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Comparative antimicrobial activity of fractions of *Vernonia glaberrima* against selected human pathogens

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Abstract
The increasing resistance of most microorganisms to existing armamentarium of antibiotics has made the search for new antimicrobial agents particularly from natural products more imperative. This study was aimed at evaluating the antimicrobial activity of the chloroform, ethyl acetate, n-hexane and n-butanol fractions of *Vernonia glaberrima* against some selected human pathogens with a view to identify the most active fraction from the plant and to ascertain the veracity of the use of the plant in ethno-medicinal practice. The antimicrobial activity of the fractions of *V. glaberrima* against eight selected human bacterial pathogens- Methicillin resistant *Staphylococcus aureus*, Vancomycin resistant enterococci, Methicillin Susceptible *Staphylococcus aureus* (MSSA), *Listeria monocytogenes, Helicobacter pylori, Campylobacter fetus, Pseudomonas fluorescens, Proteus vulgaris* and two fungal pathogens- *Candida stellatoidea* and *Candida tropicalis* was investigated using the agar well diffusion and broth dilution methods. The fractions exhibited good antimicrobial activity against the pathogens with the exception of *L. monocytogenes, C. fetus, P. vulgaris* and *C. tropicalis*. The Minimum Inhibitory Concentration (MIC) of the n-hexane, chloroform and n-butanol fractions was 0.25 mg/mL while that of ethyl acetate fraction ranged between 0.25-0.125 mg/mL against all the test microorganisms. The Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) of the n-hexane and n-butanol fractions was found to be 1 mg/mL, chloroform fraction had 0.5-1 mg/mL and the MBC/MFC of ethyl acetate ranged between 25-0.5 mg/mL. The ethyl acetate fraction possessed the most significant antimicrobial activity among the four fractions tested against the pathogens.

Keywords: *Vernonia glaberrima*; Antimicrobial activity; Fractions

INTRODUCTION

The discovery and eventual introduction of antibiotics in clinical practice heralded a new era of hope of eradication of microbial infections [1,2]. This era is accompanied by overuse and misuse of these agents, which inadvertently lead to emergence of strains of microorganisms resistant to almost all existing class of antimicrobials [3,4]. This has become a global public health problem with substantial health and economic burden [3,5]. The annual cost of infections caused by antibiotic-resistant bacteria was estimated to be $4 to 5 billion [6]. This

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coupled with high mortality rate associated with the infection, substantial morbidity and extended length of hospital stay has necessitated the search for alternative antimicrobial agents with ability to kill or inhibit the growth of these resistant strains of microorganisms [4,6,7].

Medicinal Plants have served from time immemorial as a potential source of new therapeutic compounds and have been demonstrated in several literatures to possess significant antimicrobial activity against a wide range of microbial infections with little or no side effect [7–9]. Plants-derived compounds exhibit antimicrobial activity by mechanisms other than the well-studied conventional methods and may therefore have clinical value in the treatment of infections caused by resistant strains of microorganisms.

The plant, *Vernonia glaberrima* Welw. Ex O. Hoffm, is known in northern Nigeria as Shìwaákár-ján-gágári [10]. It is an erect shrub, 2 m high, found on hillside grassland in Guinea to Northern Nigeria, Western Cameroon and Central Africa to Angola belonging to the family, Asteraceae [10,11]. The plant has been reported to be used against malaria, migraine, psoric and dysmenorrhea [10,11]. Previous antimicrobial study of the methanol leaf extract of the plant has justified the use of the plant in traditional medicine as it was reported to contain bioactive principles with good antimicrobial activity [10]. This study is therefore aimed at evaluating the antimicrobial activity of the n-hexane, chloroform, ethyl acetate and n-butanol soluble fractions of the methanol leaf extract of *V. glaberrima* against some selected human pathogens with the view to identify the most potent fraction that can serve as lead for development of novel antimicrobial agent.

EXPERIMENTAL

Collection and identification of plant material. The whole plant material of *V. glaberrima* was collected from Nasarawa State, Northern-Nigeria in June 2012 during the rainy season. It was authenticated by Mallam U. S. Gallah of the herbarium section, Biological Sciences Department, Ahmadu Bello University, Zaria. A voucher specimen (No. 899) was deposited at the herbarium for future reference.

Preparation of extract. The leaves were removed, shade dried, pulverized, labelled and stored at room temperature in an airtight container prior to extraction. The powdered leaves (2500 g) were extracted with 70 % methanol using maceration method for 10 days with occasional shaking. The extract was evaporated *in vacuo* using rotary evaporator at 40 °C to obtain a gummy greenish product (400 g) subsequently referred to as the crude methanol leaf extract (ME). The crude methanol extract (150 g) was suspended in 600 mL distilled water and successively extracted with the organic solvent of increasing polarity (600 mL two times each) to obtain n-hexane (HF), chloroform (CF), ethyl acetate (EF), n-butanol (BF) and the aqueous (AF) soluble fractions, respectively.

Test organisms. The pathogenic microbes used in this study were obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital, Zaria. The organisms include eight bacterial species- Methicillin Resistant *Staphylococcus aureus (*MRSA), Vancomycin Resistant *Enterococci* (VRE), Methicillin Susceptible *Staphylococcus aureus* (MSSA), *Listeria monocytogenes, Helicobacter pylori, Campylobacter fetus, Pseudomonas fluorescens, Proteus vulgaris* and two fungal species- *Candida stellatoidea* and *Candida tropicalis.* These microorganisms were maintained on nutrient agar slant in a refrigerator at 4 °C.

Antimicrobial screening. Antimicrobial screening was carried out using the agar diffusion method as described by

Wahyuningrum *et al*. [12]. This was done by adding 0.1 mL of 1 mg/mL solution of the different fractions into a well bored by the use of a standard 6 mm cork borer on a Mueller Hinton agar plate already seeded with the standard 0.5 McFarland turbidity of the test organisms. The plates were then incubated at 37 ^{0}C for 24 h after which the plates were observed for the zone of inhibition of growth. The total diameter of zone of inhibition was measured with a transparent ruler and the result recorded in millimeters. A control tube containing sparfloxacin and fluconazole as positive controls were maintained.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentration (MBC/MFC). Minimum Inhibitory Concentration (MIC) of the fractions against the pathogens was determined by the Broth dilution method using serially diluted plant extracts/fractions as previously described [13]. The fractions were serially diluted into different concentrations ranging from 1, 0.5, 0.25, 0.125 and 0.063 mg/mL in nutrient broth. Then, into each of the tubes containing the fractions, 0.1 mL of broth culture of the test organism (1.5×10^8 CFU/mL) was added and the tubes incubated at 37ºC for 18-24 h. The MIC is the lowest or minimum concentration of the fraction in a tube with no visible growth of bacteria. Cultures from the tubes with no visible growth were sub-cultured into a fresh recovery media, Mueller Hinton agar, and incubated at 37^{o}C for 24 h, after which the plates of the medium were observed for colony growth. The lowest concentration at which no colony/turbidity was observed was interpreted as Minimum Bactericidal Concentration (MBC)/Minimum Fungicidal Concentration (MFC).

RESULTS

The results of the antimicrobial screening revealed that the isolates were significantly inhibited by the various fractions of the methanol extract of *V. glaberrima* with the exception of *L. monocytogenes, C. fetus, P. vulgaris* and *C. tropicalis*. The ethyl acetate fraction (EF) exhibited a remarkable antimicrobial activity when compared to the n-hexane, chloroform and n-butanol fractions. EF showed inhibition range of 25 – 30 mm, which was almost comparable to that of the standard antibiotic drug, Sparfloxacin having 29–37 mm. CF had 20 – 26 mm while HF and BF showed inhibition range of 20 - 22 mm. The antifungal drug, Fluconazole was active against *C. stellatoidea* and *C. tropicalis* with inhibition range of 32 - 34 mm. The Minimum Inhibitory Concentration (MIC) of the n-hexane, chloroform and n-butanol fractions was 0.25 mg/mL while that of ethyl acetate fraction ranged between 0.25-0.125 mg/mL against all the test microorganisms. The Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) of the n-hexane and n-butanol fractions was found to be 1 mg/mL, chloroform fraction had 0.5-1 mg/mL and the MBC/MFC of ethyl acetate ranged between 25-0.5 mg/mL as indicated in (Tables 1 – 3).

DISCUSSION

The frequency of life threatening diseases due to resistant microorganisms has increased worldwide with possibility of an impending post-antibiotic era where common infections could kill [3,14]. This research is therefore timely as attempt was made to identify plant derived antimicrobial agents with therapeutic value against resistant pathogens with least or no adverse effects. This is necessary so as to influence the preservation, conservation and sustainable management of the plant for use in drug research and development [9].

The results obtained from this study showed that the various fractions of the methanol leaf extract of *V. glaberrima* possess good antibacterial and antifungal activities against the tested pathogens. This

concurs with the result of an earlier study on the antimicrobial activity of the crude methanolic extract of the plant [15]. Related species, *Vernonia amygdalina*, *Vernonia adoensis* and *Vernonia polyanthes* have also been reported to exhibit good antimicrobial activity [4,16–18].

Table 1: Zone of inhibition (mm) of the fractions of *V. glaberrima* against the test microbes

Test Organisms	HF	CF	EF	BF	SF	FZ
Methicillin Resistant *S. aureus*	21	23	25	20	32	0
Vancomycin Resist. *Enterococci*	20	24	28	22	0	0
S. aureus	22	26	30	20	37	0
L. monocytogenes	0	0	0	0	30	0
Helicobacter pylori	21	25	29	20	35	0
Campylobacter fetus	0	0	0	0	0	0
P. fluorescens	20	20	26	21	29	0
Proteus vulgaris	0	0	0	0	32	0
Candida Stellatoidea	20	22	28	20	0	32
Candida tropicalis	0	0	0	0	0	34

Key= HF=Hexane fraction; CF=Chloroform fraction; EF=Ethyl acetate fraction; BF=n-Butanol fraction; SF= Sparfloxacin; FZ= Fluconazole

Table 2: Minimum Inhibition Concentration of the fractions against the test microbes

Fraction	Concn.	MRSA	VRE	SA	HP	PF	CS
	1 mg/mL	-	-	-	-	-	-
	0.5 mg/mL	-	-	-	-	-	-
HF	0.25 mg/mL	O*	O*	O*	O*	O*	O*
	0.125 mg/mL	+	+	+	+	+	+
	0.063 mg/mL	++	++	++	++	++	++
	1 mg/mL	-	-	-	-	-	-
	0.5 mg/mL	-	-	-	-	-	-
CF	0.25 mg/mL	O*	O*	O*	O*	O*	O*
	0.125 mg/mL	+	+	+	+	+	+
	0.063 mg/mL	++	++	++	++	++	++
	1 mg/mL	-	-	-	-	-	
	0.5 mg/mL	-	-	-	-	-	
EF	0.25 mg/mL	O*	-	-	-	O*	
	0.125 mg/mL	+	O*	O*	O*	+	
	0.063 mg/mL	++	+	+	+	++	
	1 mg/mL	-	-	-	-	-	-
	0.5 mg/mL	-	-	-	-	-	-
BF	0.25 mg/mL	O*	O*	O*	O*	O*	O*
	0.125 mg/mL	+	+	+	+	+	+
	0.063 mg/mL	++	++	++	++	++	++

Key: **-** = No turbidity (No growth); O*=MIC; +=Turbid (light growth); ++=Moderate turbidity
MRSA = Methicillin-resistant *S. aureus*; VRE = Vancomycin-resistant *Enterococci*; SA = *S. aureus*; HP = *Helicobacter pylori*; PF = *P. fluorescens*; CS = *Candida stellatoidea*

Table 3: Minimum Bactericidal/Fungicidal Concentration of the fractions against the test microbes

Fraction	Concn.	MRSA	VRE	SA	HP	PF	CS
HF	1 mg/mL	O*	O*	O*	O*	O*	O*
	0.5 mg/mL	+	+	+	+	+	+
	0.25 mg/mL	++	++	++	++	++	++
	0.125 mg/mL	+++	+++	+++	+++	+++	+++
	0.063 mg/mL	++++	++++	++++	++++	++++	++++
CF	1 mg/mL	--	--	--	--	O*	O*
	0.5 mg/mL	O*	O*	O*	O*	+	+
	0.25 mg/mL	+	+	+	+	++	++
	0.125 mg/mL	++	++	++	++	+++	+++
	0.063 mg/mL	+++	+++	+++	+++	++++	++++
EF	1 mg/mL	-	-	-	-	-	-
	0.5 mg/mL	O*	-	-	-	O*	-
	0.25 mg/mL	+	O*	O*	O*	+	O*
	0.125 mg/mL	++	+	+	+	++	+
	0.063 mg/mL	+++	++	++	++	+++	++
BF	1 mg/mL	O*	O*	O*	O*	O*	O*
	0.5 mg/mL	+	+	+	+	+	+
	0.25 mg/mL	++	++	++	++	++	++
	0.125 mg/mL	+++	+++	+++	+++	+++	+++
	0.063 mg/mL	++++	++++	++++	++++	++++	++++

Key: - = No Colony growth; O*=MBC/MFC; +=Scanty Colonies growth; ++= Moderate Colonies growth; +++= Heavy Colonies growth. MRSA = Methicillin-resistant *S. aureus*; VRE = Vancomycin-resistant *Enterococci*; SA = *S. aureus*; HP = *Helicobacter pylori*; PF = *P. fluorescens*; CS = *Candida stellatoidea*

In a study on the phytochemical screening of the methanolic leaf extract of *Vernonia glaberrima*, the presence of flavonoids, saponins, tannins, steroids, glycosides, terpenes and alkaloids in the crude methanolic extract of the plant has been reported [10]. The broad antimicrobial activity recorded in this study may be due to the presence of these potent secondary metabolites. These phytochemicals have been demonstrated in several literatures to exert antimicrobial activities via a number of diverse mechanisms like intercalation of DNA, destruction of cell membrane, inactivation of microbial adhesions and enzymes [19].

The ability of the fractionation process to concentrate an active compound in some fractions more than the other as a result of varying solubility of the phytochemicals may explain why the ethyl acetate fraction of the extract exhibited a remarkable activity than the other three fractions [4,14,19]. The antimicrobial activity of the fractions of *V. glaberrima* was in the order EF>CF>BF & HF which indicates that the active phytochemical constituents with antimicrobial property are found in a moderately polar solvent. Hence, these constituents (flavonoids, saponins, etc.) have been reported to have antimicrobial activity [20].

Further studies will be conducted on the mechanisms underlying the antimicrobial activity of the various fractions of the plant, especially the most potent ethyl acetate fraction. In addition, the activity of the various fractions of the plant extract will be investigated *in vivo* as the scope of the current study was only limited to the *in vitro* evaluation of the antimicrobial activity of the fractions. In a similar vein, inhibitory activity of the fractions against microbial biofilms and drug efflux pumps will be investigated as co-formulation of conventional antibiotics with plant-derived adjuvants may serve as a novel mechanism for countering antimicrobial resistance.

Conclusion. The potential use of *Vernonia glaberrima* as an antimicrobial agent is

promising as the fractions exhibited excellent antimicrobial activity against the selected human pathogens with the ethyl acetate fraction being the most potent. This validates the ethno-medicinal use of the plant in the management of microbial infections.

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