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STEM BARK EXTRACTS OF *MAESOBOTRYA BARTERI*: PHYTOCHEMICAL ANALYSIS AND INVITRO ANTIMICROBIAL STUDIES

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ABSTRACT

The methanol and aqueous extracts of the stem bark of *Maesobotrya barteri* were assessed for their chemical constituents and antimicrobial activity. The phytochemical screening showed the presence of alkaloids, terpenes, flavonoids, tannins, saponins and cardiac glycosides. Evaluation of antimicrobial activity of the extracts on clinical isolates (*Escherichia coli, Staphylococcus aureus, Klebsiella spp, Pseudomonas aecroginosis, Shigella dysenteriae, Salmonella typhi, Microsporum spp, Trichophyton spp, Epidermophyton spp, Aspergillus flavus and Candida albicans*) showed activity against most of the microorganisms tested. The minimum inhibition concentration (MIC), minimum bactericidal concentration (MBC)/ minimum fungicidal concentration (MFC) of the extracts showed that some microorganisms were responsive to the extract at some given concentrations. Methanol extract exhibited the highest activities against bacteria; *Staphylococcus aureus, Shigella dysenteriae, Salmonella typhi* and fungi *Microsporum canis, Candida albicans.* The bioactivity recorded compares favourably with the standard drug and therefore validates the ethnomedicinal uses of the plant.

Keywords: *antimicrobial activity, minimum bactericidal concentration, phytochemical screening, Bioactivity*

INTRODUCTION

The medicinal usage of plant is as old as human history and it has been mostly traditionally without sufficient data to confirm their efficacy [1]. In search for food for sustenance, the early man discovered that some food have special characteristics of relieving certain diseases and maintaining good health in addition to having ornamental and poisonous properties too [2]. Research in 2001, identified 122 natural products used in modern medicine which all come from ancient plant sources; 80 % of these were already used traditionally for the same or related purpose to the current use of active element of the plant [3]. The interest in the study of medicinal plant and their curative properties has led to wide usage of plant materials in the development of therapeutic remedies in Africa [4]. Dry or sometimes fresh parts of herbal substance may be used for preparation of herbal drugs, food, processed products and the herbal substance may also be utilized for the manufacture of homeopathic drug. Also besides their importance in the health care system of rural communities, medicinal plant also improves the economic state of people involved in the sale in addition to providing affordable health care to rural dwellers [5][6]. The ideas related to the usage of medicinal plants and the evolution of awareness have increased the ability of Pharmacists and Physicians to respond to the challenges that have emerged as a result of decreased efficacy of synthetic drugs and the attendant increased contradictions thereby making the usage of natural drugs tropical again [7]. In recent years, a significant revival of interest in natural products as a potential source for new medicines has been observed among academia as well as pharmaceutical companies. Several modern drugs (~40 % of the modern drugs in use) have been developed from natural products. According to [8]. `39 % of the 520 new approved drugs between 1983 and 1994 were natural products or their derivatives, and 60–80 % of antibacterial and anticancer drugs were from natural origins´.
Natural products contribute to the search for new drugs in different ways

a.) By acting as new drugs that can be used in an unmodified state (e.g., vincristine from *Catharanthus roseus*) [9].
b.) By providing chemical ‘‘building blocks’’ used to synthesize more complex molecules (e.g., diosgenin from *Dioscorea floribunda* for the synthesis of oral contraceptives) [9].
c.) By indicating new modes of pharmacological action those allow complete synthesis of novel analogs (e.g., synthetic analogs of penicillin from *Penicillium notatum*) [9].

Plant secondary metabolites are unique source for pharmaceuticals, food additives, flavours and industrially important biochemical [10].

Maesobotrya barteri is a shrub with a simple indumentum up to 10m high. It is found in the rainforest occurring in Sierra Leone, Southern Nigeria, Western Cameroun and Congo Basin [11]. It bears fruit from April to June has leaves which are mostly long-petiolate, alternate, stipulate simple and penninerved [12]. The aim of this work is to study the phytochemical and antimicrobial activity of extracts of *Maesobotrya barteri.*

Table 1: Botanical classification of *Maesobotrya barteri*

Kingdom	Plantae
(unranked)	Angiosperms
(unranked)	Endicots
(unranked)	Rosids
Order	Malpighiales
Family	Phyllanthaceae
Genus	Maesobotrya
Species	*Maesobotrya barteri*
Trinomial name	*Maesobotrya barteri var.sparsiflora*

[13].

MATERIALS AND METHODS

Collection and identification

The bark of fresh stem of *Maesobotrya barteri* (bush cherry) were collected from a forest in Ikot Akpan Udo II-Ibesit Nung Ikot, Oruk Anam Local Government Area of Akwa Ibom State, Nigeria. The time of collection coincided with the rainy season in Nigeria. The plant was identified at the Pharmacognosy and Natural medicine unit in the Faculty of Pharmacy, University of Uyo. The stem´s bark was air dried for 7 days and ground into uniform powdery form and stored in an airtight container prior to analysis.

Extraction of plant materials

1 Kg of pulverized sample was thoroughly extracted with methanol (5 L) and distilled water (5 L) using cold extraction for 7 days at room temperature (maceration) [14]. The mixture was filtered using a filter paper. The filtrate was then concentrated under reduced pressure using rotary evaporator at 40 ^{o}C to near dryness over a water bath before drying to constant weight in an oven at 40 ^{o}C. The extracts obtained were stored in sealed bottles.

Pytochemical analysis

Qualitative test for alkaloids, flavonoids, saponins, tanins, philobatnnins, deoxy-sugars, anthraquinones, terpenes and cardiac glycoside were carried out using standard procedures as described by [4].

Antimicrobial analysis of stem bark of *m. Barteri*

Collection of isolates

Clinical samples were collected from St Luke's Hospital-Anua, Microbiology laboratory of University of Uyo; fungal isolates were collected from environment and Dermatophyte from school children all in Uyo metropolis. A total of 11 isolates were collected which were purified by sub-culturing into their selective medium and thereafter sub-cultured into nutrient agar. The isolates were 1 Gram positive, 4 Gram negative and 5 fungi (*Staphylococus aureus, Klebsiella sp, Pseudomonas aeroginosa, Shigella dysentery, Salmonella typhi, Esherichia coli, Microsporum sp, Trichophyton sp, Epidermophyton sp, Aspergillus fumigatus and Candida albicans.* The test organisms were selected based on the growth rate and susceptibility to the extract.

Standardization of microbial isolates before inoculation

All isolates were inoculated with sterile peptone water and incubated for 24hours. Gram positive and fungal isolates were serially diluted to factor 3 using 10-fold dilution while Gram negative isolates were serially diluted up to factor 5 using 10-fold dilution. These were carried out to standardize the number of cells inoculated into the medium for antimicrobial activities. The cells contain in 0.5ml that will be inoculated will be equivalent to Mcfarlend Standard [15].

Preparation and inoculation of Muller-Hinton plates with the test organism

The Muller-Hinton agar used were prepared according to the manufacturer's specification, dissolved in appropriate volume of water and heated to gel in hot plate. The medium was autoclaved at 121^{o}C for 15 minutes at 10 psi. Thereafter, on cooling about 25ml of the molten agar was poured into sterile petri dishes and left on the bench to set. Plates were dried over 70 ^{o}C to remove water of condensation on the surface.
Each diluted isolates (0.5 ml) was introduced aseptically into the plates and spread evenly on the surface of the plates and left to acclimatized on the medium.

Grading of the extracts preparation of Muller-Hinton plates

Different concentrations (mg/ml) of each extract were prepared as follows: 250, 350, 450 and 550 and were stored in different test tubes and labelled accordingly. 5mm wells were bored on the surface of the medium that were seeded with the test organism. The different concentration of Aqueous and Methanol extract (550mg/ml, 450mg/ml, 350mg/ml and 250 mg/ml) were introduced into the wells such that each extract fraction has to contain on each isolate used. The same procedure was used for the standard drugs, Streptomycin (250 mg and Nystatin (80 mg). Plates were incubated for 24 and 72 hours for bacterial and fungi respectively. Zones of inhibition were measured and recorded as shown in Table 4.

Determination of minimum inhibitory concentration (MIC)

Antimicrobial activities of the extracts were first screened by agar diffusion technique. The MIC testing was carried out using broth dilution method, Aloopful of the standardized isolates were inoculated into tubes of the sterile peptone broth supplemented with 0.5ml of each extract concentration. Tubes were incubated for 24 hours. The MIC for the extract were determined by visual method and comparing it with the uninoculated broth (control).

RESULTS AND DISCUSSION

The results of the phytochemical screening of methanol and aqueous extracts stem bark of *Maesobotrya barteri* are shown in Table 2. The inhibition zone diameter, IZD (mm) of the methanol and aqueous stem bark extract of *M. barteri* against tested clinical isolates is shown in Table 3. The Minimum inhibitory concentration (MIC) of the methanol and aqueous of the stem bark extract of *M. barteri* against the tested clinical isolates is also presented in Table 4. The Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) of the methanol extract of *M. barteri* against the tested clinical isolates are presented in Table 5.

Table 2. Phytochemical compositions of methanol and aqueous stem bark extract of *Maesobotrya barteri.*

Phytoconstituents	**methanol extract**	**aqueous extract**
Alkaloids	Present	Present
Philobatannins	Absent	Absent
Tanins	Present	Present
Terpenes	Present	Present
Deoxy sugar	Absent	Absent
Cardiac glycosides	Present	Present
Flavonoids	Present	Present
Saponins	Present	Present
Anthraquinones	Absent	Absent

Table 3: Inhibition zone diameter, IZD (mm) of methanol and aqueous extracts of *Maesobotrya barteri* on some test organisms

Test Organisms	Zones of inhibition (mm) with different concentration of methanol and aqueous extract								Streptomycin	Nystatin
	550 mg/ml		450 mg/ml		350 mg/ml		250 mg/ml		250 mg/ml	50 mg/ml
	ME	AE	ME	AE	ME	AE	ME	AE		
S. aureus	18	-	14	-	12	-	10	-	23	-
Klebsiella spp	-	-	-	-	-	-	-	-	16	-
P. acroginoss	-	-	-	-	-	-	-	-	12	-
S. dysenteriae	15	10	12	7	10	-	8	-	7	-
S. typhi	10	7	8	-	6	-	6	-	7	-
E. coli	-	-	-	-	-	-	-	-	19	-
Microsporum spp	10	-	-	-	-	-	-	-	-	15
Trichophyton spp	-	-	-	-	-	-	-	-	-	22
Epidermophyton spp	-	-	-	-	-	-	-	-	-	29
A. flavus	-	-	-	-	-	-	-	-	-	20
C. albicans	20	10	17	7	14	12	12	-	-	24

Key: ME = methanol extract and AE = aqueous extract

Table 4: Result of minimum inhibitory concentration (MIC) of stembark extract of *M. barteri.*

Test Organisms	Extract Concentration (mg/ml)	
	Methanol	Aqueous
S. aureus	200	-
S. dysenteriae	200	450
S. typhi	200	-
Microsporum spp	500	-
C. albicans	150	450

Table 5: Result of minimum bacteriostatic and bactericidal concentrations/MFC of methanol extract on the isolates.

Test Organisms	Bacteriostatic Concentration (mg/ml)	Bactericidal Concentration (mg/ml)
S. aureus	200	450
S. dysenteriae	200	550
S. typhi	200	-
Microsporum spp	500	-
C. albicans	150	450

Out of the two extracts methanol provided more consistent and prominent antimicrobial activity as compared to the aqueous extract. The reasons for minimal antimicrobial activity in aqueous extracts could be a low concentration of antimicrobial compounds in these extracts or all of the identified components from plants active against microorganisms were aromatic or saturated organic compounds which are most often obtained through ethanol or methanol extraction [16]. The result of the phytochemical screening of the methanol and aqueous extracts of stem bark of *M. barteri* revealed the presence of alkaloids, flavonoids, tannins, terpenes, saponins and cardiac glycosides by qualitative methods. The zone of inhibition of methanol and aqueous extract in Table 3 showed remarkable activities against five of the eleven organisms tested. The zones of inhibition were compared with these drugs; streptomycin and Nystatin. The MIC studies (Table 4) of the methanol extract stem bark of *M. barteri* inhibited growth of *Staphylococcus aureus Shigella dysenteriae,* and *Salmonella typhi* at a concentration of 200 mg/ml while *Microsporum canis* and *Candida albican* were 500 and 150 mg/ml respectively whereas MIC studies of the aqueous extract inhibited the growth of *Shigella dysenteriae and Candida* albicans at 450 mg/ml this is similar to what was reported by [17] for *Rhus glabra*. MBC/MFC studies (Table 5) of the methanol extract showed the *Staphylococcus aureus, Shigella dysenteriae, Salomella typhi, Microsporum canis and Candida albicans* were bacteriostatic (were in stationary phase growth) at those concentration whereas *S. aureus, S. dysenteriae* and *C. albicans* showed no growth(MBC/MFC) at (450,550 and 450) respectively.

Conclusion

The phytochemical screening of the methanol and aqueous extracts of stem bark of *M. barteri* revealed the presence of alkaloids, flavonoids, tannins, terpenes, saponins and cardiac glycosides and antimicrobial activities showed remarkable activities against five of the eleven organisms tested. From the antimicrobial activity of the plant collaborate its frequent usage in the treatment of several diseases including diarrhea, stomachache, dysentery, urethral discharge, venereal disease, jaundice and cough in traditional medicine.

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