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Full Length Research Paper

Preliminary phytochemical and antimicrobial investigations of leaf extracts of *Ochna schweinfurthiana* (*Ochnaceae*)

M. I. Abdullahi[1], I. Iliya[2], A. K. Haruna[1], M. I. Sule[1], A. M. Musa[1]* and M. S. Abdullahi[3]

[1]Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.
[2]National Institute for Pharmaceutical Raw Materials Research and Development, Abuja, Nigeria.
[3]National Research Institute for Chemical Technology, Zaria, Kaduna State, Nigeria.

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The antimicrobial activity of the acetone and methanol extracts of the leaves of *Ochna schweinfurthiana* F Hoffm *(Ochnaceae*) obtained through maceration was evaluated using disc diffusion and Nutrient broth dilution techniques. The microorganisms tested were: *Staphylococcus aureus* ATCC 021001, *Bacillus subtilis* NCTC 8236, *Escherichia coli* NCTC 10418, *Salmonella typhi* ATCC19430, *Klebsiella pneumoniae* ATCC 10031, *Pseudomonas aeruginosa* NCTC 6750, *Candida albicans* ATCC 10231; local hospital isolates: *Streptococcus pyogenes*, *Corynebacterium ulcerans*, *Neisseria gonorrhoea* and methicillin-resistant *S. aureus.* Susceptibility test results showed that both extracts (600 g/disc) inhibited growth with a mean zone of inhibition range of 15 - 21 mm against *Staphylococcus aureus*, *S. typhi, K. pneumoniae and P. aeruginosa*; no activity was however observed against methicillin resistant *S. aureus, N. gonorrhea, C. ulcerans, B. subtilis, E. coli* and the only fungus, *C. albicans*. Sparfloxacin (100 g/disc) a standard antibiotic inhibited the growth of all the organisms tested with the exception of *C. albicans*. Preliminary phytochemical screening revealed the presence of flavonoids, saponins and steroids/terpenes. The results suggest that the plant contains bioactive constituent(s) with modest antimicrobial activity and validates the ethno-medical use in wound dressing and other forms of bacterial infections.

Key words: Antimicrobial, *Ochna schweinfurthiana* leaves extract, preliminary phytochemical.

INTRODUCTION

Antimicrobial agents are among the most commonly used and misused of all drugs. The inevitable consequences of the wide spread use of anti-microbial agents has been the emergence of antibiotic resistant pathogens, fuelling an ever increasing need for new drugs (Chambers, 2006). Herbal remedies used in traditional folk medicine provide an interesting and still largely unexplored source for the creation and development of potentially new drugs for chemotherapy which might help to overcome the growing problem of drug resistance and also the toxicity of currently available commercial antibiotics (Al- wadh-Ali et al., 2001).

*Corresponding author: E -mail address: alimsa69@yahoo.com.

Ochna schweinfurthiana F. Hoffm. (*Ochnaceae)* is a shrub or small tree up to 4 m tall mostly occurring in African woodlands; from Guinea to North and Southern Nigeria, across central Africa to Sudan, Uganda, Zimbabwe and Mozambique. The plant grows into a decorative shrub bearing bright-yellow flowers (Burkill, 1997). In ethnomedicine, the powdered bark is used as antimalarial and antihelmintic while the decoction of the root, leaves or bark is used in dressing wounds. In Northern Nigeria, the plant is said to be used for the treatment of measles, typhoid fever and fungal skin infections.

Literature review on this plant revealed that no previous phytochemical and pharmacological investigations have been reported. This study was, therefore, carried out to evaluate the antimicrobial effect of the methanol and

acetone extracts of the leaves of the plant on some selected pathogens so as to establish a scientific basis for its use in treatment of conditions related to bacterial infections. There are few reports on chemical investigations of some species of Ochnaceae and many compounds were isolated, most of which are phenolic derivatives such as biflavonoids and chalcones, (Murakami et al., 1992; Ngo mbing et al., 2006); these compounds have been reported to have anti-tumor (Murakami et al., 1992), anti- inflammatory (Viana et al., 2003) anti-malarial (Li et al., 1995) activities. Relatively few biological studies were reportedly conducted on plants in the *Ochna* genus and the results showed that *Ochna macrocalynx* has cytotoxic activity (Tang et al., 2003) while *Ochna afzelii* was reported to have anti beta lactamase activity (Gangoué-Piéboji et al., 2007).

MATERIALS AND METHODS

Collection, identification and preparation of plant material

The whole plant material of *O. schweinfurthiana* was collected from Samaru-Zaria, Northern-Nigeria in June 2007. It was authenticated by Mallam Musa Muhammad of the herbarium section of Biological Sciences Department, Ahmadu Bello University, Zaria, where a voucher specimen (number 900229) was deposited for future reference. The leaves were removed, air-dried, powdered, labelled and stored in air-tight container.

Extraction

Powdered leaves (200 g) were continuously extracted with acetone by maceration for 3 days (600 ml - three times); the extract was filtered and the filtrate dried in vacuum to afford a greenish product (12 gm) coded OSLA. The residue (marc) was then similarly macerated with methanol, filtered and concentrated under vacuum to yield a gummy dark- greenish product (13 gm) coded OSLM. The 2 extracts were kept in refrigerator prior to use.

Phytochemical screening

The extracts were subjected to phytochemical screening for the presence of alkaloids, flavonoids, saponins, tannins and steroids/ triterpenes, according to standard procedures (Trease and Evans, 1983).

Bioassay studies

Test organisms

The organisms used were obtained from the Medical Microbiology Department, Ahmadu Bello University Teaching Hospital, Zaria-Nigeria. All bacterial cultures were checked for purity and maintained in a blood agar slant while the fungus was maintained on a slant of Sabraud dextrose agar (SDA). The organisms tested include *Staphylococcus aureus* ATCC 021001, *Bacillus subtilis* NCTC 8236, *Escherichia coli* NCTC 10418, *Salmonella typhi* ATCC 19430, *Klebsiella pneumoniae* ATCC 10031, *Pseudomonas aeruginosa NCTC* 6750, *Candida albicans* ATCC 10231 and hospital isolates: *Streptococcus pyogenes*, *Corynebacterium ulcerans*, *Neisseria gonorrhoae* and methicillin-resistant *S. aureus.*

Susceptibility testing

The disc diffusion method (Sardari et al., 1998) was used for the test. Filter paper disc (7 mm in diameter) impregnated with sample solutions were placed on blood agar plates which have been inoculated with test organisms according to standard protocol described by National Committee of Clinical laboratory standard (1993).The extracts dissolved in their respective extraction solvents were tested at a concentration of 600 g/disc. The plates were incubated at 37°C for 24 h, after which the diameters of the inhibition zones were measured and recorded in millimetres using a transparent ruler. Filter paper disc containing extraction solvents without any test extract served as control and no inhibition was observed. The reference antibacterial drug Sparfloxacin (100 g/disc) was used as a positive control.

Determination of minimum inhibitory concentration (MIC)

Determination of the MIC was carried out on the extracts that showed inhibitory effect on the test micro-organism. Broth dilution method was used (Ibekwe et al., 2001). Nutrient broth was prepared according to the manufacturers instructions. 2mls of the medium was dispensed in screw-capped test tubes and sterilized at 121°C for 15 min.

Mc-Farland's turbidity standard scale (0.5) was prepared by adding 9.95 ml of 1% H_2SO_4 and 0.05 mls of 1% $BaCl_2$ to give a turbid solution. Ten mls sterile normal solution was used to make a turbid suspension of the micro-organism. Dilution of the organism suspension was done continuously using normal saline until the turbidity marched that of Mc-Farland's scale by visual comparison.

At that point, the concentration of the micro-organisms was about 1.5×10^8 cfu/ml. Two-fold dilution of the extracts with nutrient broth was done to give concentrations of 40, 20, 10, 5 and 2.5 mg/ml. 0.2 mls of the micro-organism suspension was inoculated into the different concentration of the extract in a test tubes. The tubes were incubated at 37°C for 24 h and at 25°C for 48 h for bacteria and fungi respectively after which the plates were observed for growth. The MIC was defined as the lowest concentration of the extract inhibiting the visible growth of each micro organism.

Determination of minimum bactericidal concentration (MBC)

Blood agar plates were prepared according to the manufacturers instructions. The contents of the MIC tubes and the following tubes in the serial dilution were sub-cultured into appropriately labeled blood agar plates by dipping a sterile wire loop into each test tube and streaking the surface of the labeled blood agar plates. The plates were then incubated at 37°C for 24 h after which they were observed for growth. The MBC was the plate with the lowest concentration of the extract in serial dilution without growth.

RESULTS

Preliminary phytochemical

The results of preliminary phytochemical tests of the acetone and methanol leaves extracts of *O. schweinfurthiana* are as shown on Table 1. The results indicated the presence of flavonoids, saponins and steroids/terpenes.

Table 1. Phytochemical tests on the acetone and methanol extracts of the leaves of *Ochna schweinfurthiana*.

Tests	Acetone extract	Methanol extract
Steroid/terpenes	+	-
Flavonoid	+	+
Saponins	+	+

Key: + = present, - = Present.

Table 2. Susceptibility test of leaves extracts of *Ochna schweinfurthiana* against the test organisms (mm).

Test	Zone of inhibition(mm)		
	OSLA (600 g/disc)	OSLM (600 g/disc)	Sparfloxacin (100 g/disc)
S. aureus	20	19	22
S. pyogenes	-	17	24
C. ulcerans	-	-	19
B. subtilis	-	-	27
E. coli	-	-	14
S. typhi	19	15	27
K. pneumoniae	21	20	14
P. aeruginosa	17	17	17
N. gonorrhea	-	-	20
C. albicans	-	-	-
MRSA	-	-	19

Key: mean zone of inhibition measured in millimeter (mm), - = activity not detected , MRSA ; Methicillin resistant *S. aureus.*

Table 3. Minimum inhibitory concentration of leaves extracts of *Ochna schweinfurthiana* against the test organisms (mg/ml) MIC.

Test organism	Minimum inhibitory concentration mg/ml	
	Acetone	Methanol
S. aureus	10	20
S. pyogenes	X	20
S. typhi	20	20
K. pneumoniae	10	10
P. aeruginosa	20	20

x = MIC greater than 40 mg/ml.

Antimicrobial activity

The results of susceptibility test were as shown in Table 2 while that of MIC and the MBC have been summarized in Tables 3 and 4, respectively. The two extracts (OSLA and OSLM) were active against *S. aureus*, *S. typhi*, *K. pneumoniae* and *P. aeruginosa* but showed no activity against Methicillin-resistant *S. aureus*, *N. gonorrhea, C. albicans*, *C. ulcerans*, *B. subtilis* and *E. coli*. Sparfloxacin a standard antibiotic was active on all the organisms tested with exception of *Candida albicans.* The results of MIC and MBC showed ranges of 10 - 20 mg/ml and 10 - 40 mg/ml respectively.

DISCUSSIONS

Preliminary phytochemical screening of the extracts revealed the presence of flavonoids, steroids/terpenes and saponins. Each of this group of compounds has been reported to possess antimicrobial activity (Ragasa et al., 2005; Cuhnie and Lamb, 2006; Soetan et al., 2006) and reportedly exert their effects by affecting the cell membrane integrity of the bacteria (Hendrich, 2006; Trombetta et al., 2005; Killeen et al., 1998).

Both acetone and methanol extracts showed broad spectra of activity against the tested organisms. Except for *S. pyogenes*, the acetone extract showed more activity

Table 4. Minimum Bactericidal Concentration of leaves extracts of *Ochna schweinfurthiana* against the Test organisms (mg/ml) MBC.

Test organism	Minimum bactericidal concentration mg/ml	
	Acetone	Methanol
S. aureus	20	40
S. pyogenes	X	40
S. typhi	40	40
K. pneumoniae	20	20
P. aeruginosa	40	40

x = greater than 40 mg/ml.

activity against the tested organisms as indicated by the susceptibility, MIC and MBC tests suggesting that more of the bio-active chemical constituents were extracted by acetone. These could probably be non-polar or moderately-polar compounds such as flavonoids. The leaves extract of the plant generally had moderate activity at the concentrations tested with mean zone of inhibition diameter > 18 mm (Tania Maria et al., 2000). The selective effects of the extracts on gram-positive (*S. aureus and S. pyogenes*) and gram-negative- *K. Pneumoniae, S. typhi* and *P. aeruginosa* suggest that the extracts may serve as source of compound(s) that can be used to combat infection caused by these organisms. It is of interest to note that, the growth of Methicillin-resistant *S. aureus*, *N. gonorrhea* , *C. ulcerans* , *B. subtilis* and *E. coli* were not inhibited at concentration tested, this implies that these organisms are resistant to these extracts. The traditional use of the leaves of *O. schweinfurthiana* in dressing wounds is apt as *S. aureus, K Pneumoniae, P. aeruginosa* and *S. pyogenes* are all implicated in bacterial wound infections (Mark, 2004).

Conclusion

The result of this work has rationalized the ethno-medicinal use of the plant *O. schweinfurthiana* as an antimicrobial agent. More work is however required to isolate and characterize the active ingredients and possibly their mechanism of biological action.

REFERENCE

Al- wadh AN, Julich WD, Kusmik C, Lendequist U (2001). Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities. J. Ethnopharmacol., 74: 173-179.

Burkill HM (1997). The useful plants of West Tropical Africa, Royal Botanical Gardens, Kew. 4: 275.

Chambers FH (2006). General Principle of antimicrobial therapy in: *The Pharmacolgical basis of Therapeutics* Bruton, L. L., Lazo, J. S. and Parker, LL. (Eds.) McGraw-Hill publishers, New York. U.S.A. 1095p.

Cuhnie TP, Lamb AJ (2006). Antimicrobial activity of flavonoids. Int. J. Antimicrob. Agents. 26: 342-356.

Gangoué-Piéboji J, Stéphane B, Jean-Marie F, Ngassam P, Ngameni B, Azebaz A, Pegnyemb DE, Watchueng J, Goffin C, Galleni M (2007): Screening of some medicinal plants from Cameroon for - Lactamase inhibition activity. Phytother. Res. 21 (3): 284-287.

Hendrich AB (2006). Flavonoid-membrane interactions: possible consequences for biological effects of some polyphenolic compounds. Act. Pharmacol. Sin. 27(1): 27-40.

Ibekwe VI, Nnayere NF, Akujobi CO (2001). Studies on Antimicrobial Activity and Phytochemical Qualities of Extracts of Orange peels. Int. J. Environ. Health. Hum. Dev. 2(1): 41-46.

Killeen G, Madigan C, Connolly C, Walsh G, Clark C, Hynes M, Timmins B, James P, Headon D, Power R (1998). Antimicrobial saponins of *Yucca schidigera* and the implications of their in vitro properties for their *in- vivo* impact. J. Agric. Food Chem. 46(8): 3178–3186.

Li R, Konyon GL, Cohen FE, Chen X, Gong B, Dominguez JN, Davidson E, Kuzban G, Miller RE, Nuzam EO (1995). In Vitro Antimalarial Activity of Chalcones and Their Derivatives, J. Med. Chem. 38: 5031-5037.

Mark C (2004). Recognition and Management of wound infection, http://www.worldwidewounds.com/2004/january/Collier/Management-of-Wound-infections.html.

Murakami A, Tanaka S, Ohigashi H, Hirota M, Irie R, Takeda N, Tatematsu A, Koshimizu K (1992). Chalcone tetramers, lophirachalcone and alatachalcone, from *Lophira alata* as possible anti-tumor promoters. Biosc. Biotech. Biochem. 56(5): 769-72.

NCCLS (1993). Performance Standards Antimicrobial Disc Susceptibility Tests. Approved Standard Fifth Edition. NCCLS Document M2-A5, Villanova, PA, USA.

Ngo MJ, Ngweherd GC, Delheodore AA, Allouhghi H, Gangoue-pieboji J, Mbafor JT, Ghogonu TR (2006). Two biflavonoids Ouarantine A and B from *Ouaratea nigroviolacea.* Phytochen., 67(2424): 2666-2770

Ragasa CY, De Luna RD, Hofilena JG (2005). Antimicrobial terpenoids from *Pterocarpus indicus*. Nat. Prod. Res., 19(4): 305-309.

Sardari SA, Gholameraza RG, Daneshtalab M (1998). Phytopharmaceuticals. Part 1: antifungal Activity of Selected Iranian and Canadian plants. Pharm. Biol. 36: 180-188.

Soetan k, Oyekunle MA, Aiyelaagbe OO, Fafunso MA (2006). Evaluation of the antimicrobial activity of saponins extract of *Sorghum Bicolor* L. Moench. Afr. J. Biotech. 5(23): 2405-2407.

Tang S, Bremner P, Kortenkamp A, Schlage C, Gray AI, Gibbons S, Heinrich M (2003). Biflavonoids with cytotoxic and antibacterial activity from *Ochna macrocalyx*. Plant. Med., 69(3): 247-253.

Tania MAA, Axréia FS, Mitzi B, Telma SMG, Elza AS, Artur SJ, Carlos LZ (2000). Biological Screening of Brazilian Medicinal Plants. Mem Inst Oswaldo Cruz, Rio de Janeiro. 95(3): 367-373.

Trease G E, Evans W C (1983). Textbook of Pharmacognosy, 12th (Ed) Balliers Tindall, London. pp. 343-384.

Trombetta D, Astelli F, Grazia-Sarpietro M, Venuti V, Cristanl M, Daniele C, Saija A, Mazzantl G, Bisignano G (2005). Mechanisms of antibacterial action of three monoterpenes. Antimicrob. agents Chemother. 49: 2474-2478.

Viana GSB, Bandeira MAM, Matos FJA (2003). Analgesic and anti-inflammatory effects of chalcones isolated from *Myracrodruon urudeuva* Allemao. Phytomed. 10 (2-3): 189-197.