Journal of Pharmacy & Pharmacognosy Research
E-ISSN: 0719-4250
editor@jppres.com
Asociación de Académicos de Ciencias Farmacéuticas de Antofagasta
Chile

Mukhtar Danmusa, Umar; Abdullahi Nasir, Idris; Abdullahi, Musa I.; Alkasim Ahmad, Auwal; Abdulkadir, Ibrahim S.
Phytochemical analysis and antimicrobial activities of methanolic stem extracts of Ochna schweinfurthiana F.Hoffm.
Journal of Pharmacy & Pharmacognosy Research, vol. 3, núm. 6, noviembre-diciembre, 2015, pp. 171-182
Asociación de Académicos de Ciencias Farmacéuticas de Antofagasta
Antofagasta, Chile

© 2015 Journal of Pharmacy & Pharmacognosy Research, 3 (6), 171-182
ISSN 0719-4250
http://jppres.com/jppres

Original Article | Artículo Original

Phytochemical analysis and antimicrobial activities of methanolic stem extracts of *Ochna schweinfurthiana* F.Hoffm.

[Análisis fitoquímico y actividad antimicrobiana de extractos metanólicos del tallo de *Ochna schweinfurthiana* F. Hoffm.]

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Abstract

Context: Medicinal plants are an enormous source of alternative antimicrobial therapy, particularly in this era of emerging resistance against orthodox antimicrobial agents.

Aims: To evaluate the phytochemical and antimicrobial activities of methanolic stem extracts of *Ochna schweinfurthiana* F.Hoffm. and various fractions (chloroform, n-hexane, ethyl acetate, and n-butanol) obtained through liquid-liquid partition.

Methods: The basic phytochemistry assay and disc diffusion/broth dilution techniques were used. The microorganisms tested were pure isolates of Methicillin Resistance *Staphylococcus aureus*, *Staphylococcus aureus, Streptococcus pyogenes, Salmonella typhi, Shigella dysenteriae, Klebsiella pneumonia, Neisseria gonnorhea, Pseudomonas aeruginosa, Corynebacterium ulcerans, Bacillus subtilis, Escherichia coli, Proteus mirabilis, Candida albicans, Candida tropicalis, Candida stellatoidea and Candida krusei*.

Results: Thin layer chromatography results showed 13 prominent coloured spots from chloroform extract using dichloromethane/methanol 10:1 as the solvent system. The crude extract revealed the presence of flavonoids, saponins, tannins, glycosides and steroids/terpenes. Antimicrobial susceptibilities and zones of inhibition (ZI) findings showed that stem extracts inhibited growth of all microbes at ZI range of 22 - 29 mm except *C. ulcerans, B. subtilis, E. coli, P. mirabilis, C. stellatoidea and C. krusei*. It was observed that chloroform fraction had the highest antimicrobial activities with minimum inhibitory concentration of 1.25 mg/mL against all susceptible pathogens except *P. aeruginosa* (2.5 mg/mL).

Conclusions: *Ochna schweinfurthiana* F.Hoffm. stem contains bioactive constituents with potent antimicrobial activities at low MIC, especially in the chloroform soluble fraction. This study validates and encourages the ethnomedicinal use of this plant in treating infections caused by these susceptible microbes.

Keywords: Antimicrobial; bioactive constituents; *Ochna schweinfurthiana*; Nigeria; pathogens.

Resumen

Contexto: Las plantas medicinales son una enorme fuente para la terapia antimicrobiana alternativa en esta era de resistencia emergente contra los agentes antimicrobianos ortodoxos.

Objetivos: Evaluar la fitoquímica y la actividad antimicrobiana de extractos metanólicos de tallos de *Ochna schweinfurthiana* F.Hoffm. y varias fracciones (cloroformo, n-hexano, acetato de etilo y n-butanol) obtenidas a través de la partición líquido-líquido.

Métodos: Se utilizaron ensayos básicos fitoquímicos y técnicas de difusión en disco/caldo. Los microorganismos probados fueron aislados de *Staphylococcus aureus resistentes a meticilina*, *Staphylococcus aureus, Streptococcus pyogenes, Salmonella typhi, Shigella dysenteriae, Klebsiella pneumonia, Neisseria gonnorhea, Pseudomonas aeruginosa, Corynebacterium ulcerans, Bacillus subtilis, Escherichia coli, Proteus mirabilis, Candida albicans, Candida tropicalis, Candida stellatoidea y Candida krusei*.

Resultados: Los resultados de la cromatografía en capa fina mostraron 13 manchas coloreadas en el extracto clorofórmico mediante un sistema de disolventes diclorometano/metanol 10:1. El extracto crudo reveló la presencia de flavonoides, saponinas, taninos, glicósidos y esteroides/terpenos. El extracto de tallos inhibió el crecimiento de todos los microorganismos con zonas de inbición entre 22 – 29 mm, excepto *C. ulcerans, B. subtilis, E. coli, P. mirabilis, C. stellatoidea y C. krusei*. La fracción clorofórmica tuvo una mayor actividad antimicrobiana con una concentración inhibitoria mínima de 1,25 mg/mL contra todos los patógenos susceptibles, excepto *P. aeruginosa* (2,5 mg/mL).

Conclusiones: El tallo de *Ochna schweinfurthiana* F.Hoffm. contiene constituyentes bioactivos con potente actividad antimicrobiana, especialmente en la fracción clorofórmica soluble. Este estudio valida y promueve el uso ethnomedicinal de esta planta en el tratamiento de infecciones causadas por estos microorganismos susceptibles.

Palabras Clave: Antimicrobiano; constituyentes bioactivos; *Ochna schweinfurthiana*; Nigeria; parógenos.

ARTICLE INFO
Received | Recibido: September 25, 2015.
Received in revised form | Recibido en forma corregida: December 21, 2015.
Accepted | Aceptado: December 21, 2015.
Available Online | Publicado en Línea: December 23, 2015.
Declaration of interests | Declaración de Intereses: The authors declare no conflict of interest.
Funding | Financiación: The authors confirm that the project has not funding or grants.
Academic Editor | Editor Académico: Gabino Garrido.

INTRODUCTION

From time immemorial, many plants have been used by man as a source of treatment of various disease conditions, particularly at the community level. These covered a wide variety of therapeutic practices that varied from country to country. These plants are referred to as alternative or complementary medicines (WHO, 2009).

The World Health Organization estimated that 80% of the world population relied on medicinal plants for their health care needs. Out of estimated 350,000 known plant species, about 35,000 were used for medicinal purposes and less than 0.5% of these have been investigated for their phytochemical and pharmacological potentials (Hostettmann and Marston, 2002). Several active principles such as tannins, flavonoids, anthraquinones, terpenes, alkaloids and steroidal glycosides have been isolated, purified and standardized from plants and used for orthodox medicine. Similarly, medicinal plants are major sources of antimicrobial drugs (Sofowora, 1993).

Ochnaceae or wild plane, a family of the order malpighiales, comprised of 53 genera and some 600 species of tropical trees, shrubs and herbs. Species of the *Ochnaceae* are found from subtropical to tropical Nations. Members of the Ochnaceae have evergreen petiolate leaves, which are sometimes leathery (in the genus *Ochna*). The genus *Ochna* comprises about 86 species of evergreen shrubs and occurs widely in woodlands of Africa, Asia, and Madagascar (Verdcour, 2005). Species of the *Ochna* are usually called mickey-mouse plant, named after shape of the drupelet fruit (Makhafola and Eloff, 2012). The name of this genus comes from Greek "Ochna" a word used by Homer and means wild pear (Burkill, 1997).

The family is characterized by the presence of flavonoids and biflavonoids and terpenoids as main secondary metabolites (Oliveira et al., 2002). Several studies on most *Ochna* species revealed that the phytochemical contained within this genus constitute mainly glycosides, steroids, saponins, flavones and fatty acids (Agra et al., 2007). This family of plants has been used as food and for medicinal purposes for many centuries; several members of genus are also used for the extraction of edible oils, and as decorative plants (Makhafola and Eloff, 2012).

Ochna schweinfurthiana named after Dr. Georg August Schweinfurth (1836-1925), German botanical collector and taxonomist (Drummond, 1981), is a shrub or small tree up to 4 m tall mostly occurring in African woodlands; from Guinea to Northern and Southern Nigeria, across central Africa to Sudan, Uganda, Zimbabwe and Mozambique, the plant grows as a decorative shrub bearing bright yellow flowers (Burkill, 1997).

In ethnomedicine, the powdered bark is used as antimalarial, febrifuges, and antihelminthic, while the decoction of the root, leaves or bark is used in dressing wounds (Abdullahi et al., 2010). The leaves are used in eye treatments and as laxatives and for social, religion, superstition and magic (Abdullahi et al., 2010). In Northern Nigeria, the plant is said to be used for the treatment of measles, typhoid, fever and fungal skin infections (Abdullahi et al., 2010).

Resistance to antimicrobial agents has become an increasingly global health problem. Microbes will continue to develop resistance once exposed to any antimicrobial agent, thereby imposing the need for a permanent search and development of newer drugs especially from natural plant products (Silver and Bostian, 1993). Most of the available orthodox antimicrobial agents are implicated with serious side effects and adverse reactions, which limit their utilization as medicines, these necessitate research on safer plant sources of antimicrobial therapy (Maureer-Grimes et al., 1996). Very few studies were done regarding the activity of *Ochna schweinfurthiana*. The antimicrobial effect of the methanol and acetone extracts of the leaves of *O. schweinfurthiana* have been reported (Abdullahi et al., 2010; 2011; 2014).

This study sought to investigate; the phytochemical, thin layer chromatography and antimicrobial activities of the various fractions of the crude methanol extract (chloroform, n-hexane, ethyl acetate, n-butanol) on some selected pathogens.

MATERIAL AND METHODS

Collection, identification and preservation of the plant sample

The whole plant material of *O. schweinfurthiana* was collected at Samaru town, Zaria, Kaduna State, Nigeria in June 2012. It was authenticated by Musa Muhammad of the herbarium section of Biological Sciences Department, Ahmadu Bello University, Zaria, Nigeria. A voucher specimen (number 900229) was deposited for future reference purpose. The stems were removed, air-dried, powdered, labeled and stored in the air-tight container before extraction.

Extraction

The powdered stems (800 g) were continuously extracted with methanol by maceration for seven days (1000 mL - three times); the extract was filtered and the filtrate dried under vacuum with a rotary evaporator at 45°C to obtain a brownish product (78 g) of *O. schweinfurthiana* methanolic stem extract, coded (OSMSE). It was stored in a refrigerator pending further investigations.

Phytochemical screening

Phytochemical analysis of methanol stem extract of *O. schweinfurthiana* was performed using different chemical tests to check the presence of active metabolites such as sterols, steroids, fatty acid, alkaloids, glycosides, flavonoid, and tannins in the plant by standard methods (Harbone, 1998).

Test for sterols/terpenes

Liebermann-Buchard test: Anhydrous acetic acid (1 mL) was added to 1 mL of chloroform and cooled to 0°C then a drop of concentrated sulphuric acid was added to the cooled mixture followed by the stem extract. The resultant mixture was observed for blue, green, red or orange color that changes with time.

Salkowski test: A little quantity of the stem extract was dissolved in 1 mL of chloroform, thereafter; 1 mL of concentrated sulfuric acid was added down the test tube to form two phases. Formation of red or yellow coloration was taken as an indication for the presence of sterols.

Test for flavonoids

Shinoda test: To methanolic solution of the stem extract, 3 pieces of magnesium chips were added followed by a few drops of concentrated hydrochloric acid. Appearance of an orange, pink or red to purple color indicates the presence of flavonoids.

Sulphuric acid test: Stem extract (0.5 g) was dissolved in concentrated sulfuric acid and notable color change was observed (if any).

Ferric chloride test: Stem extract (0. 5 g) was boiled in distilled water and filtered to 2 mL of the filtrate, two drops of freshly prepared ferric chloride solution was added; green, blue or violet coloration indicated the presence of phenolic hydroxyl group.

Sodium hydroxide test: The extract (0.5 g) was dissolve in 2 mL of 10% aqueous sodium hydroxide solution and filtered to give yellow color, a change in color from yellow to colorless on addition of dilute hydrochloric acid indicated the presence of flavonoids.

Test for alkaloids

The extract (0.5 g) was stirred with 5 mL of 1% aqueous hydrochloric acid on a water bath and filtered. 3 mL of the filtrate was divided into three. To the first 1 mL of freshly prepared Dragendoff's reagent was added and observed for formation of orange to brownish precipitate. To the second, 1 mL of Mayer's reagent was added and observed for formation of white to yellowish or cream colored precipitate. To the third 1 mL of Wagner's reagent was added to give a brown or reddish or reddish-brown precipitate.

Test for tannins

Ferric chloride test: A small quantity of the extract was boiled in water and filtered. Two drops of ferric chloride was added to the filtrate, the formation of a blue-black, or green precipitate was considered for the presence of tannins.

Test for saponins

Frothing test: The stem extract (0.5 g) was shaken with water in a test tube. Frothing which persisted for 15 min indicates the presence of saponins.

Fractionation of the crude extract using liquid-liquid partition method

Partition or liquid-liquid extraction is a method in which compounds are pulled from solvent A to solvent B where solvents A and B are not miscible. The most common method of liquid-liquid extraction was performed using a separator funnel. The crude methanolic extract was partitioned using different solvents based on their relative polarity, the solvent employed for this purpose were: n-hexane, chloroform, ethyl acetate and n-butanol. The separator funnel method was employed.

The crude methanol extract was first suspended in 500 mL distilled water and successively extracted with the organic solvent of increasing polarity (400 mL three times each) beginning with n-hexane (the least polar), chloroform, ethyl acetate, n-butanol, yielding n-hexane, chloroform, ethyl acetate, n-butanol and the aqueous soluble fractions respectively. The fractions were dried using rotary evaporator and kept in a refrigerator for subsequent future tests.

Thin Layer Chromatography (TLC)

The TLC profile of the various fractions of the plant was determined using different solvent systems (i.e. chloroform/methanol 10:1, 13:1; hexane/ethyl acetate 5:1, 5:2, 5:5; ethyl acetate/chloroform/methanol/water 15:8:4:1; butanol/acetic acid/water upper layer 4:1:5).

A baseline was drawn on one side of silica gel coated TLC plate (0.25 mm layer). The plates after being spotted with the respective fractions with the aid of a capillary tube were developed in a TLC tank.

The plates were removed and dried, the plate was then sprayed with 10% sulphuric acid and dried in an oven at the temperature of 105°C for 10 min, the colors of the spots were recorded and their respective Rf values calculated.

Antimicrobial assays

Antimicrobial susceptibility testing

The antimicrobial activities of *O. schweinfurthiana* crude methanol extract, *O. schweinfurthiana* n-butanol, *O. schweinfurthiana* chloroform and *O. Schweinfurthiana* ethyl acetate soluble fraction was determined using some pathogenic microbes; the microbes were obtained from Department of Medical Microbiology Ahmadu Bello University Teaching Hospital, Zaria. All the isolates were checked for purity, and they were maintained in slants of nutrient agar for the bacteria and in slants of Sabouraud dextrose agar for fungi.

Each extract/fraction (0.1 g) was measured and dissolved in 10 mL of DMSO to obtain the concentration of 10 mg/mL. It was the initial concentrations used to check the antimicrobial activities. The growth medium used for the microbes was Mueller-Hinton agar. The medium was prepared according to the manufacturer's instructions and sterilized at 121°C for 15 minutes. The sterilized medium was then poured into a sterile Petri dishes, and the plates were covered, allowed to cool and solidify. The extract/fractions were screened using the plate diffusion method.

The sterilized medium was seeded with 0.1 mL of standard inocula of the test microbes. The inoculum was spread evenly over the surface of the medium by the use of a sterile swab. A sterile cork borer of 6 mm in diameter was used to cut a well at the center of each inoculated medium. The solution of the extract (0.1 mL) was then introduced into the well at the center of the inoculated medium. The inoculated medium was then incubated at 37°C for 24 h, after which each medium was observed for the zone of inhibition of growth, the zone was then measured with a transparent ruler and the result recorded in millimeters. There were four replicates for both extract concentration and control against the bacteria.

Determination of minimum inhibitory concentration (MIC)

The MIC of the stem extract was carried out using broth dilution method. Mueller-Hinton broth was prepared, and 10 mL were dispensed into test tubes and was sterilized at 121°C for 15 min, the

tube was allowed to cool. McFarland's standard turbidity scale number (0.5) was prepared to give a turbid solution.

Normal saline was prepared, and 10 mL were dispensed each into a sterile test tube and the test microbes were inoculated and incubated at 37°C for 6 hours. Dilution of the test tube was done using normal saline until the turbidity reached that of the McFarland's standard scale by visual comparison, at this point, the test microbe has a concentration of 1.5 x 10^8 CFU/mL.

Two-fold serial dilutions of the extract in the sterile broth were made to obtain the concentrations of 0.625, 1.25, 2.5, 5, and 10 mg/mL. The initial concentrations were obtained by dissolving 0.1 g of the *O. schweinfurthiana* extracts in 10 mL of sterile broth.

Having obtained the different concentrations of the stem extracts in broth, 0.1 mL of standard inoculum of test microbes in the normal saline was then inoculated into these various concentrations of the extracts in the broth, incubated at 37°C for 24 h and at 25°C for 48 h for bacteria and fungi respectively. Thereafter, each test tube was visually observed for turbidity (growth); the lowest concentration of the extract in the broth, which shows no turbidity, was recorded as the minimum inhibitory concentration. There were performed four replicates for both extract concentration and control against the bacteria.

Determination of minimum bactericidal concentrations/ minimum fungicidal concentration (MBC/MFC)

MBC/MFC were carried out to check whether the test microbes were killed or their growth inhibited. Mueller Hinton agar was prepared according to the manufacturer's instructions, sterilized at 121°C for 15 min, poured into sterilized Petri dishes; the plate were allowed to cool and solidify.

The content of the MIC in the serial dilution were then cultured onto the prepared media, they were incubated at 37°C for 24 h, and at 25°C for 48 h for bacteria and fungi respectively. After that, the plates were observed for colonial growth, the MBC/MFC is the plates with lowest concentrations of the extract in serial dilution without microbial growth, thus determined. There were performed four replicates for both extract concentration and control against the bacteria

Statistical analysis

The zones of inhibition demonstrated from this experiment were presented as mean ± SD (standard deviation) of the four replicates for both extract concentration and control against the bacteria. The comparisons between the control group (ciprofloxacin, erythromycin and sparfloxacin or bacteria isolates or fluconazole for the fungal isolates) and the test groups (i.e. antimicrobial activities of the stem extracts against test microorganisms) were performed by one-way analysis of variance (ANOVA) with non-parametric post-hoc Dunnett's test using GraphPad Prism (version 6.0). P-values < 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Percentage yield of the plant extract

The percentage yield of the plant extract after extraction by methanol using maceration method of extraction was calculated to be 9.75%. The total weight of the extract was 78 g, and it was extracted from 800 g of the powdered stem.

Phytochemical findings

The results of preliminary phytochemical tests conducted on methanol stem extract of *O. schweinfurthiana* are as shown in Table 1. The results indicated the presence of flavonoids, saponins, glycosides, tannins and steroids/terpenes.

Thin Layer Chromatography

Preliminary phytochemical screening of the stem extract revealed the presence of flavonoids, steroids/terpenes, glycosides, tannins, and saponins (Table 1). Each of this group of compounds has previously been reported to possess antimicrobial activity (Pegnyemb et al., 2003; Runyoro et al., 2006; Reddy et al., 2008). The mechanisms of actions of these compounds have been proven to be through cell membranes perturbations (Esimone et al., 2006). This alongside with the action of β-lactams on the transpeptidation of the cell wall could lead to an

enhanced antimicrobial effect of the combinations (Singh and Bhat, 2003). In this study, it was found that the stem extract does not contain an alkaloid, this is in consonance with the study of Abdullahi et al. (2010).

Flavonoids and tannins have been reported to possess antimicrobial activity, the antimicrobial activity of flavonoids is due to their ability to complex with extracellular, soluble protein and to complex with bacterial cell wall while that of tannins may be related to their ability to inactivate microbial adhesions, enzymes and cell envelope proteins. The presence of alkaloids, tannins and flavonoids in plants extracts has also been shown to enhance antimicrobial properties as reported by Singh and Bhat (2003) and Sato et al. (2004).

By using dichloromethane/methanol in the ratio of 10:1 as a solvent system for chloroform soluble fraction, 13 colored spots comprising of seven major and six minor spots, but while using chloroform/methanol 10:1 on the same extract seven colored spots were produced on the plate, which contained one highly dense spot at the apex of the chromatogram ahead of the solvent (Tables 3 and 4). This conspicuous spot indicates that the condensed compounds are non-polar, but by reducing the polarity of the solvent using chloroform/methanol 13:1, eight visible spots where found with similar Rf values but the condensed compounds dispersed. Therefore, it can be inferred that the solvent system DCM/MET 10:1 is the best solvent system for the separation of soluble chloroform extracts.

The TLC results for n-hexane soluble fraction using 10:1 chloroform/methanol as solvent system shows four spots, two major and two minor, some of which are colored, this indicates that n-hexane soluble extracts are slightly non-polar (Table 5). Result for ethyl acetate fraction shows that 10:1 chloroform/methanol solvent system was the best for its separation by providing four different spots. While n-butanol extract shows only one spot using butanol/acetic acid/water 4:1:5 (upper layer) solvent system, and no visible spot using the above different solvent system, this indicates that the compounds present in this portion are highly polar (Table 6). Based on findings from TLC, it can be deduced that the chloroform soluble fraction contained the highest number of compounds. Some of the spots were yellow when viewed in daylight and light blue when viewed under UV light (365 nm). This difference may be as a result of the presence of a strong chromophoric group, which is often observed with plant phenolics (especially flavonoids) (Makhafola and Eloff, 2012).

Table 1. Preliminary phytochemical screening from methanol stem extract from *O. schweinfurthiana*.

Test	Result
Flavonoids	
Shinoda test	+
Ferric chloride test	+
Sulphuric acid test	+
Sodium hydroxide test	+
Sterols/terpenes	
Liebermann-Buchard test	+
Salkowski's test	+
Saponins	
Frothing test	+
Tannins	
Ferric chloride test	+
Alkaloids	
Mayer's reagent	-
Dragendoff's reagent	-
Wagner's reagent	-
Glycosides	
Fehling's solution test	+

+ present, - not detected

The 78 g of crude methanolic extracts partition gave the following soluble extractives (Table 2).

Table 2. Results for liquid-liquid partition of crude methanolic extracts from *O. schweinfurthiana*.

Extracts	Quantity (g)	Percentage
OSCHP	22.64	29.03
OSEAP	30.65	39.29
OSNHP	1.19	1.53
OSNBP	20.62	26.43
OSAP	2.90	3.72
	78.00	100.00

OSAP- *O. schweinfurthiana* aqueous portion; OSNBP- *O. schweinfurthiana* n-butanol portion; OSNHP- *O. schweinfurthiana* n-hexane portion; OSEAP- *O. schweinfurthiana* ethylacetate portion; OSCHP- *O. schweinfurthiana* chloroform portion.

Table 3. TLC results for *O. schweinfurthiana* chloroform soluble fraction DCM/MET 10:1.

N° of spots from the base-line	Color of the spots after spraying with 10% sulphuric acid	Type of spots (minor/major)	Rf values
1	Brown	Major	0.20
2	Bright yellow	Major	0.30
3	Bright	Minor	0.34
4	Light	Minor	0.40
5	Bright yellow	Minor	0.44
6	Pink	Major	0.50
7	Yellow	Major	0.54
8	Dark	Major	0.60
9	Brown	Major	0.68
10	Yellow	Major	0.70
11	Dark	Minor	0.80
12	Dark	Minor	0.84
13	Dark	Minor	0.88

Solvent system used dichloromethane/methanol ratio (DCM/MET) 10:1. Solvent front 5.0 cm.

Table 4. TLC results for *O. schweinfurthiana* chloroform soluble fraction.

N° of spots from the baseline	Color of the spots after spraying with 10% sulphuric acid	Type of spots (minor/major)	Rf values
Solvent system used chloroform/methanol in the ratio 10:1			
1	Pink	Major	0.17
2	Brown	Minor	0.25
3	Red	Major	0.35
4	Yellow	Major	0.36
5	Pink	Major	0.46
6	Red	Major	0.56
7	Red	Major	0.83
Solvent system used chloroform/methanol in the ratio 13:1			
1	Dark	Minor	0.11
2	Dark	Minor	0.22
3	Pink	Major	0.31
4	Yellow	Major	0.42
5	Dark	Major	0.51
6	Pink	Major	0.65
7	Yellow	Major	0.67
8	Brown	Major	0.87

Table 5. TLC results for *O. schweinfurthiana* hexane soluble fraction CFM/MET 10:1.

N° of spots from the base line	Color of the spots after spraying with 10% sulphuric acid	Type of spots (minor/major)	Rf values
1	Pink	Major	0.36
2	Bright yellow	Major	0.40
3	Light	Minor	0.83
4	Pink	Minor	0.88

Solvent system used chloroform/methanol in the ratio 10:1.

Table 6. TLC results for *O. schweinfurthiana* n-butanol soluble fraction BAWu 4:1:5.

N° of spots from the base-line	Color of the spots after spraying with 10% sulphuric acid	Type of spots (minor/major)	Rf values
1	Dark	Major	0.18

Solvent system used butanol/acetic acid/water in the ratio 4:1:5. Upper layer.

Findings from the antimicrobial screening showed that all the fractions (crude methanol extract, chloroform soluble fraction, ethyl acetate soluble fraction and n-butanol soluble fraction) had antimicrobial activities against MRSA, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Salmonella typhi*, *Shigella dysenteriae*, *Klebsiella pneumonia*, *Neisseria gonnorhea*, *Pseudomonas aeruginosa*, *Candida albican*, *Candida tropicalis* while no activity was demonstrated against *Corynebacterium ulcerans*, *Bacillus subtilis*, *Escherichia coli*, *Proteus mirabilis*, *Candida stellatoidea* and *Candida krusei* (Table 7). These findings were partly in consonance with those of Abdullahi et al. (2010) who investigated leave extracts of *Ochna schweinfurthiana*, although some differences in antimicrobial activities existed, the variation might be attributable to differences in parts of the plant used for the study.

Zones of inhibition (ZI) for the entire soluble fraction/extract measured in millimeter were found to be (20 – 29 mm) for all susceptible microbes (Table 8). More so, it was found that the zones of inhibition were highest with chloroform extracts; maximal ZI was 29 mm for *Klebsiella pneumonia* and an average zone of inhibition for all other susceptible organisms as 27 mm. Therefore this is an indication that chloroform extract contains highest concentrations of the active compounds responsible for the antimicrobial actions.

Findings from our study showed that MIC for all the extract/fractions using chloroform fractions inhibited bacterial growth of all susceptible organisms at a concentration of 1.25 mg/mL except for *Pseudomonas aeruginosa* which had MIC of 2.5 mg/mL (Table 9). The low MIC (1.5 mg/mL) and the MBC (2.5 mg/mL) as reflected by our findings suggest that our plant extract has good antimicrobial activities against the susceptible organisms considering that compounds with MICs of less than 10 mg/mL are regarded as having strong antimicrobial potential (Tang et al., 2003).

The MBC/MFC findings showed that chloroform extract killed susceptible microbes at the concentrations of 2.5 mg/mL, except for MRSA, *Shigella dysentrae, Pseudomonas aeruginosa, and Candida albicans* were MBC/MFC is 5 mg/mL. While other extracts had MBC and MFC of 5 mg/mL and 10 mg/mL respectively (Table 10). This is an indication that chloroform fraction contained the highest concentrations of the active principles in the stem of *Ochna schweinfurthiana* responsible for antimicrobial activities, and the remaining soluble fractions only had moderate antimicrobial activities. Different patterns of the sensitivity of the test organisms to the plant extracts were noted after incubation at 37°C for 12 and 36 h, which indicates that some of the bioactive compounds in the extracts are bacteriostatic while some are bactericidal.

Table 7. Results of antimicrobial susceptibility tests of stem extracts from *O. schweinfurthiana* and control antimicrobial drugs against pathogens.

Test organism	Sensitivity of stem extracts and some standard drugs to pathogens							
	Crude methanol extract	Chloro-form frac-tion	Ethyl acetate fraction	n-butanol fraction	Ciprof-loxacin	Sparflo-xacin	Erythromy-cin	Flucon-azole
MRSA	S	S	S	S	R	S	R	R
Staphylococcus aureus	S	S	S	S	S	S	S	R
Streptococcus pyogenes	S	S	S	S	S	S	S	R
Corynobacterium ulcerans	R	R	R	R	S	S	R	R
Bacillus subtilis	R	R	R	R	R	R	R	R
Escherichia coli	R	R	R	R	S	S	S	R
Salmonella typhi	S	S	S	S	S	S	R	R
Shigella dysentrae	S	S	S	S	S	S	R	R
Proteus mirabilis	R	R	R	R	R	R	S	R
Klebsiella pneumonia	S	S	S	S	S	S	S	R
Neisseria gonorrhoea	S	S	S	S	R	S	S	R
Pseudomonas aeruginosa	S	S	S	S	S	R	S	R
Candida albicans	S	S	S	S	R	R	R	S
Candida krusei	R	R	R	R	R	R	R	S
Candida tropicalis	S	S	S	S	R	R	R	S
Candida stellatoidea	R	R	R	R	R	R	R	S

S= sensitive; R= resistance; MRSA= Methicillin resistance *Staphylococcus aureus*

Table 8. Zone of inhibition of stem extracts from *O. schweinfurthiana* and some standard drugs to pathogens.

Test organism	Zone inhibition of stem extracts and some standard drugs against pathogens in millimetres							
	Crude methanol extract	Chloro-form frac-tion	Ethyl acetate fraction	n-butanol fraction	Ciprofloxa-cin	Sparfloxaci n	Erythromy-cin	Flucon-azole
MRSA	20	25	23	22	0	27	0	0
S. aureus	21	26	23	21	34	26	30	0
S. pyogenes	24	28	24	23	30	31	29	0
C. ulcerans	0	0	0	0	31	29	27	0
B. subtilis	0	0	0	0	0	0	0	0
E. coli	0	0	0	0	27	30	26	0
S. typhi	20	27	24	22	29	27	0	0
S. dysentrae	21	26	23	22	37	32	0	0
P. mirabilis	0	0	0	0	0	0	27	0
K. pneumonia	22	29	26	23	32	34	30	0
N. gonorrhoea	20	27	24	21	0	26	27	0
P. aeruginosa	22	25	22	20	29	0	26	0
C. albicans	21	25	22	22	0	0	0	22
C. krusei	0	0	0	0	0	0	0	27
C. tropicalis	22	26	23	20	0	0	0	30
C. stellatoidea	0	0	0	0	0	0	0	21

* Statistical significance determined by one-way ANOVA using Dunnett's Multiple Posttest. MRSA= Methicillin resistance *Staphylococcus aureus.*

Table 9. Minimum inhibition concentrations (MIC) of stem extracts from *O. schweinfurthiana* on pathogens.

Test Organism	MIC of stem extracts against pathogens in (mg/mL)			
	Crude methanol extract	Chloroform fraction	Ethyl acetate fraction	n-butanol fraction
MRSA	2.5	1.25	2.5	2.5
S. aureus	2.5	1.25	2.5	2.5
S. pyogenes	1.25	1.25	1.25	2.5
Corynobacterium ulcerans	R	R	R	R
Bacillus subtilis	R	R	R	R
Escherichia coli	R	R	R	R
Salmonella typhi	2.5	1.25	2.5	2.5
Shigella dysentrae	2.5	1.25	2.5	2.5
Proteus mirabilis	R	R	R	R
Klebsiella pneumonia	2.5	1.25	1.25	1.25
Neisseria gonorrhoae	2.5	1.25	2.5	2.5
Pseudomonas aeruginosa	2.5	2.5	2.5	2.5
Candida albicans	2.5	1.25	2.5	2.5
Candida krusei	R	R	R	R
Candida tropicalis	1.25	1.25	2.5	2.5
'Candida stellatoidea	R	R	R	R

R = Resistant

Table 10. Minimum bactericidal/fungicidal concentrations of stem extracts from *O. schweinfurthiana* on pathogens.

Test Organism	MBC/MFC of stem extracts against pathogens in (mg/mL)			
	Crude methanol extract	Chloroform fraction	Ethyl acetate fraction	n-butanol fraction
MRSA	10	5	5	10
S. aureus	10	2.5	10	10
Streptococcus pyogenes	5	2.5	5	5
Corynebacterium ulcerans	R	R	R	R
Bacillus subtilis	R	R	R	R
Escherichia coli	R	R	R	R
Salmonella typhi	10	2.5	5	10
Shigella dysentrae	5	5	10	10
Proteus mirabilis	R	R	R	R
Klebsiella pneumonia	5	2.5	2.5	5
Neisseria gonorrhoae	10	2.5	5	10
Pseudomonas aeruginosa	10	5	10	10
Candida albicans	10	5	10	10
Candida krusei	R	R	R	R
Candida tropicalis	10	2.5	10	10
Candida stellatoidea	R	R	R	R

R = Resistant

CONCLUSIONS

Methanolic extract of stem bark of *Ochna schweinfurthia* was found to contain some potent phytochemical. These compounds were responsible for its antimicrobial activities. This study thus supports the ethnomedicinal use of stem extracts of *O. schweinfurthiana* in treating infections caused by these susceptible microbes such as typhoid fever, skin, pharyngitis, nosocomial and sepsis in preference to synthetic antimicrobials due to the side and adverse effects they may provoke *in vivo*. Since there are very limited pharmacognostic studies on *O. schweinfurthiana,* there is a need to refine, standardize and conduct more detailed phytochemical analyzes on this plant.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENT

The authors thank the staff of Medical Microbiology Department of the Ahmadu Bello University Teaching Hospital for providing us with pure isolates of pathogens used for this experiment.

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