ml} \times 4}{\text{Original Weight of Powder}} \times 100$$

Water-Soluble Extractive Value

This is the amount of extraction in percentage of a plant sample with water. Same procedure as in alcohol-soluble extractive value was repeated here for the two plants, but solvent for extraction here was water.

$$\% \text{ Water Extractive Value} = \frac{\text{Weight of Extract in 25ml} \times 4}{\text{Original Weight of Powder}} \times 100$$

Qualitative Phytochemical screening of Methanolic extract of *Ficus sycomorus* Stem bark and Root

The plant extracts (methanol) were subjected to phytochemical screening in order to identify the phytochemical constituents of the plant using the method described below.

Tests for carbohydrates

Molish's (General) Test for Carbohydrates: To 1 ml of the filtrate, 1 ml of Molish's reagent was added in a test tube, followed by 1 ml of concentrated sulphuric acid down the test tube to form a lower layer. A reddish colour at the interfacial ring indicates the presence of carbohydrate (Evans, 2009).

Tests for Saponins

Frothing test: About 10ml of distilled water was added to a portion of the extract and was shaken vigorously for 30seconds. The tube was allowed to stand in a vertical position and was observed for 30mins. A honeycomb froth that persists for 10-15mins indicates presence of saponins (Evans, 2009).

Test for Flavonoids

Shinoda Test: A portion of the extract was dissolved in 1-2ml of 50% methanol in the heat metallic magnesium chips and few drops of concentrated hydrochloric acid were added. Appearance of red color indicates presence of flavonoids (Evan, 2009).

Test for Alkaloid

Wagner's Test: Few drops of Wagner's reagents were added to a portion of the extract, whitish precipitate indicates the presence of alkaloid (Evans, 2009).

Test for Steroid and Triterpenes

Liebermann-Burchard's test: To a portion of the extract, equal volume of acetic acid anhydride was added and mixed gently. 1ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer. A colour change observed immediately and later indicates the presence of steroid and triterpenes. Red, pink or purple colour indicates the presence of Triterpenes while blue or blue green indicates steroids (Evans, 2009).

Test for Cardiac Glycoside

Kella-killiani's test: A portion of the extract was dissolved in 1ml of glacial acetic acid containing traces of ferric chloride solution. This was then transferred into a dry test tube and 1ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer at the bottom. Observed carefully

at the interphase for purple-brown ring, this indicates the presence of deoxy sugars and pale green colour in the upper acetic acid layer indicates the presence of cardiac glycosides (Evans, 2009).

Test for Tannins

Ferric chloride test: To a portion of the extract, 3-5 drops of ferric chloride was added. A greenish black precipitate indicates presence of condensed tannins while hydrolysable tannins give a blue or brownish blue precipitate (Evans, 2009).

Test for Anthraquinones

Bontrager’s test: To a portion of the extract in a dry test tube, 5ml of chloroform was added and shaken for at least 5mins. This was filtered and the filtrate shaken with equal volume of 10% ammonium solution, bright pink colour in the aqueous upper layer indicates the presence of free anthraquinones (Evans, 2009).

Antioxidant Activity Procedure

The antioxidant activity of the plant extracts was measured in terms of radical scavenging ability, using a stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to the modified method adopted from (Sani and Dailami, 2015). 200µl of 100µM methanol solution of DPPH were added to 100µL of various concentrations of the sample fractions in methanol (1000, 500, 250, 125, 62.5, 31.25, and 15.63µg/ml) and made to react in dark for 30mins time at room temperature. Absorbance of the blank, test and control were recorded at 517 nm. The experiment was performed in triplicate and scavenging activity was calculated by using the following formula and expressed as percentage of

inhibition.

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test} \times 100}{\text{Absorbance of control}}$$

The concentration corresponding to the 50%inhibition (IC₅₀) was determined using probit analysis by means of SPSS 16.0 software. The IC₅₀values obtained are compared with that of ascorbic acid as a standard antioxidant.

Acute Toxicity Studies (LD₅₀)

The LD₅₀ of the extract was determined using Lorke’s method (1983). The study was carried out in two phases and animals (mice) were deprived of food for 16-18h prior to administration of the extract. In phase 1, three groups of three animals per group were used. The extract was administered orally in geometrically increasing doses (10mg/kg, 100mg/kg and 1000mg/kg). The treated animals were observed for four hours post administration for signs of toxicity. After 24 hours, phase 2 was initiated. In phase 2, four groups of one animal each were given the extract orally in geometrically increasing doses (1500 mg/kg, 2250 mg/kg, 3250 mg/kg and 5000 mg/kg). The animals were then observed for signs of toxicity for the first 4 hours and mortality for 24hours.

RESULTS

Chemo-microscopical studies on the powdered stem bark and root of *F. sycomorus* were found to have cellulose cell wall, lignin, calcium oxalate crystals, tannins, starch and mucilage while calcium carbonate was absent in the powdered stem bark but present in the root.

Table 1. Chemo-microscopic Studies of Powdered Stem bark and Root of *F. sycomorus*

Constituents	Inference	
	Stem bark	Root
Starch	+	+
Gum and Mucilage	+	+
Cellulose cell walls	+	+
Lignin	+	+
Aleurone grain	+	+
Calcium oxalate crystals	+	+
Calcium carbonate	-	+
Suberized/Cuticular cell wall	+	+
Inulin	+	+

Key: + Present, - Not detected

The result of average moisture contents using loss on drying method was calculated to be 6.40% and 7.82% in stem bark and root respectively. The percentage yield of total ash, acid insoluble and water soluble matter were recorded in percentage values as 7.20%, 2.70% and 5.80% respectively in powdered stem bark while 9.30%, 3.10% and 5.90% in the powdered root. The extractives obtained were 16.80%, 20.60% and 21.40%, 24.90% for water and ethanol/alcohol solvents in stem bark and root respectively

Table 2. Physicochemical Constituents of Powdered Stem bark and Root of *F. sycomorus*

Parameters	Values (% w/w) ± SEM*		B.H.P Standard
	Stem bark	Root	
Moisture content	6.40±0.33	7.82±0.33	12-14%
Ash content	7.20±0.58	9.30±0.58	6-19%
Acid insoluble ash	2.70±0.33	3.10±0.33	>1
Water soluble ash	5.80±0.42	5.90±0.42	-
Water extractive value	16.80±0.33	20.60±0.33	-
Ethanol extractive value	21.40±0.58	24.90±0.58	-

Key: SEM (Standard Error Mean), B.H.P (British Herbal Pharmacopeia)

Alkaloid, flavonoids, tannins, triterpenes, cardiac glycoside, steroids, saponins, phenol and carbohydrates were detected in both stem bark and root methanol extracts while anthraquinones was absent in stem bark but present in root extract.

Table 3. Qualitative Phytochemical Screening of Methanol extracts of *F. sycomorus* Stem bark and Root

Metabolite	Inference	
	Stem bark	Root
Alkaloid	+	+
Flavonoid	+	+
Saponins	+	+
Cardiac glycoside	+	+
Tannins	+	+
Steroid	+	+
Triterpenes/terpenoids	+	+
Phenol	+	+
Anthraquinones	-	+
Carbohydrate	+	+

Key: + Detected, - Not detected

The antioxidant activity of the methanol stem bark and root extracts of *F. sycomorus* and ascorbic acid used as control were used found to be dose dependent. The higher the dose of the extracts, the lower the absorbance and consequently the higher the percentage inhibition of the free radicals (Table 2).

Table 4. Antioxidant activities of Methanol Stem bark and Root extract of *F. sycomorus*

Analyte	Concentration ($\mu\text{g/ml}$) / % Inhibition						
	1000	500	250	125	62.5	31.25	15.6
Ascorbic acid	99.7	99.3	98.7	97.4	95.4	92.2	87.5
Root extract	95.9	95.7	95.5	95.2	95.0	94.8	94.5
Stem bark extract	98.0	96.7	94.8	92.1	88.3	83.7	77.8

The trend of the inhibition of DPPH radical by the extract was concentration dependent with respect to the IC_{50} values (concentration of the extract to cause 50% inhibition), the DPPH radical scavenging ability of the extract showed the following trend Ascorbic acid < Root extract > Stem bark extract.

Table 5. IC_{50} values of DPPH Scavenging Effects of Ascorbic acid, Methanol Stem and Root extract of *F. sycomorus*

Analytes	IC_{50} value ($\mu\text{g/ml}$)
Ascorbic acid	0.79
Root extract	0.001
Stem bark extract	1.312

No death was recorded in the first phase of the study in rats. In the second phase, doses of 1500mg/kg, 2250mg/kg, 3250mg/kg and 5000mg/kg were used and no death was also recorded. The oral median lethal dose (LD_{50}) for the methanol stem bark and root extract of *F. sycomorus* was therefore estimated to be greater than 5000mg/kg and no sign of behavioural changes were also observed.

Table 5. Acute Toxicity Study of Methanol extracts of *F. sycomorus* Stem bark and Root

Plant species	Group	Number of Animals	Dose (mg/kg)	Mortality recorded after 24hrs
Phase I	I	3	10	0/3
	II	3	100	0/3
	III	3	1000	0/3
Phase II	I	1	1500	0/1
	II	1	2250	0/1
	III	1	3250	0/1
	IV	1	5000	0/1

DISCUSSION

The studies carried out on the stem bark and root of *F. sycomorus* have established some pharmacognostic standards that will guide its utilization as crude drug in pharmacy and other fields. The microscopic studies on the stem bark and root showed very important parameters which are unique to *F. sycomorus* as compared to other members of the Moraceae family. These anatomical features of the internal structures of plant drugs provide salient diagnostic characteristics for the identification of both entire and powdered crude drugs and detection of adulterants in plant materials (Ghani, 1990). Macro and microscopical evaluation of crude drugs are targeted at identification of precise variety and search for contaminants in plant materials (WHO, 1996). Chemo-microscopical examination of the powdered stem bark and root of *F. sycomorus* revealed the presence of cellulose, tannins, starch, lignin, calcium oxalate, suberin, aleurone grain and mucilage but calcium carbonate was absent in the stem bark but present in the powdered root.

The average moisture content of the powdered plant material using loss on drying method was found to be 6.4% and 7.82% in stem bark and root respectively and this value is within the permissible limits because British Herbal Pharmacopeia (1990) and WHO, (2011) recommend the percentage of moisture content in any crude drug to be within 12-14 %. Low or permissible moisture in crude drugs may discourage the growth of bacteria, yeast, mould and fungi and will stand for long period of time during storage without spoilage or suggesting better stability against degradation of product (WHO, 1996). Ash values obtained include total ash as 7.2% and 9.3% in the powdered stem bark and root respectively. These Ash values indicate the presence of various impurities such as carbonate, oxalate, sand and silicate in plant materials (Kaneria and Chanda, 2011). These values are useful as criteria to evaluate the identity and purity of crude drugs (Evans, 2009; WHO, 1996). It also indicates the presence of various inorganic materials like carbonate, oxalate and silicate in plant materials.

From the ash values 7.2% and 9.3% of stem and root respectively. The total ash value represented both the physiological and non-physiological ash from the crude drugs upon incineration. The non-physiological ash is the inorganic residues in water soluble ash after the plant drug is burnt while the acid insoluble ash indicated that the plant was in good physiological condition and it contained little extraneous matters compared to the total ash content. The total ash value is used as a standard to assess the identity and purity of crude drugs (WHO, 1996, WHO, 2011).

Preliminary phytochemical screening of the stem bark and root extracts of *F. sycomorus* revealed the presence of some phytochemicals such as carbohydrate, alkaloids, tannins, flavonoids, cardiac glycosides, saponins and triterpenes. The ethanolic stem bark extract of *Ficus sycomorus* as reported to contained alkaloids, tannins, anthraquinones, phlobatannins, cardiac glycosides and sugars (Ishola *et al.*, 2013), Adebayo and

Odeniyi (2012) reported that ethanolic leaves and stem bark extract contained tannins, phytates, saponins, alkaloids, terpenoids, flavonoids and phenolics. These primary and secondary metabolites in plants have numerous functions. Crude, pure and isolated alkaloids and their synthetic derivatives have been used as analgesic, antispasmodic and bactericidal agents (Okwu and Okwu, 2004). Flavonoids have been shown to provide antibacterial, anti-inflammatory, antiallergic, antimutagenic, antiviral, antineoplastic, anti-thrombotic and vasodilatory activity (Alan and Miller, 1996). Flavonoid also has immense antioxidant and anti-inflammatory activity because of its ability to scavenge hydroxyl radicals, super oxide anions and lipid peroxy radicals (Okwu and Josiah, 2006). Tanins have been used in the treatment of wounds especially those emanating from varicose ulcers and hemorrhoids (Njoku and Akumufula, 2007) and is able to stop bleeding during circumcision (Edeoga *et al.*, 2005). The phytochemical constituents especially the secondary metabolites could be useful as guide to chemotaxonomic markers (Jonathan and Tom, 2008) that will aid in chemo taxonomical classification system and further phylogenetic studies in Moraceae family.

The antioxidant activity of the *F. sycomorus* stem bark and root was successfully carried out using DPPH assay for free radical scavenging potentials of the extracts. The remarkable antioxidant activities exhibited by methanol extracts have made them potential free radicals scavenging agents, and this is probably due to their phenolic, alkaloids, flavonoids, terpenoid and vitamins constituents (Sri-sudewi *et al.*, 2014). The facts mentioned above have proven the folkloric use of this plant in traditional medicine in Nigeria for the treatment of numerous diseases and health conditions such as malaria, fever, pains, diabetes and convulsion (Akuodor *et al.*, 2015).

The findings of this study showed that both stem bark and root extracts of *F. sycomorus* had low IC₅₀ of 1.312 µg/ml and 0.001 µg/ml respectively against the H₂O₂ radicals. Thus, the low IC₅₀ values obtained from the study indicates that the two extracts have strong H₂O₂ scavenging activities. Thus supports their therapeutic use against stress-related disorders. The IC₅₀ value obtained for *F. sycomorus* was similar to one obtained by Deo *et al.* (2016) on some selected herbal extracts inhibitory properties against protein glycation and angiotensin enzyme linked to type II diabetes. Additionally, *P. amarus* and *L. pumilavar. alata* medicinal plants have been shown to possessed potent radical inhibiting properties with low IC₅₀ values of 3.4 and 5.7 µg/ml, respectively (Saputri and Jantan, 2011).

The reduction of DPPH radical is one of the popular and simpler ways to measure antioxidant activities of medicinal plants. The potential of plant extracts to inhibit DPPH radical is strongly linked to their ability to donate electrons to the radical (Daniel and Dluya, 2016). Normally, DPPH radical is stable in various solvents including methanol, ethanol and water. Therefore, the radical is usually prepared in a solution of either ethanol or methanol (Fukumoto and Mazza, 2000). In the present study, the DPPH radical was prepared in methanol. The *in vitro* DPPH

scavenging assay was preferred in this study because it is rapid, easy, reliable and less expensive since it does not require specialized device and methods.

The results obtained in this study showed dose dependent DPPH scavenging activities of the two extracts. It was, however, noted that the stem bark and root extract of *F. sycomorus* had lower DPPH scavenging abilities than ascorbic acid. This could be due to the crude nature of extract as compared to the refined standard drug. This result does not corresponded with the observations of Igbinosae *et al.* (2011), who found that *Jatropha curcas* had lower DPPH activities than ascorbic acid (standard). The results of stem bark extract of *F. sycomorus* agrees with a study by Kambli *et al.* (2014), who found that the DPPH scavenging activity of *F. racemosa* was considerable but not higher than that of the standard drug. The good antioxidant property of *F. sycomorus* stem bark extract against DPPH, corroborate well with findings of Degollado *et al.* (2014), who noted that *F. odorata* had a good antioxidant activity against DPPH radicals.

The IC₅₀ values of the root and stem bark extract of *F. sycomorus* against DPPH free radical were 0.001 µg/ml and 1.312 µg/ml respectively. The low IC₅₀ values obtained from this study, showed that both extracts had excellent antioxidants activities against DPPH radicals. This argument is in line with studies by Mbaebie *et al.* (2012), who worked on stem bark extract of *S. latifolia* against DPPH radical, and obtained an IC₅₀ value of 0.126mg/ml thereby strongly recommending the plant to be used as an antioxidant supplement.

Flavonoids and phenols naturally exhibit strong scavenging abilities for free radicals due to their hydroxyl groups (Mohamed *et al.*, 2010), which are attached to their aromatic ring structures and help to quench the radicals either by donating their electrons and thus neutralizing them or via the electron delocalization over all three ring system achieved by ortho-dihydroxyl of the B-ring and 4-oxo group of the ring C of the flavonoid, which actively reduce radicals like DPPH and Fe³⁺ to Fe²⁺ ions (Marino *et al.*, 2014). Phenols are common in diet and are divided into two classes; the benzoic derivatives like gallic acid and cinnamic derivatives like caffeic acids. Gallic acid is the best known polyphenol working efficiently in polar medium, the compound has greater ability of scavenging hydroxyl free radicals through deprotonation. Gallic acid is a prolific scavenger, with a greater ability to deactivate variety of ROS and RNS mainly via electron movement through the cellular physiological pH (Marino *et al.*, 2014). The mechanism of antioxidant action of phenolic compounds is through inactivation of lipid free radicals or by preventing the decomposition of hydroperoxides into free radicals (Kibiti and Afolayan, 2015).

In this study, the phytochemical screening of *F. sycomorus* extracts showed that in addition to flavonoids and phenols, the extracts contained saponins, terpenoid, alkaloids, steroids and cardiac glycosides, which are helpful in protecting cells against oxidative stress. Saponins are surface active group of complex compounds largely found in plants but also to some extent, in marine organisms (Chen *et al.*, 2014). The structure of saponins,

consists of two parts; a sugar oligosaccharide and a steroid (Akinpeluet *et al.*, 2014). Saponins have been shown to have an increasing use in traditional and industrial applications as anti-inflammatory, molluscicidal, antimicrobial, antispasmodic, antidiabetic, antitumor and as well as an antioxidant (Chen *et al.*, 2014). There is also growing evidence linking saponins to strong antioxidant activities through electron donation (Tapondjouet *et al.*, 2011). They are also widely used in beverage, cosmetic and confectionary industries due to their antimicrobial effects (Tapondjouet *et al.*, 2011).

Alkaloids are widely distributed among different plants families. Some reports have indicated that they have a strong antioxidant property. They express their antioxidant activities by removing the effects of hydrogen peroxide-induced oxidation, inhibit lipid peroxidation by inhibiting the cyclooxygenase enzyme, and protect neurons against toxic effects of dopamine and glutamate. Besides, other studies have shown that alkaloids exert protective effects on oxidative neuronal damage through ROS scavenging (Kim *et al.*, 2004). In addition, Moura *et al.* (2007) demonstrated significant antioxidant effects of alkaloids on yeast.

Condensed or hydrolysable tannins are naturally occurring plant polyphenol compounds composed of a central glucose molecule with galloy residues. They are found in several beverages and green leaves (Chung *et al.*, 1998). Horikawa *et al.* (1994) found that tannins have anti-mutagenic and anti-carcinogenic properties. Bouchet *et al.* (1998) reported that tannins have antiradical and potent antioxidant properties. They are also known to inhibit lipid peroxidation by inhibiting the enzyme cyclooxygenase (Chen *et al.*, 2014). Tannins achieve their antioxidant properties through chelating metal ions such as Fe³⁺ and they are also known to destabilize some key steps in the Fenton reaction, thereby slowing down the oxidation process (Andrade *et al.*, 2005).

Although the *in vivo* analysis of the two plants extracts are yet to be done, results obtained in this study strongly point out that the two tested extracts exhibit strong *in vitro* antioxidant activities. No wonder these plants have been in use in African traditional medical practices in treatment and management of diseases like rheumatism, diabetes, abdominal pain, healing wounds and mouth antiseptic which are all oxidative stress-related disorders.

Acute toxicity studies of *F. sycomorus* stem bark and root was performed using the Lorke guideline and using the limit dose test of Up and Down method. With careful observations of experimental animals from the first 30 minutes up to the 24hrs, it was revealed that there were no deaths and any sign of toxicity such as loss or increase in weight, tiredness, abdominal constrict convulsion, hyperactive, weakness, diarrhea or increased diuresis within the short and long term effect in rats dosed with 5000 mg/kg body weight of the *F. sycomorus* extracts. The outcome of the study of Al-hassan *et al.*, (2014) gave an LD₅₀ of 2000 mg/kg and this guided our choice of dose used (5000 mg/kg). The LD₅₀ was found to be greater than 5000 mg/kg body weight orally, and this suggested that the extract has low acute

toxicity when administered orally. This may be attributed to the incomplete absorption brought about by inherent factors limiting absorption in the gastro intestinal tract (Dennis, 1984). The present study agrees with the work done by Prasanthet *al.*, (2015); Ugboguet *al.*, (2016); Kofi *et al.*, (2014) and Adesegun *et al.*, (2016). Bruce, (2006) reported that any substance with LD50 estimated to be greater than 2000-5000 mg/kg body weight given orally could be considered to be of low toxicity and safe. Similarly, the chemical labelling and classification of acute systemic toxicity based on oral LD50 values recommended by the organisation of Economic Cooperation and Development (OECD, Paris, France) and (Walum, 1998) are as follows: less than 5 mg/kg: very toxic, greater than 5 but less than 50 mg/kg: toxic, greater than 50 but less than 500 mg/kg: harmful, and, greater than 500 but less than 2000 mg/kg: no label. The very high LD50 observed is not a conclusive finding about the safety of the extracts of *F. sycomorus*, higher doses could be tested for better understanding of its effects if use for a long period of time and for proper recommendation on its future utilization (Ogbonniaet *al.*, 2011).

CONCLUSION

The methanol stem bark and root extract of *F. sycomorus* were found to possessed several bioactive constituents including flavonoids, saponins, tannins, cardiac glycosides among others, associated with potent pharmacological activities. The extract was found to possessed considerable antioxidant properties at doses tested. This partly justifies the claim for the traditional use of the plant in the treatment of oxidative stress and toothache. Methanol extracts of *F. sycomorus* contain other phytochemicals which are rich in antioxidant and potent DPPH radical scavenging activities.

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