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ANTIMICROBIAL POTENTIALS AND PHYTOCHEMICAL INVESTIGATION OF STEM BARK METHANOLIC EXTRACT AND FRACTIONS OF *Milletia chrysophylla* Dunn

[*1]Madumelu, M., [2] Iwuala, N. B. and [3] Uttu, J. A.

[1] Department of Chemistry, Federal College of Education Zaria Nigeria
[2] Department of Chemistry, Ahmadu Bello University Zaria Nigeria
[3] Department of Chemistry, Federal University Gashua, Yobe State Nigeria

*Corresponding authors' email: **madumelumark@gmail.com** Phone: 08034641173

ABSTRACT

The stem bark of crude methanolic extract of Milletia chrysophylla and it fractions were screened for phytochemical constituents and antimicrobial activity against Methicillin resistant Staphylococcus aureus, Staphylococcus aureus, Streptococcus pyogenes, Corynebacterium ulcerans, Escherichia coli, Neisseria gonorrhoae, Salmonella typhi, Shigella dysenteriae, Proteus vulgaris, Candida albicans, Candida krusei and Candida tropicalis using the ager-well diffusion method. The analysis showed relevant and interesting activities for all the extracts on most of the organisms. However, their sensitivities were a bit lower relative to the positive control. Zones of inhibition ranged between 20 - 31 mm for all the extracts and their MIC values were between 0.06 -2.5 mg/ml with the n hexane and chloroform fractions having the least values for some of the organisms. Similarly, the MBC/MFC values recorded ranged between 0.13 - 10 mg/ml with the chloroform fraction, also having the least value of 0.13 mg/ml for Escherichia coli, Shigella dysenteriae and Candida albicans which indicates a possible higher concentrations of the active components therein. Phytochemical studies of the crude extract, showed presence of carbohydrates, glycosides, cardiac glycoside, saponins, tannins, condensed tannins, flavonoids, alkaloids, steroids and triterpenes. The presence of these metabolites and the inhibitory effect of each of the extracts on most of the test pathogens showed the broad spectrum antimicrobial activities of the plant and justify it use in ethno medicine for treating ailments of microbial origin thus, introducing the plant as a candidate for new drug search and development for ailments due to these pathogens.

Keywords: Stem bark, *Milletia chrysophylla*, antimicrobial potentials and Phytochemical investigation

INTRODUCTION

It has been estimated globally, that almost 80% of the world population depends largely on traditional medicine from many sources including plant extracts for their primary health care (WHO, 2002; Akindele & Adeyemi, 2007a). This according to Duraipandiyan *et al.,* (2006) is mainly because plants have been reported to contain a wide range of therapeutic substances used for the treatments of chronic and infectious diseases.

Tropical and subtropical Africa countries contain well over 40,000 plant species with vast medicinal potentials and benefits which can be harnessed and developed. However, only about 5,000 species are used medicinally (Van Wyk, 2008). Also in spite of this huge diversities and medicinal potentials inherent in the region, the African continent has only contributed 83 out of the 1100 classic drugs globally (Van Wyk, 2008). This therefore, calls for increased exploration into these diversities with the hope of developing new and better drugs that could help combat the problems associated with existing and emerging health challenges that plagues the world.

The *Millettia* genus has appeared in the African pharmacopeia since centuries. A wide range of biological activities have been associated to it which include antitumoral, anti-inflammatory, antiviral, bactericidal, insecticidal and pest-destroying (Banzouzi *et al.,* 2008). Aubreville (1950) earlier reported that 20% of it approximately 260 species, mostly shared between Asia (121 species) and Africa (139 species), are medicinal. These claimed activities that are now being confirmed through pharmacological studies, brings to this genus a huge interest in traditional medicine and research studies for the discovery and development of new biologically active compounds (Banzouzi *et al.,* 2008).

Milletia chrysophylla Dunn is a member of the Fabaceae family. It is a liana, shrub or a small tree with a bushy crown. It can grow up to 15 meters tall. It is found in Nigeria, Cameroon, Guinea and Gabon. Generally, it flexible branches are used for hut building (Dominique, 2008; Ken, 2014). It is called Enampyepye in northern part of Nigeria precisely Edumoga tribe in Benue State and useful among the local healers to alleviate malaria, syphilis, tuberculosis, and liver related problems. It is also used for the management of rheumatism, waist pain, high blood pressure, diabetes, sickle cell anemia. It equally serves as a source of vitamin. Hitherto to the best of our search, there hasn't been any report on the phytochemical constituents and antimicrobial activities of this plant specie.

Supporting the on-going global efforts towards the discovery of new antimicrobial compounds that could serve as better remedy and better treatment substitute for various ailments caused by microbes and to help curb the problems associated with the disturbing issues of drug resistances on existing pathogens and their emerging new strains, the stem bark of this plant was screened for their phytochemical constituents and antimicrobial properties.

MATERIALS AND METHODS

Plant collection and identification

Fresh samples of the plant parts were collected from Ayeje Eke, Okpokwu L. G. A. of Benue state Nigeria on the 1st of July, 2016. Identification of the plant materials was done at the herbarium section, Department of Biological Science; Ahmadu Bello University Zaria, Nigeria by Mal. Namadi Sanusi and a voucher specimen number ABU090081 was deposited at the herbarium.

Plant extract preparation

The plant materials were thoroughly washed with cold tap water and shade dried at room temperature for 3 weeks and then pulverized using a wooden mortar and pestle. Cold extraction was carried out on about 1 Kg of the pulverized plant sample using methanol for 48 hrs. The process was repeated several times until the plant material was completely extracted. The resulting crude extract was concentrated at about 40 ^{0}C in vacou under pressure with the aid of a rotary evaporator and subsequently air dried to a constant weight of 101.13 g. 90 g of the dried crude extract was then partitioned using n hexane, chloroform and ethyl acetate exhaustively and respectively. Their separate fractions were concentrated at 40 ^{0}C in vacou under pressure also with the aid of a rotary evaporator and further air dried to constant weights of 14.74, 2.52 and 2.86 g respectively. All the four extracts were then screened for antimicrobial activities.

Test organsms

Strains of pathogens subjected for the test are: *Methicillin resistant Staphylococcus aureus (MRSA), Staphylococcus aureus, Streptococcus pyogenes, Corynebacterium ulcerans, Escherichia coli, Neisseria gonorrhoae, Salmonella typhi, Proteus vulgaris, Shigella dysenteriae, Candida albicans, Candida tropicalis and Candida krusei.*

Media used

Mueller - Hinton Agar (MHA) and Potato dextrose Ager (PDA) were the growth medium used to grow the bacterial and fungi strains respectively for the sensitivity test and mueller hinton broth was used for the minimum inhibitory concentrations (MIC) and minimum bactericidal/fungicidal concentrations (MBC/MFC) determination.

Antimicrobial activity

The antimicrobial activities of the four extracts were determined by ager -well diffusion method using some clinical isolates of pathogenic microbes gotten from the Medical Microbiology unit of Ahmadu Bello University teaching Hospital Zaria, Nigeria. 1g of the crude extract was dissolved in 10 ml of dimethyl sulphuroxide (DMSO) to give a concentration of 10 mg/ml. Similarly, 0.01 g each of the n hexane, chloroform and ethyl acetate fractions was dissolved separately in 10 ml of DMSO to get a concentration of 1 mg/ml respectively. These were the initial concentrations used for the determination of the antimicrobial activities of the plant extracts.

The growth mediums were prepared based on the manufacturer's instruction, sterilized at 121°C for 15 mins and dispensed into sterile petri dishes. The contents were allowed to cool and solidify. The sterilized medium was then seeded with 0.1ml of the standard inoculum of the test organisms. The inoculum was evenly spread by the use of a sterile swab over the surface of the medium. A fine well was cut with a standard 6 mm diameter cork borer at the center of each of the inoculated medium and properly labeled. 0.1ml solution of 10 mg/ml concentration of the crude extract and 1 mg/ml each of the n hexane, chloroform and ethyl acetate fractions were then transferred into the respective bored and labeled wells of the inoculated medium. The extracts were allowed to diffuse through the medium for about an hour on a bench and then incubated at 37°C for 24 h for the bacteria and in a locker for 72 h for the fungi. The plates were observed for zones of inhibition of growth and then, measured and recorded in mm.

Minimum inhibition concentration (MIC)

The MICs of the extract and fractions were determined using the broth dilution method. Muller – Hinton broth was prepared. 10 ml was introduced into test tube and was sterilized at 121°C for 15 mins. Mc -Farland's turbidity standard number 0.5 was prepared and was used as the test criteria for comparison. Normal saline was prepared; 10 ml was transferred into each sterile test tube and inoculated with the bacterial test organisms.

Serial dilution of concentrations of the extracts that showed sensitivity against the organisms were done in the sterile broth to obtain various concentrations of 10, 5, 2.5, 1.25, 0.63 and 0.3 mg/ml for the crude extract and 1, 0.5, 0.25, 0.13, 0.063 and 0.03 mg/ml concentrations for the n hexane, chloroform and ethyl acetate fractions. To the different concentrations of the extracts in the sterile broth, 0.1 ml of the test organisms in the normal saline was then inoculated. Incubation was done at 37°C for 24 h for bacteria and 48 h for fungi after which the test tubes were checked for turbidity (growth). The least concentration in the series without any sign of growth was recorded as the MIC.

Minimum bactericidal/fungicidal concentration (MBC/MFC)

MBCs/MFCs were conducted to see if the test organisms were killed or only their growths were inhibited. Muller-Hinton agar was again prepared and sterilized at 121°C for 15 mins for this purpose. The prepared medium was transferred into sterile petri dishes and was allowed to cool and solidify. A loopful of the MIC content of each tube was then sub cultured onto the prepared medium and incubated at 37°C for 24 h for the bacteria and 48 h for the fungi, after which the plates were checked for any growth. The least concentration of the subculture with no growth was noted and recorded as the MBC/MFC.

Phytochemical screening

The crude extract was screened for some possible phytochemicals that may be present therein using the standard methods of Soforawa, 1993 and Trease & Evans, 1989.

RESULTS

The preliminary antimicrobial test, MIC and MBC/MFC results of the extracts are summarized in tables 1 to 3.

Table 1: Zones of inhibition (mm) of extracts/ positive controls

MIicroorganisms	Crude methanolic	Hexane	Chloroform	Ethyl acetate	Ciprofloxacin	Fluconazole
MRSA	0	0	0	0	0	-
Staphylococcus aureus	22	26	29	21	32	-
Streptococcus pyogenes	21	25	27	20	30	-
Corynebacterium ulcerans	0	0	0	0	35	-
Escherichia coli	24	28	30	23	37	-

Neisseria gonorrhoae	0	0	0	0	0	-
Salmonella typhi	0	0	0	0	42	-
Shigella dysenteriae	26	27	31	23	40	-
Proteus vulgaris	20	26	28	22	0	-
Candida albicans	22	25	30	24	-	35
Candida krusei	0	0	0	0	-	32
Candida tropicalis	21	25	27	20	-	34

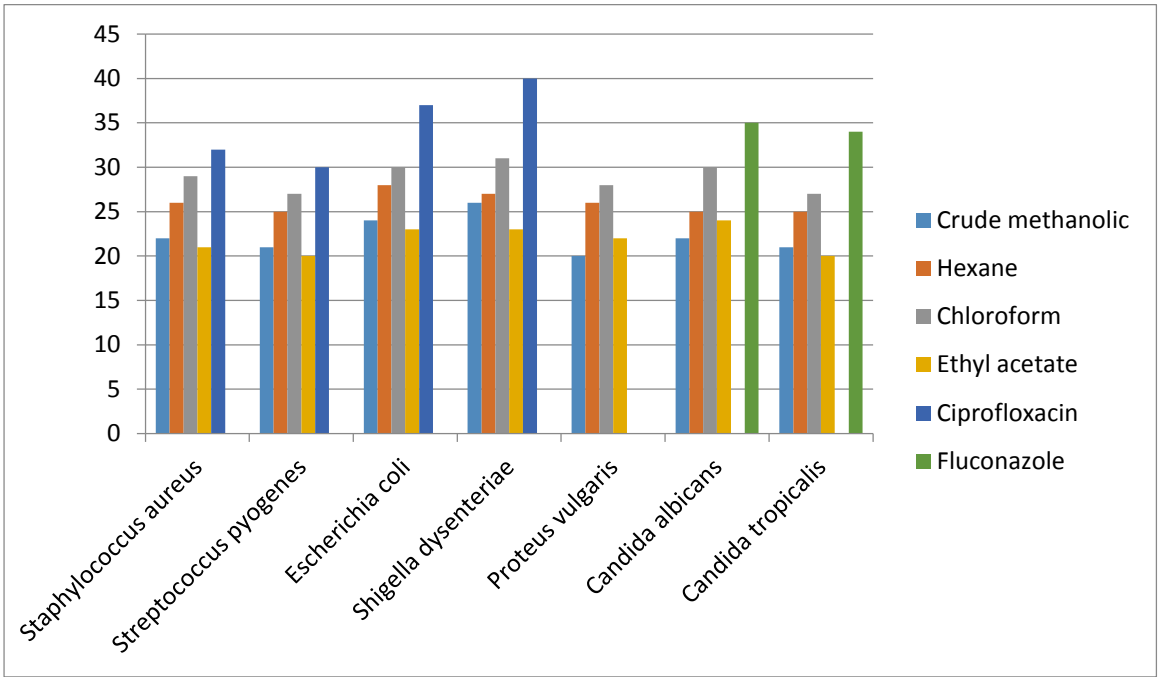

Figure 1: Graphical correlation of extract and fractions with positive controls

Table 2: The MIC of extracts in mg/ml

Microorganisms	Crude methanolic	Hexane	Chloroform	Ethyl acetate
Staphylococcus aureus	2.5	0.13	0.06	0.25
Streptococcus pyogenes	2.5	0.13	0.13	0.25
Escherichia coli	1.25	0.06	0.06	0.25
Shigella dysenteriae	1.25	0.13	0.06	0.25
Proteus vulgaris	2.5	0.13	0.06	0.25
Candida albicans	2.5	0.13	0.06	0.13
Candida tropicalis	2.5	0.13	0.13	0.25

Table 3: The MBC/MFC of extracts in mg/ml

Microorganisms	Crude methanolic	Hexane	Chloroform	Ethyl acetate
Staphylococcus aureus	10	0.5	0.25	1.0
Streptococcus pyogenes	10	0.5	0.25	1.0
Escherichia coli	5	0.25	0.13	0.5
Shigella dysenteriae	2.5	0.25	0.13	0.5
Proteus vulgaris	10	0.5	0.25	1.0
Candida albicans	10	0.5	0.13	0.5
Candida tropicalis	10	0.5	0.25	1.0

DISCUSSION

The results of the preliminary antimicrobial studies of the extracts, their MICs and MBCs /MFCs against the test microbes were summarized in tables 1 to 3.The zones of inhibitions of all the four extracts, showed relevant and interesting activities. However, they compared slightly lower than those of Ciprofloxacin and Fluconazole which were used as the positive control for the bacteria and fungi respectively (Figure 1). Their zones of inhibitions, ranged between 20 -26, 25 - 28, 27- 31 and 20 - 24 mm for the crude methanolic extract, n hexane, chloroform and ethyl acetate fractions respectively (Table 1). The highest zones were observed with the chloroform fraction. All the four extracts did not show sensitivity for *MRSA, Corynebacterium ulcerans, Neisseria gonorrhoae, Salmonella typhi* and *Candida krusei.* The MIC values for all the extract and fractions ranged between 0.06 - 2.5 mg/ml and most effective with *Staphylococcus aureus, Escherichia coli, Shigella dysenteriae, Proteus vulgaris* and *Candida albicans* for the chloroform fraction as well as *Escherichia coli* for the n hexane fraction at 0.06 mg/ml

(Table 2). This implies a higher activity of these extracts over others and could be attributed to the extracts, containing higher proportions of the secondary metabolites. The crude methanolic extract had the highest MIC value of 2.5 mg/ml for almost all the test microbes also, implying a relatively lower synergistic activity. Furthermore from the MBC/MFC values in table 3, bactericidal effects on the organisms were observed for all the four extracts with the values ranging from 0.13 - 10 mg/ml. Similarly, the chloroform fraction showed the least value of 0.13 mg/ml for *Escherichia coli*, *Shigella dysenteriae* and *Candida albicans* followed by the n hexane fraction with the value of 0.25 mg/ml for both *Escherichia coli* and *Shigella dysenteriae*, while the crude methanolic extract exhibited the highest MBC/MFC value of 10 mg/ml for virtually all the test microbes. The lower MBC/MFC concentrations exhibited by the chloroform and n hexane fractions on some of the test microbes relative to other extracts, indicated their ability to exterminate the organisms at lower concentrations and implied a better activity.

Phytochemical screening

This analysis on the crude methanolic extract of *Milletia chrysophylla* Dunn, showed the presence of carbohydrates, glycosides, cardiac glycoside, saponins, tannins, condensed tannins, flavonoids, alkaloids, steroids, triterpenes and absence of anthraquinone. The observed antimicrobial effects of this plant extracts on the organisms could be associated with the phytochemical constituents therein and has probably aided it. This view was partly corroborated by Musa *et al.*, (2019). Flavanoids are reported to possess a wide range of biological activities such as antiinflamatory, anti-allergic, antimicrobial, antiangionic, antioxidant, analgesic and cytostatic properties (Hodek *et al.*, 2002). A number of saponins and triterpenes compounds have been reported to be useful anti-inflammatory, antiulcerogenic, anti-edematous, antipyretic, analgesic and fibrinolytic agents (Ndukwe *et al.*, 2005). Perekh & Chanda, 2007 reported the reaction of tannins with proteins to provide the typical tannin effect which can aid the treatment of inflamed tissues. Also, Li *et al.*, (2003) had earlier reported the anticancer and antiviral activity of tannins (Herbone & Baxter, 1993).

CONCLUSION

The inhibitory effect of the methanolic extract and fractions of the stem bark of *Milletia chrysophylla* Dunn against most of the test organisms is a proof of it broad spectrum antimicrobial potentials, and could be due to the individual or synergistic effects of the phytochemical constituents present in the crude extract. This justifies the use of the plant in ethno medicine for the treatment of ailments of microbial origin thus, introducing the plant as a candidate for new drug search and development that may be useful for the treatment of infectious diseases due to the organisms. There is the need for more studies towards isolation, identification and characterization of the bioactive agents from the most active extract for better insight on the plant medicinal potentials.

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