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Research Article

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Preliminary phytochemical and antimicrobial evaluations of the methanolic leaf extract of *Ochna kibbiensis* Hutz and Dalz (Ochnaceae)

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ABSTRACT

The antimicrobial effect of the crude methanol leaf extract of Ochna kibbiensis obtained through maceration technique was evaluated against pathogenic microorganisms which include Staphylococcus aureus, Methicillin Resistant Staphylococcus aureus (MRSA), Vancomycin Resistant Enterococci, Listeria monocytogenes, Helicobacter pylori, Campylobacter fetus, Proteus vulgaris, Pseudomonas flourescens, Candida tropicalis and Candida stellatoidea. Agar Disc diffusion and Nutrient Broth dilution techniques were employed. Susceptibility test results showed that the extract (400µg) inhibited the growth of all the test organisms (bacteria and fungi) with a mean zone of inhibition range of 18-27mm, with the exception of Methicillin Resistant Staphylococcus aureus, Helicobacter pylori and Pseudomonas flourescens. The standard antibacterial drug, Sparfloxacin (5ug/ml) had inhibitory activity against all the organisms except H. pylori, P. flourescens, C. tropicalis and C. stellatoidea while Fluconazole, the standard anti-fungal drug showed activity only on the two fungi species tested C. tropicalis and C. stellatoidea. The Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal/Fungicidal Concentration (MBC) range for the extract was 5-20mg/ml and 10-40mg/ml, respectively. Preliminary phytochemical screening revealed the presence of flavonoids, steroids/terpenes, saponins, glycosides and alkaloids in the extract. The results of this study suggest that the leaves extract of the plant O. kibbiensis contains bioactive constituent(s) with good antibacterial and antifungal activity and, lends credence to its folkloric use in wound healing and other microbial infections.

Keywords: *Ochna kibbiensis*, extract, Antimicrobial evaluation, Phytochemical screening

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INTRODUCTION

Antimicrobial resistance (AMR) threatens the effective prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses and fungi. It is a critical health issue that has evolved to become a worldwide public health threat [1]. Infections which are increasingly resistant to antibiotics together account for a heavy disease burden often affecting developing countries, disproportionately [2]. Antibiotic-resistant bacteria are increasingly seen to be just as virulent as their sensitive counterparts, and their genetic adaptability gives bacteria a huge advantage over mankind [3].

About 2 million people become infected with bacteria that are resistant to antibiotics each year in the United States out of which 23,000 die as a direct result of these infections, while many more people die from other complications related to antibiotic resistance [4]. Most European countries similarly witness a seemingly unimpeded increase of

antimicrobial resistance in the major Gram-negative pathogens which could unavoidably lead to loss of therapeutic treatment options [5]. In Africa, although there is a scarcity of accurate and reliable data on AMR in general, recent external quality assessment of public health laboratories revealed weakness in antimicrobial susceptibility testing in many countries [1].

The emergence of drug-resistant microbes is not new or unexpected. Both natural causes and societal pressures drive bacteria, viruses, parasites, and other microbes to continually change in an effort to evade the drugs developed to kill them. Resistant strains evolve when microorganisms replicate themselves erroneously or when resistant traits are exchanged between them [6]. Theoretically, bacteria will continue to develop resistance once exposed to any antimicrobial agent, thereby imposing the need for a permanent search and development of new drugs [7]. The use and misuse of antimicrobial drugs accelerates the emergence of drug-resistant strains. Poor infection control practices, inadequate sanitary conditions and inappropriate food-handling encourage further the spread of AMR [8]. The emergence of multidrug resistance in human and animal pathogenic bacteria as well as undesirable side-effects of certain antibiotics has triggered immense interest in the search for new antimicrobial drugs of plant origin [9]. More than hundreds of plants worldwide are used in traditional medicine as treatments for bacterial infection [10].
A number of *Ochna* species have a long history of use as herbal remedies in Asia and Africa for the treatment of ailments including malaria, microbial infections, epilepsy, snake bite, asthma and gastric disorders [11]. Pharmacological studies conducted on some of these species have validated the antimicrobial [12-14]; anti-malarial [15]; analgesic and anti-inflammatory properties [16]. Some *Ochna* species have also shown promising anti-HIV-I [17] and anti-proliferative activity [12]. The family, *Ochnaceae*, has been reported to be a rich source of complex dimmers of biflavonoids and chalcones [15, 18-21].

Ochna kibbiensis Hutz and Dalz, is a shrub or small tree belonging to the family Ochnaceae found in Tropical Africa from Guinea to Southern and Northern Nigeria. It is found in Zaria, Northern Nigeria, where it could be distinguished from the closely related *Ochna* species such as *O. schweinfurthiana* and *O. rhamatosa* by its characteristic elliptical, lanceolate leaves which acuminate at the apex and the paired and axillary flowers with brilliant and large red calyx in the fruits. The plant is used traditionally to treat wound infections and for pains [22]. Literature search on the plant revealed that no previous phytochemical or pharmacological study has been reported. In continuation of our work on *Ochna* species, we hereby report on the preliminary phytochemical constituents and antimicrobial evaluation of the crude methanol leaves extract of the plant, *Ochna kibbiensis*.

EXPERIMENTAL SECTION

Collection and Identification of Plant material

The whole plant material of *Ochna kibbiensis* was collected from Samaru-Zaria, Northern-Nigeria in July 2013. It was authenticated by Mallam U. S. Gallah of the herbarium section of Biological Sciences Department, Ahmadu Bello University, Zaria, where a voucher specimen (number 573) was deposited for future reference.

Preparation of the extract

The leaves were removed, air-dried, pulverized, labelled and stored in air-tight container. Powdered leaves (430 g) were continuously extracted with methanol by maceration for 3 days (1000ml - three times); the extract was filtered and the filtrate dried in-vacuo using rotary evaporator at 40^{o}C to afford a greenish product (30g) coded OK. The extract was kept in refrigerator prior to use.

Preliminary Phytochemical screening

Portion (1g) of the extract was subjected to phytochemical screening for the presence of secondary metabolites including, flavonoids, saponins, tannins and steroids/triterpenes and alkaloids, according to standard procedures [23-24].

Test Organisms

The microbes, Clinical isolates, were obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital Zaria, Nigeria. All bacterial cultures were checked for purity and maintained in a blood agar slant while the fungi were maintained on a slant of Sabraud dextrose agar (SDA). The microbes tested include Methicillin Resistant *Staphylococcus aureus* (MRSA), Vancomycin Resistant *Enterococci*, *Listeria monocytogenes, Helicobacter pylori, Campylobacter fetus, Staphylococcus aureus, Proteus vulgaris, Pseudomonas flourescens, Candida tropicalis* and *Candida stellatoidea.*

Antimicrobial evaluation

Susceptibility test

Preliminary antimicrobial activity of the methanol leaf extract of *Ochna kibbiensis* was first determined through susceptibility test using agar diffusion method. The stock concentration of the extract (40 mg/ml) was prepared with dimethyl sulfoxide (DMSO). Mueller Hinton agar, the growth medium, was prepared according to Manufacturer's instructions and sterilised for 15 minutes at 121^{O}C; it was poured into sterile petri dishes and allowed to cool and solidify. The inocula were prepared by inoculating the test organisms in nutrient broth and incubating them for 24 hours at 37°C for the bacteria, while for fungi, Sabouraud Dextrose broth was used and incubated for 48 hours at 25°C. The sterile medium was seeded with 0.1 ml standard inoculum of the test microbe at 45°C, swirled gently and allowed to cool and solidify. Wells were bored into the solidified inoculated nutrient agar plates using cork borer of 6 mm diameter. The wells were filled with 0.1 ml (400 µg) DMSO solution of the extract. Sparfloxacin (5 µg) and Fluconazole (5 µg) discs were also placed on the agar plates and served as the standard drugs for bacteria and fungi, respectively. 1 hour was allowed for the extract and the standard compounds to diffuse into the agar after which the plates were incubated overnight at 37 and 25°C for bacteria and fungi, respectively. At the end of incubation period, diameter of inhibition zone was measured using transparent ruler and recorded. The zones of inhibition of microbial growth were tested in duplicates and the mean of the results was recorded in millimetres (mm).

Minimum Inhibitory Concentration (MIC)

The MIC of the extract was carried out using Broth dilution method [25]. Mueller Hinton broth was prepared of which 10ml was dispensed into test tubes, sterilised at 121^{O}C for 15 minutes and allowed to cool; MC-Farlands standard turbidity scale number 0.5 was prepared. Dilution of the organism suspension was done continuously using sterile normal saline until the turbidity marched that of Mc-Farland's scale by visual comparison At that point, the concentration of the test microbe was about 1.5 x 10^{8}cfu/ml. Two-fold serial dilution of the extract in the sterile broth was made to obtain the concentrations of 40 mg/ml, 20 mg/ml, 10 mg/ml, 5 mg/ml and 2.5 mg/ml. 0.1ml of the standard inoculum of the test microbe was then inoculated into the different concentrations of the extract in the broth. The tubes were incubated at 37°C for 24 h and 25°C for 48 h for bacteria and fungi respectively after which the plates were observed for turbidity (growth). The MIC was defined as the lowest concentration of the extract inhibiting the visible growth of each micro-organism.

Minimum Bactericidal Concentration/Minimum Fungicidal Concentration (MBC/MFC)

The MBC/MFC was carried out to determine whether there is complete death of test microbes or just growth inhibition. Mueller Hinton agar broth was prepared, sterilized at 121^{o}C for 15mins, and transferred into sterile petri dishes to cool and solidify. The contents of the MIC in the serial dilution were sub-cultured into the prepared medium and incubated at 37^{o}C for 24 hrs; the plates were observed for colony growth; the MBC/MFC was the plate with lowest concentration of the extract in serial dilution without colony growth [25].

RESULTS AND DISCUSSION

The results of preliminary phytochemical tests and the antimicrobial activity conducted on the methanol extract of the leaves of *Ochna kibbiensis* are presented in Tables 1- 4.

Table 1: Preliminary Phytochemical Screening of *Ochna kibbiensis*

Constituents	Test	Inferences
Steroids & Triterpenes	Lieberman-Buchard Salkowski test	+ +
Alkaloids	Mayer's test Dragendorf's test	+ +
Flavonoids	Ferric chloride test NaOH test	+ +
Saponins	Frothing test	+
Tannins	Lead Sub-acetate	+
Glycosides	Fehling's test	+

Key: *+ presence of constituent*

Table 2: Susceptibility test of Methanolic Leaf Extract of *Ochna kibbiensis*

Zone of Inhibition (mm)			
Test Organisms	**OK (400 µg/ml)**	**Ciprofloxacin(5µg/ml)**	**Fluconazole(5µg/ml)**
Staphylococcus aureus	25	35	
Methicillin Resistant *S. aureus*	-	35	
Vancomycin Rest *Enterococci*	22	32	
Listeria monocytogenes	27	37	
Helicobacter pylori	-	-	-
Campylobacter fetus	20	38	-
Proteus vulgaris	18	32	
Pseudomonas fluorescence	-	-	-
Candida tropicalis	18	-	37
Candida stellatoidea	22	-	35

Key: mean zone of inhibition measured in millimetre (mm), - = activity not detected, OK= O. kibbiensis extract

Table 3: Minimum Inhibitory Concentration of Methanolic Leaf Extract of *O. kibbiensis* against the test organisms

Test Organisms	**Minimum Inhibitory Concentration (mg/ml)**				
	40	**20**	**10**	**5**	**2.5**
Staphylococcus aureus	-	-	-	OA	+
Vancomycin Rest *Enterococci*	-	-	OA	+	++
Listeria monocytogenes	-	-	-	OA	+
Campylobacter fetus	-	OA	+	++	+++
Proteus vulgaris	-	OA	+	++	+++
Candida tropicalis		OA	+	++	+++
Candida stellatoidea			OA	+	++

Key: - = no turbidity (no growth), OA= MIC, + = turbid (light growth), ++ = moderate turbidity, +++ = heavy growth

Table 4: Minimum Bactericidal/Fungicidal Concentration of Methanolic Leaf Extract of *O. kibbiensis* against the test organisms

Test Organisms	**Minimum Bactericidal/Fungicidal Concentration (mg/ml)**				
	40	**20**	**10**	**5**	**2.5**
Staphylococcus aureus	-	-	OA	+	++
Vancomycin Rest *Enterococci*	-	OA	+	++	+++
Listeria monocytogenes	-	-	OA	+	++
Campylobacter fetus	OA	+	+	++	+++
Proteus vulgaris	OA	+	+	++	+++
Candida tropicalis	OA	+	+	++	+++
Candida stellatoidea	-	OA	+	+	++

Key: - = no colony growth, OA= MBC/MFC, + = scanty colonies growth, ++ = moderate colonies growth, +++ = heavy colonies growth

Preliminary phytochemical investigation

The Preliminary phytochemical screening of *O. kibbiensis* revealed the presence of flavonoids, steroids/terpenes, saponins, glycosides and alkaloids. These secondary metabolites have been reported to possess antimicrobial activity [26].

Antimicrobial Activity

The results of the susceptibility tests, MIC and MBC/MFC have been summarized as shown on Tables 2, 3 and 4 respectively. The methanol leaves extract of *Ochna kibbiensis* exhibited activity against both gram positive and negative bacteria and the two fungi tested. It was however inactive against MRSA, *helicobacter pylori* and *pseudomonas flourescens.* Ciprofloxacin, the standard antibacterial drug used showed activity against all the bacterial microbes with the exception of *Helicobacter pylori,* and *Pseudomonas flourescens, w*hile fluconazole, the standard antifungal drug, exhibited activity on the two fungi, *Candida tropicalis and Candida stellatoidea.* The most sensitive organism was *Listeria monocytogenes* (27 mm) and the least was *Candida tropicalis* (18 mm). The concentration ranges for the MIC and MBC/MFC were 5-20mg/ml and 10-40mg/ml, respectively. The leaves extract of the plant can be said to have a good broad spectrum of activity at the concentrations tested with mean zone of inhibition diameter > 18 mm [27].

The increased incidence of multidrug resistant bacteria strain has added yet another dimension to the already confounded nightmare of antimicrobial treatment. Paradoxically, the antibiotic pipeline has almost run dry, with no new classes of agents expected to be in use in the next 20 years [28]. There has been a tremendous research in plant-derived antimicrobial compounds as useful alternative strategy to finding lead compounds for the control of

infectious diseases [29-30]. Several *Ochna* species have been investigated and found to contain phenolic-related antimicrobial compounds, most especially flavonoids, biflavonoids and chalcones [21, 31-34].

The *O. kibbiensis* extract was not sensitive (at the tested doses) against MRSA, *H. Pylori* and *P. Flourescens* suggesting that it cannot be used to cure infections caused by those bacteria. It showed greatest activity on *Listeria monocytogenes*, one of the most virulent food-born gram positive pathogen causing as high as 30% mortality [35]. Similarly, the extract showed varying degree of activity against bacteria causing nosocomial transmission of opportunistic infections; *P. Vulgaris*, *Staph. aureus* and *Campylobacter fetus* have been implicated in urinary tract infections (UTI), wound abscesses and thrombophelebitis, respectively [36-38]. The modest antifungal effect recorded by the extract is worthy of note in view of increasing cases of fungal infections due to immunocompromised HIV patients. Candida tropicalis is particularly an emerging pathogenic fungus responsible for candidiasis resistant to a variety of currently available antifungal drugs [39].

CONCLUSION

The results of this study suggest that the leaves extract of *Ochna kibbiensis* contains bioactive constituent(s) with good antibacterial and antifungal activity and lends credence to its folkloric use in wound healing and other microbial infections.

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