African Journal of Microbiology Research Vol. 4 (8), pp. 598-601, 18 April, 2010
Available online http://www.academicjournals.org/ajmr
ISSN 1996-0808 © 2010 Academic Journals

Full Length Research Paper

Preliminary *in-vitro* antibacterial activities of ethanolic extracts of *Ficus sycomorus* Linn. and *Ficus platyphylla* Del. (Moraceae)

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Accepted 23 March, 2010

Antibacterial therapeutic failure due to emergence of resistant bacterial strain is a world wide phenomenon. The search for effective antibacterial substances from sources such as plants has become a necessity to overcome emergent of bacterial resistant in clinical practice. The dried leaves and stem barks of *Ficus sycomorus* and *Ficus platyphylla* were collected in Samaru-Zaria, Nigeria in July 2006 and extracted with 70% aqueous ethanol at room temperature. The antibacterial activities such as susceptibility, Minimum inhibitory concentrations (M.I.C.) and the minimum bactericidal concentrations (M.B.C.) were determined using appropriate methods. Using the same concentration of the two test plants extracts, the zones of inhibition showed by *F. sycomorus* ranged between 11.5 - 21.5 mm while that of *F. platyphylla* was from 17.0 - 22.0 mm. The values of the M.I.C and M.B.C of *F. sycomorus* were 1.95, 31.3 and 3.91, 250 mg/ml, respectively. Similarly, *F. platyphylla* displayed 1.95 and 7.81 mg/ml M.I.C. values and 3.91 to 62.5 mg/ml M.B.C. values against the test organisms. The observed antibacterial activities in this study proved that the leaves and stem bark extracts of *Ficus* spp. obtained in Zaria support the forcloric claims of the use of Ficus plants in the treatment of ailment such as wound dressing.

Key words: Antibacterial activity, medicinal plants, bacterial resistance, Ficus spp.

INTRODUCTION

The *Ficus* species belong to the mulberry family, Moraceae which consists of about forty (40) genera and over one thousand four hundred (1,400) species of trees, shrubs, vines and herbs, often with milky latex juices (Zerega et al., 2005). They are usually found near streams in the savannah area. *Ficus sycomorus* have been suspected to possess anti-diarrhoeal activities (Ahmadu et al., 2007). The sedative and anticonvulsant properties of this plant have also been reported (Sandabe et al., 2003). The extracts of *Ficus platyphylla* have been reported to inhibit gastro-intestinal motility (Amos et al., 2001). It has been reported to possess analgesic (Wakeel et al., 2004), anti-inflammatory and anti-conceptive (Amos et al., 2002) activities. (Kubmarawa et al., 2007) reported the use of the stem bark in treating tuberculosis. The extracts of these plants are used in Hausa ethno medicine of Northern Nigeria for the treatment of various ailments such as mental illness, dysentery, cough, diarrhoea, chest condition, tuberculosis, convulsive disorder and pain relief (Sandabe and Kwari, 2000; Wakeel et al., 2004). About 70% of the human population is dependent (wholly or partially) on plant-based medicines (Pravin, 2006). 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 ((Lewis and Ausubel, 2006; Adeshokan et al., 2007; Oyeleke et al., 2008; Udobi et al., 2008). Some of these compounds have been reported to be present in the *Ficus species* such as tannins, saponins, flavonoids, steroids, anthraquinone glycosides and reducing sugars (Hassan, 2005; Sandabe et al., 2006). Medicinal plants also

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represent a rich source from which antimicrobial agents can be obtained (Kubmarawa et al., 2007).

Infectious diseases continue to represent a critical problem to human health and a leading cause of morbidity and mortality worldwide despite the extensive use of antibiotics and vaccination programs (Bloom, 2000; Eswarappa, 2009). Antibacterial resistance has been a problem for nearly as long as antibacterial agents have been in used. *Staphylococcus aureus* began developing penicillin resistant strains not long after the introduction of penicillin. Recently, resistant strains of *S. aureus* as well as other enterobacteriaceae such as *Salmonella species* that colonize the intestines are common phenomenon that poses a global health problem in hospitals (NIAID, 2006). Therefore, this study attempts to investigate the phytochemical constituents and the antibacterial activities of ethanolic extracts of *F. sycomorus* and *F. platyphylla* leaves and stem barks against sensitive and resistant species of *Salmonella typhi* and *S. aureus* in order to ascertain forcloric claim of its antibacterial therapeutically uses.

MATERIALS AND METHODS

Collection and preparation of plants materials

The leaves and stem bark of the two test plants were collected in Samaru, Zaria, Nigeria in July 2006. The plants were authenticated at the herbarium unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria, where voucher specimens 1446 for *F. sycomorus* and 730 for *F. platyphylla* were deposited. The air-dried Powdered leaves and stem barks were extracted with 70% aqueous ethanol at room temperature. The extracts were concentrated, dried and weighed (Brain and Turner, 1975; Sofowora, 1993).

Quantitative estimation of flavonoid content of the extracts

The test extracts were prepared to 50 mg/ml concentration. One mililitre (1.0 ml) of 0.1 M ferric chloride in 0.1 N hydrochloric acid was mixed with 1.0 ml of 8 µM potassium Ferro Cyanide ($K_3Fe(CN)_6$). Thereafter, 1.0 ml of the prepared crude extract was added to the reaction mixture and was gently mixed to give a homogenous solution. The optical density was read spectrophotometrically at 720 nm (Omidiji and Ehinmidu, 1990).

Antibacterial susceptibility test

The antibacterial activity was determined by the cup diffusion method according to NCCLS (2002). The test bacteria were clinical isolates obtained from the Department of Pharmaceutics and Pharmaceutical Microbiology, Ahmadu Bello University, Zaria, Nigeria. Twenty micro litres (20 µl) of washed and standardized overnight culture containing approximately 10^6 cfu/ml was inoculated into sterile Mueller-Hinton agar at 45°C. The bacterial-agar mixture was allowed to set and dry at room temperature. Wells of 6 mm diameter were bored in the agar aseptically in each plate. Each concentration of the test extract was examined for antimicrobial activity by dispensing 100 µl of test extract into the well in duplicates. The plates were allowed to stand at room temperature for 1 h for the extracts to diffuse into the agar and then, they were incubated at 37°C for 18 h. Positive and negative controls were also set up. Subsequently, the plates were examined for bacterial growth and the diameter of zones of inhibition measured appropriately.

Minimum inhibitory concentrations (M.I.C.) and minimum bactericidal concentrations (M.B.C.) determination

The M.I.C. was determined by agar dilution method. Ten milliliter (10 ml) volume of double strength melted Mueller-Hinton agar at 45°C was diluted with equal volume of the test extract in graded concentrations. These were poured aseptically into sterile Petri-dishes and dried at 37°C for 1 h with the lid slightly raised. Twenty microlitre (20 µl) of standardized test bacteria (10^6 cfu/ml) were aseptically inoculated on the sterilized paper discs placed on the agar surface at equidistance in triplicates for each concentration of the test plant extract (Ehinmidu, 1993). These were incubated at 37°C for 18 h. The M.I.C. value was taken as the least concentration of the extract showing no detectable growth. Gentamicin was used as standard antibiotic. The M.B.C was determined by transferring inoculated discs into a sterile 10 ml recovery nutrient broth (Nutrient broth containing 3% v/v Tween 80 plus 5% w/v yeast extract) from the concentrations that showed no visible growth from the growth of the M.I.C. determination. These were incubated at 37°C for 72 h. The least concentration of the extract that showed no bacterial growth in the recovery liquid medium was taken as the M.B.C.

Statistical analysis

Results were expressed as mean ± standard deviation. The data was analyzed using Student's t-test. $P < 0.05$ was considered significant and $P > 0.05$ not significant.

RESULTS

The result of the spectrophotometric estimation of the flavonoid content of the crude extracts of *F. sycomorus* and *F. platyphylla* showed that *F. platyphylla* contained the highest amount of flavonoid than the other test extracts (Table 1). From the result of the susceptibility testing, all the extracts exhibited antibacterial activity against all the test organisms investigated (Table 2). The M.I.C. and M.B.C. values obtained for the extracts against the test organisms varied from one plant extract to the other and also dose-dependent (Tables 3 and 4).

DISCUSSION

The phytochemical analysis of *F. sycomorus* and *F. platyphylla* revealed the presence of secondary metabolites such as tannins, anthraquinones, flavonoid, saponins, steroids, alkaloids (Adeshina et al., 2009), which have been previously reported for their antimicrobial activities (Amos et al., 2001; Hassan et al., 2006; Ahmadu et al., 2007; Kubmarawa et al., 2007). Many plants have been reported for therapeutic purposes because of the chemical compounds synthesized in these plants. Geographical location has been reported to

Table 1. Flavonoid quantitative assay of *F. sycomorus* and *F. platyphylla* ethanol extracts.

Plants	Flavonoid content in 50 mg extracts (µg/ml)	
	Leaves	Stem bark
F. sycomorus	48.78	45.12
F. platyphylla	78.05	58.54

Table 2. Susceptibility of the test bacterial isolates to the plant extracts (100 mg/ml).

Test organisms	Zones of inhibition (mm)			
	F. sycomorus		*F. platyphylla*	
	LE	SBE	LE	SBE
Sensitive *S. aureus*	17.5 ± 0.5	17 ± 0.0	21.3 ± 0.4	21.5± 0.5
Resistant *S. aureus*	13.0 ± 0.0	11.5 ± 0.5	17.5 ± 0.5	17.0 ± 0.0
Sensitive *S. typhi*	21.5 ± 0.5	21.5 ± 0.5	21.5 ± 0.5	21.0 ± 1.0
Resistant *S. typhi*	NIL	16.0 ± 0.0	22.0 ± 0.0	17.0 ± 1.0

The results are expressed as mean ± standard deviation, LE = Leaf extract, SBE = Stem bark extract, NIL = No inhibition.

Table 3. Minimum inhibitory concentration (M.I.C.) of the test plants leaf and stem bark extracts and standard antibiotics against the test organisms.

Test organism	M.I.C (mg/ml)				
	F. sycomorus		*F. platyphylla*		Gentamicin (µg/ml)
	LE	SBE	LE	SBE	
Sensitive *S. aureus*	7.81	15.6	1.95	1.95	15.63
Resistant *S. aureus*	15.6	31.3	7.81	31.3	500.00
Sensitive *S. typhi*	1.95	3.91	1.95	1.95	1.00
Resistant *S. typhi*	15.6	15.6	1.95	1.95	256.00

LE = leaf extract, SBE = stem bark extract, gentamicin peak plasma concentration is 4 µg/ml.

Table 4. Minimum bactericidal concentration (MBC) (mg/ml) of the test plants leaf and stem bark extracts against the test organisms.

Test organism	*F. sycomorus*		*F. platyphylla*	
	LE	SBE	LE	SBE
Sensitive *S. aureus*	15.6	31.3	3.91	31.3
Resistant *S. aureus*	62.5	250.0	15.6	62.5
Sensitive *S. typhi*	3.91	15.6	15.6	31.3
Resistant *S. typhi*	31.3	62.5	15.6	31.3

LE = Leaf extract, SBE = Stem bark extract.

influence the chemical constituents of plant extracts of the same genus found in different environment, hence the phytochemical analysis of *F. platyphylla* obtained in Zaria, Nigeria exhibited the presence of tannin known for its antimicrobial activity which is contrary to the report of Kubmarawa et al. (2007) on the same *F. platyphylla* extracts collected in Adamawa State, Nigeria.

Thus, the difference observed in the antimicrobial activities of *F. sycomorus* and *F. platyphylla* stem bark extracts against *S. aureus* reported in this study when compared to the reports of Kubmarawa et al. (2007) on the same plants against the same organism might be

attributed to difference in geographical location. In the present study, the M.I.C. values showed that all the crude ethanolic extracts of the two medicinal plants commonly used by the traditional medical practitioners in Northern Nigeria were active against Ciprofloxacin-resistant *S. typhi* and *S. aureus* with M.I.C. values ranging from 1.95 to 31.3 mg/ml. The M.I.C. (1.95 - 7.81 mg/ml) and MBC (3.91 - 15.60 mg/ml) of *F. platyphlla* extracts against ciprofloxacin-resistant test organisms in this study were found to be lower than that of the *F. sycomorus* extracts examined.

The leaf extract of *F. sycomorus* was observed to be more potent against ciprofloxacin-sensitive and resistant test organisms than the stem bark extract of the same plant. Similarly, the leaf extract of *F. platyphylla* displayed a better antibacterial activity against the test bacteria. The extract with the greatest antibacterial activity was that of *F. platyphylla* leaf (inhibition zone 22.0 mm, M.I.C. 1.95 mg/ml and M.B.C. 15.6 mg/ml for ciprofloxacin-resistant *S. typhi*). The ciprofloxacin-resistant *S. typhi* was found to be more susceptible than ciprofloxacin-resistant *S. aureus* irrespective of the cell wall of gram-negative test organism which indicates that these extracts have a mechanism of overcoming the barriers of the gram-negative cell wall. The higher flavonoid contents in the leaf than the stem bark extracts probably account for high antibacterial activity of the *Ficus spp* tested. The flavonoid content of the leaf extract of *F. platyphylla* was higher than the *F. sycomorus* investigated, hence the better antibacterial activity of the leaf extracts of *F. platyphylla* than *F. sycomorus* leaf extract. The presence of flavonoid in all the plant extracts tested, could probably be responsible for the observed antibacterial activity. Flavonoids have been reported to display strong antimicrobial activity (Cushnie, 2005; Özçelik et al., 2008). Similarly, they have been reported to inhibit *Streptococcus mutans* and other bacteria (Koo et al., 2002). Thus, these test plants present a potential novel and cheap source of potent antimicrobial agents against ciprofloxacin resistant *S. typhi* which could justify them been claimed for ethno medicinal uses.

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