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Phytochemical Screening and Antimicrobial Activity of Leaves and Fruits Extract of *Ficus sycomorus*

[1]Mudi, S. Y., [1]Muhammad, A., [2]Musa, J. and [3]Datti, Y.
[1]Department of Pure and Industrial Chemistry, Bayero University, P.M.B. 3011, Kano, Nigeria
[2]Union Diagnostic and Clinical Services, No. 53 Airport Road, Kano
[3]Department of Chemistry, Northwest University, Kano
Email: whydee@yahoo.co.uk

ABSTRACT

The leaves and fruits of *Ficus sycomorus* were collected, air dried and grounded. Each of the samples (100g) was extracted with 400ml each of n-hexane, chloroform, ethyl acetate, and methanol sequentially, using Soxhlet extraction technique. They were labeled as FS1-1 to FS1-4 for leaves extract and FS2-1 to FS2-4 for fruit extract. Each of these fractions was phytochemically screened to investigate the presence of certain class of secondary metabolites. The extracts obtained were subjected to brine shrimp larvae test and antimicrobial bioassay. Some of the fractions were found to be active against the brine shrimp larvae and the tested organisms, with FS1-1 being the most active.

Keywords: Antimicrobial, Cytotoxicity, *Ficus sycomorus*, Phytochemicals,

INTRODUCTION

Traditional herbal remedies are known to be of great importance in the management of diseases (Lehane, 1977). *Ficus sycomorus* (belong to the mulberry family moraceae) have been reported in some literature to contain bioactive substances in leaves, roots, fruits and flowers that are used alone or in combination with other plants for the treatment of diseases like diarrhea, mental illness, epilepsy, dysentery, convulsive disorder and vomiting (Sandabe and Kwari,2000; Wakeel *et al.*, 2004). The leaves when fresh are cooked and mixed with groundnut cake and eaten as food (Bernnaman, 1982). In Tanzania, especially in the rural area, the leaves of the plant are used to treat snake bite and jaundice, also its latex is said to be effective for the treatment of chest diseases, cold, and dysentery. The stem bark of the same plant is used for the remedies of cough, throat infection and chest pains (Don Maydell, 1982).

In addition, *Ficus sycomorus* together with the leaves of *Daniellia oliveri* (Fabaceae) are effective in treating diarrhea in Hausa traditional medicine of Northern Nigeria (Ahmadu *et al.*, 2002). Igoli (2005) reported that the ethanol extract possesses analgesic activity. Igbokwe *et al.*, (2010) reported the effect of prolonged oral administration of aqueous *Ficus sycomorus* stem bark extract on testicular size of growing albino rat. Decoction of *Ficus sycomorus* stem bark is used to treat infertility involving low sperm counts but the reproductive risk associated with its use is uncertain. Adoum *et al.*, (2011) reported the hypoglycemic effect of methanol extract of stem bark of *Ficus sycomorus* which was investigated in alloxan induced type-2 diabetic on albino Wister rats.

The sedative and anticonvulsant properties of this plant have also been reported (Amos *et al.*, 2002). It has also been reported to possess analgesic, anti-inflammatory and anti-conceptive activities (Amos *et al.*, 2002 and Wakeel *et al.*, 2004). Kubmarawa *et al.*, (2007) reported the use of stem bark in treating tuberculosis. This plant was found to have partial inhibition on bacterial growth (Sandabe *et al.*, 2006). Extracts obtained from the plant's stem bark, using organic solvents, were found to possess higher antifungal activity compared to aqueous extracts (Hassan *et al.*, 2006). The aqueous stem bark extracts were also reported to have inhibitory effect on smooth and skeletal muscle contractions in laboratory animals (Sandabe *et al.*, 2006). *Ficus Sycomorus* also has been shown to possess antioxidant, antibacterial, hypolipidemic, and hypoglycemic activities (Lansky *et al.*, 2008; Abdel-Hameed, 2009; Ao *et al.*, 2008).

Phytochemical and toxicity evaluation on the stem bark of *F. sycomorus* L (Moraceae) was carried out by Ibrahim *et al.*, (2006) on mice with LD_{50} value of 471.1 mg/kg. Its chemical constituents were found to include tannins, resins, steroid glycoside, reducing sugars and saponins. The extract is said to be moderately toxic to mice

and therefore can be safely used ethno-medically at lower doses (Bello *et al.*, 2015).

MATERIALS AND METHODS

Sampling of Plant Material

The leaves and fruits of *Ficus sycomorus* were collected from Hotoro, Kano on 11th May, 2013. They were identified and authenticated at Biological Sciences Department, Bayero University, Kano. The plant materials were air dried and grounded into fine powder using mortar and pestle.

Extraction

Each of the dried samples was weighed (100g) and extracted with n-hexane, chloroform, ethyl acetate and methanol gradient; each at the ratio of 1:4 (m/v) using Soxhlet extraction process, at temperature of 50-60^{o}C (Sheba, 2009, Venkatesan and Karrunakaran, 2010). Time interval of 5-6 hours was set as the duration of the extraction depending on the relative extraction strength of the solvents respectively. The extracts were concentrated using rotavapour to recover some of the solvent at 40^{o}C.

All the concentrated extracts were collected in separate weighed beakers and allowed to dry up, so they can be used for the study. The extracts were coded and labeled as FS1-1 to FS1-4 (for leaves extracts) and FS2-1 to FS2-4 (for seed extracts).

Phytochemical Screening

All the extracts obtained were screened for the presence or absence of alkaloids, flavanoids, steroids, tannins, and saponins in the fractions according to standard protocols.

Test for Alkaloids

Hager's Test: Each of the fractions obtained was treated with Hager's reagent (saturated solution of picric acid). Formation of a yellow color precipitate indicates the presence of alkaloids.

Test for Flavanoids

Alkaline Reagent Test: To each test solution few drops of sodium hydroxide solution was added. The formation of an intense yellow color, which turns to colorless on addition of few drops of dilute acid, indicates the presence of flavonoids.

Test for Sterols

Salkowski's Test: Each fraction was treated in chloroform and few drops of conc. sulfuric acid. This was well shaken and allowed to stand for 5 minutes. Any red color appearance at the lower layer indicates the presence of sterols.

Test for Tannins

Ferric Chloride Test: Each fraction was treated with ferric chloride ($FeCl_3$). Formation of blue green color indicates the presence of tannins.

Test for Saponins

Froth Test: Each of the fractions was vigorously shaken with distilled water and allowed to stand for 5 minutes. A persistent frothing indicates the presence of Saponins. (Soforowa, 1984).

Brine Shrimp Lethality Test

Investigation of the cytotoxicity of the extracts obtained was evaluated using lethality test on brine shrimp larvae (*Artemia Salina*). The brine shrimp eggs were added in a hatching chamber containing ocean sea salt water. The hatching chamber was kept under fluorescent light for 48hours for the eggs to hatch into shrimp larvae.

The extracts (20mg) were separately dissolved in methanol (2ml) from which 500, 50, and 5ml of each solution was transferred into vials corresponding to 1000, 100, and 10mg/ml respectively. Each dosage was tested in triplicate. The vials (9 per test sample and one control) were allowed to evaporate to dryness at room temperature. Two drops of dimethyl sulphoxide (DMSO) and ocean sea salt water solution (4.5ml) were added to each vial. Ten larvae of *Artemia salina* (48-72 hours after initiation of hatching) were counted into each vial. Each vial was then adjusted to a 5ml volume with sea salt water solution immediately after adding the shrimps. After 24 hours the number of surviving shrimps at each dosage was counted and recorded.

ANTIMICROBIAL BIOASSAY

Preparation of Test Solution and Disc Concentration

The stock solutions were prepared by dissolving 0.5g of each extract in 1ml of DMSO. Concentrations of 60 µg/disc, 30 µg/disc, and 15 µg/disc were prepared for the disc diffusion test.

Microorganisms

The cultures of Gram positive organisms; *Staphylococcus aureus, Aspergillus flavus* and *Mucor aspergillus* and Gram negative organisms; *Escherichia coli* and *Pseudomonas aeruginosa* were obtained from Department of Microbiology, Aminu Kano Teaching Hospital, Kano.

Inoculums Standardization

As described by standard sensitivity test (National Committee for Clinical Laboratory Standards, NCCLS), loops of the confirmed isolates were introduced in peptone water in separate sterilized bottles and kept overnight in an incubator (37^{o}C). Few colonies of the overnight growth of the isolates to be tested were dispersed in

sterile normal saline to form a turbid culture suspension that match 0.5 McFarland turbidity (NCCLS, 2000).

Preparation of Media

Nutrient Agar (28g) in 1 liter of distilled water) was autoclaved for 15minutes at 121^{o}C. The sterilized Nutrient Agar (20 cm^{3}) was transferred into a petri-dish under septic condition. The petri-dish was then allowed to cool and solidify.

In vitro Antimicrobial Susceptibility Test (AST)

Antibacterial activities of the extracts were determined using Disc Diffusion method of Antimicrobial Susceptibility Test (AST) as described by (NCCLS, 2000). Standardized inocula of isolates were swabbed onto the surface of the solidified and oven-dried Nutrient Agar in separate Petri-dishes under sterilized environment.

The discs of different concentrations (60 µg/disc, 30 µg/disc, 15 µg/disc) of the respective extracts were then placed onto the surface of the inoculated media at intervals in a clockwise direction. The positive control discs were placed at the center of the bacteria and fungi inoculated media, respectively. The plates were incubated for 24hours after which antimicrobial activity was observed by measuring the width of the clear inhibition zone around the discs and the values obtained recorded.

RESULTS AND DISCUSSION

Air dried leaves and fruits of *Ficus sycomorus* were extracted using four different solvents in order of increasing polarity; n-hexane, chloroform, ethyl acetate and methanol which yielded extracts as shown in Table 1. The Phytochemical screening (Table 2) of the various fractions of the plant materials, showed the presence or absence of alkaloids, flavanoids, tannins, saponin and steroids. Brine shrimp lethality test was employed in determining the cytotoxicity of the extracts after testing their microbial activity. The LC_{50} was calculated and presented in Table 3.

Table 1:Physical Appearance of extracts Obtained from *Ficus sycomorus*

Plant parts	Fractions	Code	Weight	Colour	Texture
Leaves	n-Hexane	FS1 – 1	5.4g	Green	Gummy
	Chloroform	FS1 – 2	2.2g	Dark Green	Gummy
	Ethyl acetate	FS1 – 3	1.3g	Dark Green	Sticky
	Methanol	FS1 – 4	3.9g	Dark Green	Gummy
Fruits	n-Hexane	FS2 – 1	3.5g	Brownish	Oily
	Chloroform	FS2 – 2	1.3g	Brownish	Gummy
	Ethyl acetate	FS2 – 3	1.6g	Brownish	Gummy
	Methanol	FS2 – 4	1.2g	Brownish	Gummy

Table 2: Phytochemical Screening Results of the Fractions

		Phytochemicals				
Plant parts	**Fractions**	**Alkaloids**	**Tannins**	**Saponins**	**Flavonoids**	**Steroids**
Leaves	FS1 – 1	+	+	+	+	+
	FS1 – 2	+	-	+	+	+
	FS1 – 3	-	-	+	+	-
	FS1 – 4	+	+	+	+	+
Fruits	FS2 – 1	+	-	+	-	+
	FS2 – 2	+	-	-	-	-
	FS2 – 3	+	+	+	+	+
	FS2 – 4	+	+	+	-	-

Key: + = Present and - =Absent

Table 3: Brine Shrimp Lethality Test Result

Plant parts	Fractions	Code	LC_{50}
Leaves	n-Hexane	FS1 – 1	0.12
	Chloroform	FS1 – 2	27.3
	Ethyl acetate	FS1 – 3	7.6
	Methanol	FS1 – 4	>1000
Fruits	n-Hexane	FS2 – 1	1.5
	Chloroform	FS2 – 2	0.44
	Ethyl acetate	FS2 – 3	0.00
	Methanol	FS2 – 4	464.5

The antimicrobial activities of the extracts, the leaves and the fruits of *Ficus sycomorus* were tested against some microbial isolates and found to possess bioactivity against some of the tested microorganisms. The n-hexanes fraction of the leaf at 60µg/disc shows a remarkable zone of inhibition against the tested microorganisms. The result is presented in Table 4.

Table 4: Antimicrobial results of all the Fractions from the Leaves and Fruits of *Ficus sycomorus*

Plant parts	Fractions	Concentration (µg/disc)	Zone of Inhibition (mm) against the Microrganisms				
			STPA	*E. coli*	PSA	ASPF	MSPP
Leaves	FS1 – 1	60	12	19	16	11	13
		30	10	10	12	10	09
		15	08	00	11	09	08
	FS1 – 2	60	11	14	15	13	10
		30	09	11	12	11	08
		15	00	00	10	09	00
	FS1 – 3	60	12	12	18	11	12
		30	09	09	12	09	10
		15	00	00	00	07	08
	FS1 – 4	60	12	15	15	12	14
		30	10	00	12	10	10
		15	00	00	00	09	08
Fruits	FS2 – 1	60	14	13	15	12	12
		30	11	11	10	08	09
		15	00	09	09	00	00
	FS2 – 2	60	12	16	13	12	12
		30	09	12	10	09	09
		15	07	08	09	00	00
	FS2 – 3	60	13	15	18	09	15
		30	11	11	11	07	11
		15	00	09	00	00	10
	FS2 – 4	60	11	12	28	09	12
		30	09	00	10	00	09
		15	07	00	00	00	00

Key: STPA = *Staphylococcus aureus*, *E.coli* = *Escherichia coli*, PSA = *Pseudomonas aeruginosa*, ASPF = *Aspergillus flavus* and M.SPP = *Mucor aspergillus*

The extracts were found to be gummy in their texture. However, colours of the fruit extracts were found to be brownish while the leaf extracts were dark green except for the n-hexane extract which is green.

The leaves extracts indicate the presence saponins and flavonoids, while the fruits extracts showed the presence of alkaloids (Table 2). These classes of compounds are known to be biologically active and are associated with the antimicrobial activities of *Ficus sycomorus* (Mohamed *et al.*, 2013). Alkaloids have been associated with medicinal applications in plants, among which is their toxicity against cells of foreign organisms. These bioactivities have been widely studied for their potential use in the inhibitory activities of human cancer cell lines (Nobori *et al.,* 1994; Akinpelu *et al.,* 2008). Flavonoids also exhibit a wide range of biological activities such as antimicrobial, anti-inflammatory, analgesic, and cystostatic, hypoglycemic and antioxidant properties (Scalbert, 1991 and Hodek *et al.,* 2002). Saponins are considered a key ingredient in Chinese medicine and are thought to be responsible for most of the observed biological activity (Liu and Henkel, 2002; Ferguson, 2001). They are known to produce inhibitory effect on inflammation (Just *et al.,* 1998).

CONCLUSION

The phytochemical analysis of the plant revealed the presence of alkaloids, tannins, saponins, flavanoids and steroids in both the aqueous extracts of the leaves and the fruits. These bioactive agents may contribute to the medicinal efficacy of the plant. From the results obtained from this research, it can be concluded that extract FS1-1 is the most active against the tested organism and the brine shrimp larvae. Based on these results, further work should be donet for the isolation and characterization of the active compound in FS1-1.

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