JMPHTR 6 (2018) 1-8 ISSN 2053-1826

Journal of Medicinal Plant and Herbal Therapy Research
www.bluepenjournals.org/jmphtr

Phytochemical properties, toxicological screening and antibacterial qualities of various parts extracts of *Ficus sycomorus*

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Article History
Received 20 January, 2018
Received in revised form 10 February, 2018
Accepted 13 February, 2018

Keywords:
Anti-nutritional analysis, Bactericidal properties, Sycamore plant.

Article Type:
Full Length Research Article

ABSTRACT

Anti-nutritional analysis and bactericidal properties of various parts of *Ficus sycomorus* were determined using standard methods. The extracts of *F. sycomorus* were subjected to bacterial susceptibility tests while the fruit was fed to Wistar rats to determine toxicological effects. The results obtained revealed the presence of tannin, hydrogen cyanide, flavonoid, saponine, alkaloid, oxalate and vitamin C in various proportions in the plant parts examined. The fruit extract showed bactericidal effect on some multi drug resistant bacteria such as *Escherichia coli*, *Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa* and *Proteus vulgaris*. Toxicological analysis of the fruit therapy on Wistar rats showed positive results as enzymatic examination of the liver, kidney and blood of the Wistar rats did not indicate any serious damage. Histological study carried out on the Wistar rats showed that the liver, kidney and blood did not suffer any damage in both 400 and 800 mg/kg test groups. Antimicrobial qualities of *F. sycomorus* conferred on it an alternative source of treatment for pathogenic infection. Therefore, it is recommended that the plant parts of *F. sycamorus* be consumed as food; for their numerous health benefits and their great potentials as antimicrobial agents.

INTRODUCTION

Currently, all over the world, research work is going on to find effective remedy against drug resistant bacteria. The medicine quest focuses on the drug of the future that will be derived from natural product (Fabricant and Fansworth, 2001). The search for unfamiliar plants in the wild regions with potential valuables as human and animal food as well curative medicine is gathering momentum (Okorondu et al., 2015). The derivatives of these plants are claimed to have several medicinal and other desirable properties (Farouk et al., 2008; Calixto et al., 1984; Evans, 2005; Obadini and Ochuko, 2001; Leandro et al., 2014).

Ficus sycomorus, also called sycamore fig or the fig Mulbern belong to the mulberry family, Moraceae and consist of about four genera and over one thousand four hundred species of trees (Zerega et al., 2005). The plant is indigenous to Africa and grows along South of Sahel and North of the tropic of Capricorn (Dale, 2007). *F. sycomorus* stem bark had been reported to have effect against tuberculosis as well as the sedation and anti convulsion properties of this plant have also been reported (Sandabe et al., 2003).

This study reports on the phytochemical and antinutritional properties of *F. sycomorus* plant. The antimicrobial and toxicological properties of the plant

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were also evaluated.

MATERIALS AND METHODS

Preparation of the treatments

Various *F. sycomorus* plant parts, that is (Leaves, stem bark, root, seed and edible fruit) were obtained from Ndikpa Alaeuyi, Ogwa Mbaitoli Local Government Area of Imo State, Nigeria. The leaves were plucked washed with water and dried at room temperature. The stem bark and roots were chopped into small pieces and also left to dry at a room temperature. The seeds were removed from the fruits and dried separately at room temperature. The dried samples were blended separately to fine powder (AOAC, 2010).

Determination of phytochemicals

Tannins were determined using Felin-Dennis Spectro-photometer method as described by (Mitra et al., 2000). Saponin was determined by method described by Obadini and Ochuko (2001). Flavonoids quantitative analysis was carried out using Harbone (1993) method as described by Okwu (2005). Alkaloids were determined by alkaline precipitation gravimetric method of Harbone (1993) as being described by Obadoni and Ochuko (2001) and Okwu (2004, 2005). Oxalate was determined by method described by Okwu (2005). Hydrogen cyanide was determined by method described by Odoemelam (2005).

Preparation of plant extract for antibacterial activity

The plant extraction procedure was carried out according to the method of AOAC (2010). The different parts of the plant were dried under shade at room temperature for at least 7 days, segregated and pulverized by mechanical grinder to form coarse powder. The course powder was air dried and samples were macerated in 800 ml of methanol for 72 h in the ratio of 1:20 (w/v). The methanol supernatants obtained was filtered in cotton wool and Whatman No 1 filter paper and evaporated until dryness under reduced pressure (204 mbar) at the temperature of 40°C. The residues were collected for further analysis.

Disc preparation

One gram of the extract was added to 2 ml of sterile distilled water. Thirty microliters of this extract was taken with pipette and delivered onto 6 mm sterile Whatman No 1 filter paper disc in drops and allowed to dry for few minutes before another drop of the extract was added. This was repeated until 30 µl were fully absorbed according to Cheesbrough (2005).

Source of test organisms

Test organisms that is, *E. coli*, *K. pneumoniae*, *S. aureus*, *P. aeruginosa* and *P. vulgaris*, were isolated from patients with confirmed clinical cases of urinary tract infection, wound and gastrointestinal tract infections at Fedicon Medical Laboratory, at Owerri, Imo State, Nigeria. All the isolates were resistant to multiple drug therapy.

Preparation of standard inoculums of test organisms

Broth culture of test organisms that were 24 h old were standardized using 0.5 McFarland standard before inoculating onto the surface of Muller Hinton agar by streaking method (Cheesbrough, 2005). The experiment was carried out in triplicate.

Discs impregnated with extracts were then placed on the Muller Hinton at distance of 5 mm from each other. Standard antibiotics were placed alongside as positive control. Zone of inhibition was measured and recorded in millimeter after 24 h incubation (Cheesbrough, 2005).

Toxicology study

Healthy Wistar rats used for the study were obtained from the animal farm of University of Agriculture, Umudike, Abia State, Nigeria and kept for 10 days to acclimatize under laboratory condition.

Thirty rats were divided into three groups. Control rats received the vehicle (distilled water) only. Rats in group 2 and 3 were administered 400 mg/kg fruit extract each for 21 days and 800 mg/kg for 10 days. The toxicological effect of the fruit extract on the rats was compared to that of the control. At the end of the experiment, rats were sacrificed by cervical dislocation. Blood was collected by heart puncture for serum analysis. Liver and Kidney tissues were excised, rinsed in physiological saline and stored in 10% neutral buffer formalin saline until used for histological analysis.

Measurement of serum enzyme activities

Serum was prepared from the whole blood by centrifugation at 3000 rpm for 10 min at room temperature (Farombi et al., 2009). Aspertate serum transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), sugar, urea and creatinine

Table 1. Phytochemical composition of *F. sycomorus* extracts.

Phytochemical parameters	Leaf	Stem	Root	Seed	Fruit
Flavonoid (%)	7.33	5.32	2.90	5.80	0.747
Saponin (%)	0.994	1.668	5.40	2.90	0.611
Alkaloid (%)	18.27	27.90	2.50	2.60	1.195
Tannins (%)	0.25	0.742	2.579	1.814	0.210
Oxalate (g/g sample)	0.0372	0.1386	1.005114	0.4400	0.10645
MgHCN/100g	10.13	21.87	2.43	1.94	3.674
Mg Vit. C/100g	0	0	0	0	284.608

Table 2. Antimicrobial susceptibility test of plant extract and commercial antibiotics.

Test organism	Fruit extract (mm)	Stem extract (mm)	Root extract (mm)	Leaf extract (mm)	Seed extract (mm)	GRA 30 mg	CH 30 mg	Ref 10 mg	SPP 10 mg	STP 30 mg	GAX 5 mg	OFX 30 mg	SXT 30 mg	CPX 10 mg
E. coli	18	0	0	0	0	24 mm	26 mm	24 mm	0	0	0	0	0	0
Klebsiella sp.	7	0	0	0	0	20 mm	0	0	26 mm	13 mm	0	0	0	0
S. aureus	17	0	0	0	0	0	0	0	0	0	20 mm	15 mm	0	0
P. aeruginosa	10	0	0	0	0	0	0	0	0	0	0	0	22 mm	23 mm

GRA, Cefrazone; **CH,** Chloramphenicol; **Ref,** Reflaxacin; **SPP,** Sparfloxacin; **STP,** Streptomycin; **GAX,** Taravid; **OFX,** Ofloxacin; **SXT,** Septrin; **CPX,** Ceprofloxacine.

were determined using commercially available Kits (Randox).

Hydrogen carbonate, sodium and potassium were determined using the method described by Cheesbrough (2005). White blood cells (WBC) were assessed by method described by (Brown, 1993).

Histological studies

Liver and kidney tissues were fixed in 10% neutral buffered formalin embedded in paraffin wax and sectioned. After deparafinization and dehydration, the paraffin blocks were stained with hacmatoxylin and eosin for microscopic examination. The histology analysis was carried out at the Department of Anatomical Pathology, University of Port-Harcourt Teaching Hospital, Nigeria.

RESULTS

The quantitative phytochemical analysis is presented in Table 1. The results showed the presence of flavonoids, saponins, alkaloids, tannins, oxalates, hydrogen cyanide at various concentrations, with vitamin C occurring only in the fruit sample.

Table 2 shows the antimicrobial susceptibility test of *F. sycomorus* extracts compared with standard antibiotic sensitivity disc. Fruit extract and antibiotics showed various degrees of inhibition. The toxicological effect of *F. sycomorus* fruits extracts on the liver tissues of rats was evaluated by determining the levels of AST, ALT, unconjugated bilirubin (TB) and conjugated bilirubin (CB) as shown in Table 3. *F. sycomorus* fruits extract administered at doses of 400 and 800 mg/kg insignificantly increased the activities of AST, ALT and ALP which were dose dependent while TB and CB showed no change in

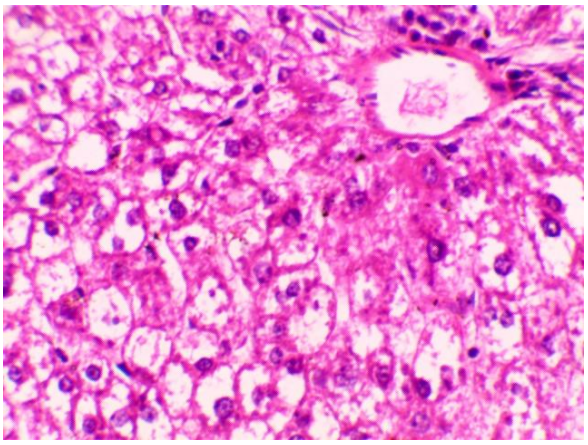

Figure 1. Micrograph of the liver tissue of the Wistar rat in the normal control group.

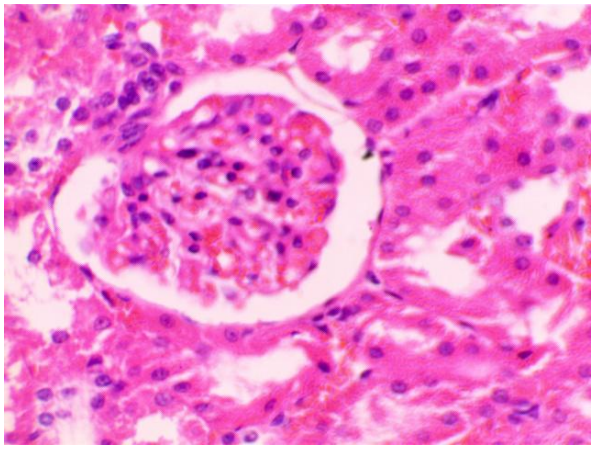

Figure 2. Micrograph of the liver tissue of the Wistar rat in the sub-acute test group.

Table 3. Hepatic toxicity test (Mean ± STD).

Parameters	Control	400 mg/kg 21 days	800 mg/kg 10 days
AST	35.0 ± 2.18 ul	39.5 ± 1.95 µl	44.3 ± 2.03 µl
ALT	15.0 ± 1.85 µl	20.2 ± 0.96 µl	24.9 ± 3.10 µl
ALP	19.3 ± 3.21 µl	201.5 ±	230 ± 3.25 µl
TB	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
CB	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1

AST, Aspertate transaminase; **ALT,** alanine transaminase; **ALP,** alkaline phosphatase; **TB,** unconjugated bilirubin; **CB,** conjugated bilirubin.

concentration at both doses.

Histological examination of liver samples corroborated the above findings. Thus the liver specimens from Wistar rats fed with 400 and 800 mg/kg of fruits extracts revealed non toxicological effect when compared with the liver in the control. In both cases, the integrity of the hepatocyte was relatively well preserved (Figures 1, 2 and 3).

Kidney functions were assessed by determining the activities of serum urea, creatinine sodium, potassium,

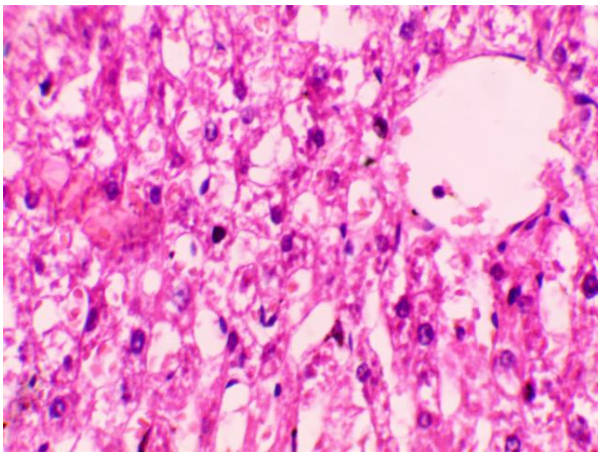

Figure 3. Micrograph of the liver tissue of the Wistar rat in the acute test group.

Table 4. Renal toxicity test (Mean ± SD).

Parameters	Control	400 mg/kg 21 days	800 mg/kg 10 days
Urea (mg)	21.5 ± 0.5	25.5 ± 0.5	36.5 ± 1.5
Creatinine (mg)	0.60 ± 0.1	0.68 ± 0.0	0.65 ± 0.4
Sodium (Na^+)	126.5 ± 2.5	121.5 ± 6.5	136 ± 0.0
Potassium (K)	8.35 ± 0.45	8.2 ± 0.45	11.3 ± 0.9
Chlorine (Cl)	94 ± 2.0	94 ± 5.0	103 ± 4.0
Hydrogen carbonate HCO_3	18 ± 1.0	16 ± 1.0	22 ± 1.0
Sugar	7.4 ± 2.0	7.4 ± 23.5	74.5 ± 0.55

chlorine, hydrogen carbonate and sugar as presented in Table 4. The result showed insignificant elevation of these parameters, accept sugar which remained stable.

Kidney histology examination also corroborated serum urea and creatinine levels. In both the fruit extracts and control samples, the integrity of the renal cells was also relatively preserved (Figures 4, 5 and 6).

The assessment of onward effect on blood toxicity of the animals relative to control was done by counting their total WBC and differential count. The results showed decrease in WBC (leucopenia) and increase in neutrophiles cells, few microcytic and hypochromic cells were observed (Table 5). The two slight changes in the blood assessment are believed to reverse back to normal after the withdrawal of the extracts.

DISCUSSION

Medicinal plants have been used for centuries as agents to combat diseases which could be dated to the origin of Man (Okorondu et al., 2015). Medicinal plants are the richest bio-resources of remedies for human diseases and offer a new source of drugs of traditional medicinal systems, modern medicines, biologically active chemical compounds as antimicrobial nutraceuticals, food supplements, folk medicines, pharmaceuticals, intermediate and chemical entitled for synthetic drugs (Hammer et al., 1999).

The plant extracts of *F. sycomorus* were screened for their phytochemical composition. Alkaloids, flavonoids, saponins, tannins, oxalates, hydrogen cynanide were detected in all the samples (leaves, stems, roots, seeds and fruits) with vitamin C occurring significantly high in the fruit extract. Flavonoids are most predominant in the leaves, alkaloids in the stems and saponins and tannins in the roots.

Also, flavonoids have been reported to possess antioxidant, antimicrobial, anticancer, anti-allergic and anti-inflammatory activity (Pier-Giorgio, 2000; Prochazkova, 2011). Though many alkaloids are toxic, some have pharmacological effects and are used as medications, recreational drugs, or in religious rites (Godlaski, 2011). In addition, alkaloids, saponins and

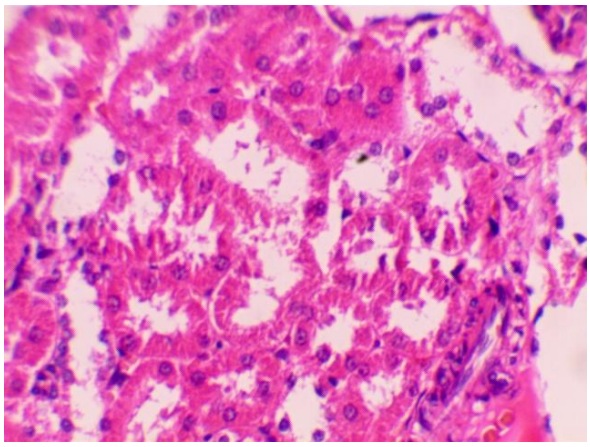

Figure 4. Micrograph of the Kidney tissue of the Wistar rat in the normal control group.

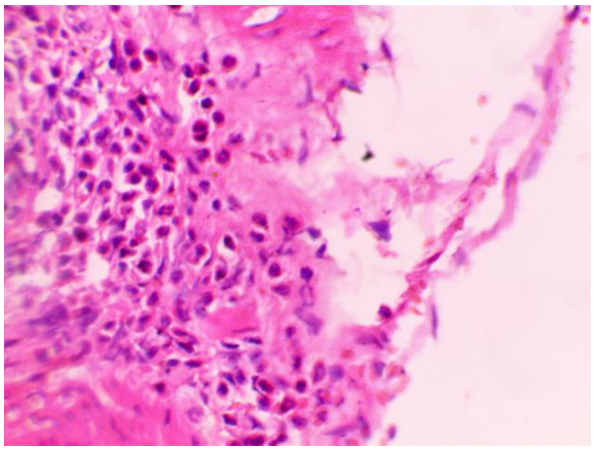

Figure 5. Micrograph of the Kidney tissue of the Wistar rat in the sub-acute group.

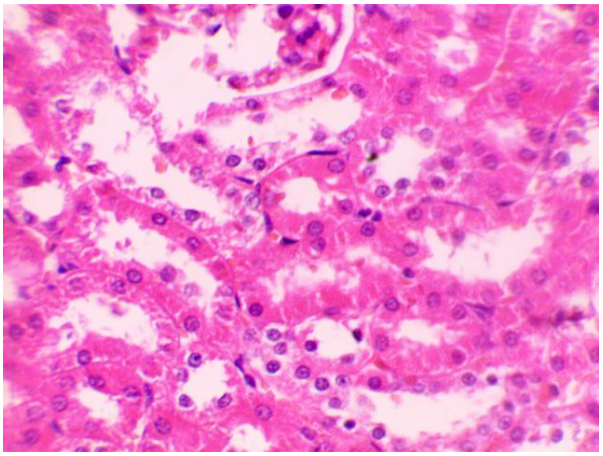

Figure 6. Micrograph of the Kidney tissue of the Wistar rat in the acute test group.

Table 5. Haematoxicity test.

Parameters	Control	400 mg/kg 21 days	800 mg/kg 10 days
White blood cells per litre	11.25 ± 0.55	5.55 ± 0.05	6.45 ± 0.55
Neutrophile per litre	13 ± 1.0	57.5 ± 1.5	20.5 ± 0.5
Lymphocyte per litre	87.5 ± 1.0	42.5 ± 1.5	79.5 ± 0.5
Monocyte	Nil	Nil	Nil
Eosinophile	Nil	Nil	Nil
Basophile	Nil	Nil	Nil
Blood film	Normocytosis	Microcytic and hypochronic	Microcytic

tannins are known to have antimicrobial activities as well as other physiological activities (Sofowora, 1993; Evans, 2005). Alkaloids are also known for their toxicity but not all alkaloids are toxic. Alkaloids inhibit certain mammalian enzymatic activities such as those of phosphodiesterase, prolonging the action of CAMP. They also affect glucagons and thyroid stimulating hormones, while some forms of alkaloids which extracted from *Rhazya stricta* have been reported to be carcinogenic (Soonham, 2015). However, some alkaloids have been used either as an analgesic, antispasmodic or bactericidal agents (Tim-Cushnie, 2014; Calixto et al., 1984; Farouk et al., 2008). Tannins have astringent properties that affect palatability, reduce food intake and consequently body growth. It also hastens the healing of wounds and inflamed mucus membrane; and prevention of decay. Tannins compounds have antimicrobial activities and are responsible for preventing and treating urinary tract infections and other bacterial infections (Leandro et al., 2014). High oxalate foods have been known to exert a negative effect on calcium and iron absorption. Oxalic acid ingestion results in corrosion of the mouth and gastrointestinal tract, gastric haemorrhage, renal failure and haematuria (Concon, 1988).

The knowledge of cyanogenic glycosides is important due to their hydrogen cyanic acid (HCN) poison in the body (Onwuka, 2005) but should not pose a problem, since the frequently used parts (leaf and fruit) in phytomedicine are free of this toxic compound. Catherine et al. (1995) reported that plant steroids were antioxidants *in vitro*, and have link with reproduction in humans. Their values in the investigated samples are appreciable and could add to their medicinal properties.

In the present study, the highly antibacterial property present in the extract of the fruit may act in synergy with the vitamin C to produce medical benefit inherent in the *F. sycomorus* fruit extract. Antibacterial assay of the plant extracts was compared with some commercial antibiotics against the drug resistant pathogens. The findings of this study demonstrated that fruit extract showed area of inhibition in the test organisms; *E. coli*, *K. pneumoniae, S. aureus, P. aeruginosa* and *P. vulgaris* as shown. However, the extracts of the stems, roots, leaves and seeds showed no significant area of inhibition.

Toxicity property may be a contributing factor in the medical usage of *F. sycomorus*. Liver and kidney organs have been reported to play a significant role in assessing toxicity. It is consented that inflammatory action of the herb on the liver and kidney tissues increases the serum level of the enzymes. The effect of the extracts on the serum levels of AST, ALT, ALP, TB and CB at $P>0.05$, there was no significant increase to cause liver damage (Berk et al., 2011). The level of AST, ALT and ALP in the 800 mg/kg group was also not significantly higher ($P>0.05$). TB and CB concentration were relatively the same in the group that ingested the herb therapy.

Similarly, renal toxicity assessment revealed a slight increase in urea and creatinine while, Na, K, Cl and HCO_3^{2-} parameters showed slight pocket of changes and the sugar level remained stable. Moreover, blood assessment also showed decrease in WBC (Leucopeania) and increase in neutrophles (netropeania) with few monocytic cells observed.

These slight changes in the tissues are believed to reverse back to normal after the withdrawal of the fruit therapy. The absence of monocyte eosinophil and basophil indicates that the fruit therapy does not cause allergic reactions (Simon and Hu, 2010).

Histology examination also corroborated the results of the biochemical assays. In both Wistar rat groups that ingested the fruits extract therapy and control group, the integrity of the renal and liver cells was relatively preserved.

The results showed that the herbal therapy is safe for ingestion at the tested dosage used. The herb therapy has immune boosting properties as indicated in the haematoxicity analysis. The basis of its antibiotics activities is based on the active antibacterial compounds in the fruit therapy.

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