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Antibacterial Activities of Leaf and Stem Bark Extracts of Ficus *Sycomorus on* Clinical Isolates of *Salmonella* Species and Its Invivo Toxicity Against Swiss Albino Mice.

KEYWORDS: Ficus sycomorus, Antibacterial, Invivo, Toxicity, Extracts, Albino mice.

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ABSTRACT *The phytochemical screening, antibacterial and toxicological activities of extracts of the leaf and stem bark of Ficus sycomorus were investigated. The phytochemical analyses according to standard screening tests using conventional protocol revealed the presence of secondary metabolites such as tannins, flavonoid, Saponins, and alkaloids. In-vitro agar diffusion of the leaf and stem bark extracts of the plant using methanol, water and hexane were investigated on Salmonella Typhi and Salmonella Paratyphi A respectively. Methanol, Aqueous and Hexane stem bark extracts showed activity on S. Typhi and S. Paprtyphi A. Hexane extract had no activity against S. Paratyphi A at the concentrations used. The study further showed that the methanol leaf extract had activity against S. Typhi but no activity was observed for the aqueous and hexane leaf extract against S. Typhi at the concentrations used. However, activity was observed for the methanol and aqueous leaf extract against S. Paratyphi A except for the Hexane leaf extract which showed no activity at the concentrations used. The minimum inhibitory concentration and minimum bactericidal concentration studies revealed that the bacterial isolates were inhibited at concentrations of about 12.5mg/ml and 25mg/ml against S. Paratyphi A and S. Typhi respectively and killed at 50mg/ml and 100mgmg/ml respectively. Toxicity studies of the methanol stem bark extracts revealed that the plant exhibited no significant toxicity. These results suggest that the plant may not be toxic to man and could be a potential source of novel antibacterial agents. It is suggested that the synergistic effect of the plant be evaluated with antibiotics and non-antibiotics respectively.*

INTRODUCTION

The active principles of many drugs found in plants are secondary metabolites. These secondary metabolites which constitute an important source of the pharmaceutical drugs have been isolated from different parts of plants. Some of these compounds have been reported to be present in the *Ficus species* such as tannins, saponins, flavonoids, steroids, glycosides and reducing sugars (Amos *et al.*, 2001; Hassan *et al.*, 2006). *Ficus sycomorus* has been suspected to possess anti-diarrhoeal activities, sedative and anticonvulsant (Olusesan *et al.,* 2010; Ahmadu *et al.*, 2010).

Ficus sycomorus (Baure or Bore in Hausa, Epin in Yoruba) belongs to *Moraceae*, a family that is reputable for its medicinal values and consist of about 40 genera and over 1,400 species of trees, shrubs, vines and herbs (Zegera, *et al.*, 2005). They are usually found near streams in the Savannah area. *Ficus sycomorus* is a tree attaining a height of 20m with widely spreading branches and a massive crown. Sheep and cattle eat its foliage (Datziel, 1953). Adeshina *et al.* (2009) reported that the leaf extracts of *F. sycomorus* was more potent than the stem bark extract against ciprofloxacin sensitive and resistant *Salmonella typhi*. Similar study reported by Bello *et al.*, 2013 revealed that the stem bark extract of *F. sycomorus* was slightly non-toxic indicating that the plant is safe for ethno medicinal uses. The Hausa and Fulani tribes of Northern Nigeria use the stem bark of the plant to treat Diabetes mellitus (Bello *et al.*, 2013).

Irrespective of the fact that antibiotics and medicinal plants are used in the management of infectious diseases, still the condition has not been fully eradicated. On the other hand most of the targeted microorganisms by such antibiotics have developed some resistance to them thus there is need for search for new drugs (Larhsini *et al.,* 2001). Antibiotics have also been found to cause side effects like nausea, diarrhea, constipation, memory loss, cardiotoxicity, hepatotoxicity, nephrotoxicity amongst many with most causing depression of the immune system (Gralla *et al.,* 2005).

Intoxication and inefficiency cases have also been on an increase on the usage of the medicinal plants, this can be due overdosing or under dosing of the medicinal plants used thus, there is need for scientific evaluation of such medicinal plants to ascertain their activity (Palombo, 2006). Lastly administration of such medicinal plants by most communities is mainly based on the patient's symptoms but not on proper diagnosis of the disease thus the underlying disease could be difficult to interpret. In addition, one plant can be used to treat different diseases or many plants can be used to treat one disease. Therefore it is important to evaluate the activity of such plants against the pathogens to ascertain there activity (Gathu, 2006). It is against this backdrop the study attempt antibacterial activities of *Ficus sycomorus* leaves and stem bark extracts against *Salmonella* Typhi and *Salmonella* Paratyphi A in order to ascertain the forcloric claim of its medicinal therapeutic uses.

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MATERIALS AMD METHODS

Collection and Identification of Plant Materials

Fresh leaves and stem bark of *Ficus sycomorus* plant were collected in Jenta Adamu, Jos, Nigeria in August 2014. The plant was authenticated by a plant Taxonomist at the herbarium unit of Federal College of Forestry, Bauchi road, Jos, Nigeria, where voucher specimens (FHJ 0195) were deposited.

Preparation of Plant Extract

The leaf and stem bark of *Ficus sycomorus* was allowed to shade dry at room temperature for four weeks. The air-dried leaf and stem-bark was pounded into fine powder. Fifty (50g) of each leaf and stem bark powder was weighed and percolated with 500ml of respective solvents (Methanol, Water and Hexane) for 8 hours in Soxhlet apparatus and then the extracts was filtered using Whatman N0 1 filter paper. Each extract was evaporated to dryness in an oven at 45°C. They were then stored in refrigerator until required for use (Shihabudeen *et al.,* 2010).

Determination of Phytochemicals

Qualitative phytochemical analysis of the extracts of the plant was determined by the methods used by Edeoga *et al.,* (2005); Jigna and Sumitra, (2007).

Preparation of Plant Extracts Concentrations

One gram of each aqueous, Methanol and Hexane extracts pre-prepared (each separately) was taken and the aqueous extract was dissolved in 10ml sterile distilled water, while the Methanol and Hexane extracts were dissolved in 10ml of DiMethyl Sulphoxide (DMSO). Thus 100 mg / ml of stock was obtained as a standard concentration of aqueous, Methanol and Hexane extracts respectively. Different concentrations of extracts were prepared using water and DMSO as solvents. Different working concentrations (100mg/l, 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml) were prepared using doubling dilution of the prepared stock solution of 100mg/ml concentration.

Collection, Preparation and identification of Isolates

Pure isolates of *Salmonella* Typhi and *Salmonella* Paratyphi A were obtained from the Bacteriological section of the Federal College of Veterinary and Medical Laboratory Technology Vom and confirmed using standard microbiological procedures. A 24h culture of the bacterial culture isolate was diluted with physiological saline solution and the turbidity corrected by adding sterile physiological saline until a McFarland turbidity standard of 0.5 (10^6 CFU/ml) was obtained (Cheesbrough, 2006).

Plant Extracts Activity Assay

Pour plate method of Bauer (1996) was adopted. Wells of 6 mm in diameter were made in the seeded Mueller-Hinton agar using a 6mm cork borer. A portion of the well was then sealed with molten nutrient agar to ensure adequate diffusion. Aliquot of 20 µl from each concentrations of extract was added into each well using a micropipette on the seeded medium and allowed to stand on the bench for 1 h for proper diffusion and thereafter incubated at 37^0C for 24 h. The resulting zones inhibition were measured in millimeters (mm) using a transparent ruler and values were tabulated.

Determination of MIC and MBC

This was determined using broth dilution method as described by Junaid (2006). The dilutions that showed no turbidity for the MIC were sub-cultured on Mueller-Hinton agar plates, well labeled for each of the extracts to determine the MBC. The minimum concentration that showed no growth when subcultured on a Mueller-Hinton agar plates was taken as the minimum bacteriocidal concentration (MBC).

Acute Toxicity Studies

The LD_{50} of the methanol stem bark and leaf extract of *F. sycomorus* was determined using modified methods of Lorke (1983) using 12 swiss albino mice in the first phase. The animals were fasted for 2 h before the study, but were given water *ad libitum*. In this phase, mice were divided into four groups of three mice each and were treated with the methanol extract of the specimen at different doses of 10, 100 and 1000 mg/kg (body weight) orally while the last group was then used as control. They were observed for 24 h for signs of toxicity such as inaction, dizziness, loss of weight and mortality. In the second phase, 12 mice were divided into four groups of three mice each and were treated with the extract doses of 1500, 2900, and 5000 mg/kg (body weight). The LD_{50} was calculated using appropriate formula if any toxicity or mortality was observed.

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

Where D_0 = Highest dose that gave no mortality,

D_{100} = Lowest those that produce mortality

RESULTS

Table 1 shows the phytochemical results of phytochemical screening of *Ficus sycomorus*. Preliminary investigation of the Methanol, Aqueous and hexane extracts of the leaf and stem bark of *F. sycomorus* were compared. Tannins were present in all the solvent's extracts. Saponins were evident in methanol, aqueous and hexane extract of the leaf and stem bark of the plant except for the aqueous stem bark extract. Similarly, Flavonoids, Alkaloids and Carbohydrates were present in all the extracts except for the aqueous and hexane stem bark extracts respectively. The result also showed that Steroids and terpenes were evident in methanol and aqueous leaf extract of the plant whereas Cardiac glycosides were absent in all the extracts but present only in the Hexane stem bark of the plant.

Table (2-3) depicts the antibacterial activity of the stem bark and leaf extracts of *F. sycomorus* at varying concentrations against *S.* Typhi and *S.* Paratyphi A respectively. The findings demonstrated that the stem bark extract was sensitive to all the test organisms and thus showed that the extract contained potential antimicrobial agents. However, the leaf extracts of *F. sycomorus* was observed to be less potent against *S.* Typhi and *S.* Paratyphi A respectively. The extract with the greatest antibacterial activity was methanol stem bark extract (13mm inhibition zone) at 100mg/ml for *S.* Typhi and *S.* Paratyphi A respectively. The higher tannin contents in the methanol stem bark extracts probably account for the high antibacterial activity of the extracts.

In the present study, the MIC values showed that the extracts were active against *S.* Typhi and *S.* Paratyphi A with MIC values ranging from 12.5mg/ml to 100mg/ml. The MBC of the leaf and stem bark extracts ranges between 25mg/ml to ≥100mg/ml against the test bacteria (Table 4 and 5).

The result of acute toxicity testing using modified locke's method showed that no death or other signs of toxicity

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was observed in both stages of the experiment. According to Hodge and Sterner, (1949) and locke, (1983), compounds of slight toxicity will have LD_{50} between 5000 and 15000mg/kg Thus, no LD_{50} was established in this study. It can thus be said that the LD_{50} of the extract is higher than 5000mg/kg body weight hence the extract is relatively non-toxic.

DISCUSSION

The phytochemical analysis of *F. sycomorus* revealed the presence of secondary metabolites such as tannins, flavonoid, Saponins, steroids, alkaloids which have been previously reported for their antimicrobial activities. This is similar to what is reported by Adeshina *et al.*, (2009) and Bello *et al.*, (2013) who asserted that many plants have been reported for therapeutic purposes because of the chemical compounds synthesized in these plants. Hence, the observed antimicrobial activity of the leaf and stem bark extracts against the test organisms may be due to the presence of phytochemical components.

The findings demonstrated that the stem bark extract was sensitive to all the test organisms and thus showed that the extract contained potential antimicrobial agents. However, the leaf extracts of *F. sycomorus* was observed to be less potent against *S.* Typhi and *S.* Paratyphi A respectively. This is similar to what was reported by Alphonsine *et* al. (2012) who reported that the leaf extract of *Ficus sycomorus* had no activity on *S.* Typhimurium but contrary to the findings of Adeshina *et al.* (2009) who reported that the leaf extracts of *F. sycomorus* was more potent than the stem bark extract against ciprofloxacin sensitive and resistant *Salmonella* Typhi. Thus, the difference observed in the antimicrobial activity of *F. sycomorus* leaf extract against *S.* Typhi reported in this study when compared to the report of Adeshina *et al.* (2009) on the same plant against the same organism may be attributed to difference in geographical location. The extract with the greatest antibacterial activity was methanol stem bark extract (13mm inhibition zone) at 100mg/ml for *S.* Typhi and *S.* Paratyphi A respectively. The higher tannin contents in the methanol stem bark extracts probably account for the high antibacterial activity of the extracts.

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CONCLUSION

From the results of the study, antibacterial activity exhibited by *F. sycomorus* leaf and stem bark extracts may be due metabolites such as tannins, flavonoids, Carbohydrates, saponins, steroids and terpenes and cardiac glycosides. The presence of tannins in all the extracts tested could probably be responsible for the observed antibacterial activity. The claims of literatures that *F. sycomorus* has antibacterial activities are hereby verified. These results also suggest that the plant extract may not be toxic to man and could be a potential source of novel antibacterial compound.

Table 1: Phytochemical constituents of the leaf and Stem bark extracts of *Ficus sycomorus*

	STEM BARK			LEAF	
Secondary metabolites	**ME**	**AE**	**HE**	**ME**	**AE**
Flavonoids	+	-	++	+	+
Saponins	-	+	+	+	++
Carbohydrates	++	+	-	++	+
Tannins	+++	++	++	+	+
Alkaloids	+	++	-	+	+
Cardiac glycosides	-	-	+	-	-
Steroids and terpenes	-	-	-	+++	+

Key: ME = Methanol extract, AE = Aqueous extract, HE = Hexane extract.

(+) = Present in small amount
(++) = Present in moderate amount
(+++) = Present in high amount
(-) = Absent

Table 2: Susceptibility Profile of *S.* Typhi to the extracts of *Ficus sycomorus*

Plant parts **Zone of Inhibition (mm)**		**Extracts**						
Concentrations (mg/ml)						Control		
		100	50	25	12.5	6.25	(+ve)	(-ve)
Stem Bark	AE	12	10	8	0.0	0.0	28	0.0
	ME	13	12	10	7	0.0	38	0.0
	HE	8	7	0.0	0.0	0.0	32	0.0
	AE	0.0	0.0	0.0	0.0	0.0	37	0.0
Leaf	ME	12	7	0.0	0.0	0.0	31	0.0
	HE	0.0	0.0	0.0	0.0	0.0	29	0.0

Key:
AE = Aqueous extract, ME = Methanol extract, HE = Hexane extract.
Reference drug = Ciprofloxacin (0.625mg/ml)

Table 3: Susceptibility Profile of *S.* Paratyphi *A* to the extracts of *Ficus sycomorus*

Plant parts **Zone of Inhibition (mm)**		**extracts**						
Concentrations (mg/ml)				Control				
		100	50	25	12.5	6.25	(+ve)	(-ve)
Stem Bark	ASE	9	8	0.0	0.0	0.0	37	0.0
	MSE	12	12	10	7	6	28	0.0
	HSE	0.0	0.0	0.0	0.0	0.0	34	0.0
	ALE	12	8	0.0	0.0	0.0	34	0.0
Leaf	MLE	10	9	9	6	0.0	31	0.0
	HLE	0.0	0.0	0.0	0.0	0.0	37	0.0

Key:
AE = Aqueous extract, ME = Methanol extract, HE = Hexane extract
Reference drug = Ciprofloxacin (0.625mg/ml).

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Table 4: Minimum inhibitory concentrations (MIC) of the extracts *Ficus sycomorus*

Test organisms	Extract	Concentrations (mg/ml)					MIC
		100	50	25	12.5	6.25	
S. Typhi	ASE	-	-	*	NA	NA	25
	MSE	-	*	+	+	NA	50
	HSE	*	+	NA	NA	NA	100
	MLE	*	+	NA	NA	NA	100
	ASE	-	*	NA	NA	NA	50
	MSE	-	-	-	*	+	12.5
S. Paratyphi A	ALE	-	*	NA	NA	NA	50
	MLE	-	-	*	+	NA	25

Key: ASE = Aqueous Stem bark extract, MSE = Methanol Stem bark extract, HSE = Stem bark extract, ALE = Aqueous leaf extract, MLE = Methanol Leaf extract.

(-) = No growth, NA = Not applicable
(+) = growth
(*) = MIC

Table 5: Minimum Bacteriocidal concentrations (MBC) of the extracts *Ficus sycomorus*

Test organisms	Extract	Concentrations (mg/ml)					MBC
		100	50	25	12.5	6.25	
S. Typhi	ASE	*	+	+	NA	NA	100
	MSE	-	*	NA	NA	NA	50
	HSE	+	NA	NA	NA	NA	>100
	MLE	-	NA	NA	NA	NA	100
S. Paratyphi A	ASE	-	*	NA	NA	NA	50
	MSE	-	-	*	+	NA	25
	ALE	*	+	NA	NA	NA	100
	MLE	*	+	+	NA	NA	100

Key:
ASE = Aqueous Stem bark extract, MSE = Methanol Stem bark extract, HSE = Stem bark extract, ALE = Aqueous stem bark extract, MLE = Methanol Leaf extract.

(-) = No growth
(+) = growth
(*) = MBC
NA = Not applicable

Table 6: Acute toxicity test of methanol Leaf and Stem bark extract of *F.sycomorus*

Leaf		Stem Bark
Doses (mg/kg)	Result	Remark
	PHASE 1	
10	No death	3/3
100	No death	3/3
1000	No death	3/3
PHASE 2		
1500	No death	3/3
2500	No death	3/3
5000	No death	3/3

Key:
3/3 = All survived

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