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Full Length Research Paper

Evaluating the Methanol Leaf Extract of Vernonia glaberrima Welw. Ex O. Hoffm (Asteraceae) for its Antimicrobial Properties

Abdullahi, M. I.[1]*, Uba, A.[1], Alebiosu, C. O.[1], Alhassan, A. M. [1], Mode, S.[1], A. Umar [1], Nasir, I.[1], S. S. Bello[1], Ibrahim, Z. Y. Y.[2], Yunusa, A.[3] and Yusuf, A. J.[1]

[1]Department of Pharmaceutical and Medicinal Chemistry, Usmanu Danfodiyo University, Sokoto, Nigeria.
[2]Department of Pharmacognosy and Ethnopharmacy, Usmanu Danfodiyo University, Sokoto, Nigeria.
[3]Department of Pharmacology, College of Health Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.

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The emergence of multi-drug resistant microbes necessitates a continuous search for newer, effective antimicrobial agents. The crude methanol leaf extract of *Vernonia glaberrima* was screened for its antimicrobial activity against pathogenic microorganisms including Methicillin resistant *Staphylococcus aureus,* Vancomycin resistant *enterococci, Listeria monocytogenes, Staphylococcusau reus, Helicobacter pylori, Campylobacter fetus, Proteus vulgaris, Pseudomonas fluorescens, Candida tropicalis* and *Candida stellatoidea* using agar diffusion and broth dilution methods. The result of the susceptibility test showed the extract (400µg) inhibited the growth of Methicillin-resistance *S. aureus*, Vancomycin resistant *enterococci*, *S. aureus, H. pylori, P. fluorescens* and *C. stellatoidea* with mean zone of inhibition range of 18 to 29 mm; the most susceptible organism was *S. aureus* (29 mm) and the least was the fungus, *C. stellatoidea* (18 mm). No activity was observed against *L. monocytogenes, C. fetus, P. vulgaris and C. tropicalis*. Sparfloxacin (5µg/ml) the standard antibacterial drug, had inhibitory activity against all the test organisms except *H. pylori, P. flourescens, C. tropicalis* and *C. stellatoidea* while the standard anti- fungal drug, Fluconazole (5µg/ml), showed activity only on the two fungi species, *C. tropicalis* and *C. stellatoidea*. The Minimum inhibitory concentration (MIC) and the minimum bactericidal/fungicidal concentration (MBC/MFC) ranges for the extract were 5 to 20 mg/ml and 10 to 40 mg/ml, respectively. The results of this study suggest that the methanol leaf extract of *V. glaberrima* contains bioactive principles that have good antibacterial and antifungal activity, validating the antimicrobial use of the plant in traditional medicine.

Key words: *Vernonia glaberrima*, extract, antimicrobial, MIC, MBC, evaluation.

INTRODUCTION

The World Health Organization (WHO) has long recognized antimicrobial resistance (AMR) as a growing

*Corresponding author. E-mail: musawara2000@yahoo.com; Tel: +2348033344511.

global health threat, and one of the important global strategies is a continuous search for effective antimicrobial agents (WHO, 2012).

AMR is driven by both appropriate and inappropriate use of anti-infective medicines for human, animal health and food production, together with inadequate measures to control the spread of infections (Goosens et al., 2005; Mathew et al., 2007; Orzech and Nichter, 2008; WHO, 2014). More than 2 million people become infected with bacteria that are resistant to antibiotics each year in the United States out of which about 23,000 die as a direct result of these infections, while many more people die from other complications related to antibiotic resistance (Centers for Disease Control and Prevention CDC, 2013). Although, the data for developing countries such as Nigeria is not very comprehensive, the mortality figures are expectedly higher (WHO, 2014).

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 (Silver and Bostian, 1993). Natural products have been the most significant source of drugs and drug leads in history (Newman and Cragg, 2007). 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 (Ahmed and Beg, 2001). *V. glaberrima* Welw. Ex O. Hoffm (Asteraceae), Shìwaákár-ján-gágári (Hausa language - N. Nigeria) is an erect shrub, 2 m high, found on hillside grassland in Guinea to Northern Nigeria, Western Cameroon and Central Africa to Angola (Burkill, 1997). It was reported to be used against malaria, migraine, psoric and dysmenorrhoea (Burkill, 1997). The leaves decoction is also employed traditionally in Nassarawa State, Northern Nigeria for treating pain, inflammation, vertigo and microbial infections (Personal communication). Preliminary phytochemical screening, acute toxicity studies and antidiabetic property of the plant have been reported (Abdullahi et al., 2015).

The aim of this study was to evaluate the antimicrobial properties of *V. glaberrima* using agar diffusion and broth dilution methods.

MATERIALS AND METHODS

Collection and Identification of Plant material

The whole plant material of *V. glaberrima* was collected from Nasarawa State 8°32'N 8°81'E, Northern-Nigeria in June, 2012 during the rainy season. It was authenticated by Mallam U. S. Gallah of the herbarium section of 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 air- tight 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 400 g of a gummy greenish product (16.0%w/w) subsequently referred to as the crude methanol leaf extract VGLE.

Test organisms

Clinical isolates of the test organisms 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*, *L. monocytogenes, H. pylori, C. fetus, S. aureus, P. vulgaris, P. flourescens, C. tropicalis* and *C. stellatoidea.*

Antimicrobial evaluation

Susceptibility test

Antimicrobial activity of the methanol leaf extract of *V. glaberrima* was determined through susceptibility test using agar diffusion method (Sardari et al., 1998) . The stock concentration of the extract (40 mg/ml) was prepared by dissolving 0.4 g of the extract in 10 ml dimethyl sulfoxide (DMSO). Mueller Hinton agar and the growth medium, was prepared according to manufacturer's instructions, and sterilized for 15 min at 121°C. Nutrient broth and sabraud dextrose broth were used for antibacterial and antifungal evaluations respectively. The test organisms were inoculated and incubated for 24 h for bacteria and 48 h for fungi. The solidified sterile medium contained in petri dish was seeded with 0.1 ml standard inoculum of the test microbe at 45°C. 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. Discs containing blank extraction solvents served as control. The reference antibacterial drug Sparfloxacin (5µg) and antifungal drug Fluconazole (5µg) were used as a positive control. The extract and standard drugs were allowed to diffuse into the agar and incubated overnight. All incubations were done 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 millimeters (mm).

Minimum inhibitory concentration (MIC)

The MIC of the extract was carried out using Broth dilution method (Volekobia et al., 2001). Mueller Hinton broth was prepared of which 10 ml was dispensed into test tubes, sterilized at 121°C for 15 min, and allowed to cool; Mc-Farland's standard turbidity scale number 0.5 was prepared. Dilution of the organism suspension was done continuously using sterile normal saline until the turbidity

Table 1. Susceptibility test (Zone of Inhibition) of Methanol Leaf Extract of *V. glaberrima.*

Test organisms	Zone of Inhibition (mm)		
	VG (400 μg/ml)	Sparfloxacin (5μg/ml)	Fluconazole (5μg/ml)
Staphylococcus aureus	29	35	-
Methicillin Resistant *S. aureus*	20	35	-
Vancomycin Rest *Enterococci*	25	32	-
Listeria monocytogenes	-	37	-
Helicobacter pylori	24	-	-
Campylobacter fetus	-	38	-
Proteus vulgaris	-	32	-
Pseudomonas fluorescens	22	-	-
Candida tropicalis	-	-	37
Candida stellatoidea	18	-	35

Key: mean zone of inhibition measured in millimeter (mm), - = activity not detected, VG= *V. glaberrima* extract.

Table 2. Minimum inhibitory concentration of methanol leaf extract of *V. glaberrima* against the test organisms.

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

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

matched that of Mc-Farland's scale by visual comparison. At that point, the concentration of the test microbe was about 1.5 x 10^8cfu/ml. Two-fold serial dilution of the extract in the sterile broth was made to obtain the concentrations of 40, 20, 10, 5 and 2.5 mg/ml, respectively. 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°C for 15 mins, 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°C for 24 h; 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 (Volekobia et al., 2001).

RESULTS AND DISCUSSION

The results of susceptibility test, MIC and MBC/MFC of the methanol leaf extract of *V. glaberrima* are shown in Tables 1, 2 and 3, respectively. The methanol leaf extract of the plant exhibited antimicrobial activity against the susceptible organisms tested.

Table 3. Minimum bactericidal/fungicidal concentration of methanol leaf extract of *V. glaberrima* against the test organisms.

Test Organisms	Minimum bactericidal/fungicidal concentration (mg/ml)				
	40	20	10	5	2.5
Staphylococcus aureus	-	-	OA	+	++
Methicillin Resistant *S.aureus*	OA	+	++	+++	+++
Vancomycin Rest *Enterococci*	-	OA	+	++	+++
Listeria monocytogenes	-	-	-	-	-
Helicobacter pylori	-	OA	+	++	+++
Campylobacter fetus	-	-	-	-	-
Proteus vulgaris	-	-	-	-	-
Pseudomonas fluorescens	OA	+	++	+++	+++
Candida tropicalis	-	-	-	-	-
Candida stellatoidea	OA	+	++	+++	+++

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

Susceptibility test result showed inhibition range of 18 to 29 mm against *Methicillin resistance S. aureus*, *Vancomycin resistant Enterococci*, *S. aureus*, *H. pylori*, *P. fluorescence* and the anti- fungi, *Candida stellatoidea*; The crude 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 (Tania et al., 2000). No activity was observed against *L. monocytogenes*, *C. fetus*, *P. vulgaris* and *Candida. tropicalis*.

The standard antibacterial drug, Sparfloxacin 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*. *S. aureus* was the most sensitive organism (29 mm) and *C. stellatoidea* was the least (18 mm). The MIC and the Minimum Bactericidal/Fungicidal Concentration (MBC) ranges for the extract were 5 to 20 mg/ml and 10 to 40 mg/ml, respectively. The low MIC value suggests that the extract has good antimicrobial activity. The highest bactericidal activity was recorded on *S. aureus* at 10 mg/ml while *C. stellatoidea* has the least activity at 40 mg/ml.

S. aureus is the most dangerous of all the many common *staphylococcal* bacteria causing skin infections, pneumonia, breast infections, endocarditis, and other fatal infections in humans (Baorto et al., 1994). The extract also exhibited inhibitory effect on Methicillin-resistant *S. aureus* (MRSA) which is a bacterium that is resistant to many antibiotics causing life-threatening bloodstream infections, pneumonia, skin and surgical site infections (Bush, 1989; CDCP, 2015).

Helicobacter pylori has been implicated in peptic and stomach ulcer (NLM, 2014) as there is a strong association between infection with *H. pylori* and gastric ulcers (Kosunen et al., 2005; Kusters et al., 2006), and antibacterial drugs are included in the prescribed gastric and peptic ulcer treatment regimen (Sung et al., 1995). The ability of the crude extract of *V. glaberrima* to inhibit this pathogenic microbe suggests its potential relevance in the management of peptic and gastric ulcers. *P. fluorescens* is an unusual cause of disease in humans, and usually affects patients with compromised immune systems (Gershman et al., 2008)

Sparfloxacin, the standard drug is a broad spectrum antibiotic with better activity on gram positive bacteria; it exhibited high activity on the tested gram positive bacteria (MRSA, VRE, *L. monocytogenes,* and *S. aureus*) but was not active against the gram negative bacteria, *H. pylori* and *P. flourescens.* It is noteworthy that the extract inhibited the growth of *H. pylori* and *P. flourescens*, the only bacteria that were resistant to Sparfloxacin.

The ability of the extract to exert its antimicrobial effects may be attributed individually or collectively to the presence of flavonoids, alkaloids, saponins, tannins, steroids and terpenes detected in the plant (Abdullahi et al., 2015). Other *Vernonia* species such as *V. amygdalina* have been reported to possess antimicrobial activity (Ibrahim et al., 2008) though with varying degrees; the result of this work for example, showed *V. glaberrima* exhibited better inhibition of *S. aureus* (5mg/ml) than 12.5 mg/ml by *V. amygdalina* (Ibrahim et al., 2008).

These observed activities will give impetus for the isolation and characterization of bioactive constituents responsible for the observed activity, thereby aiding the

attempt to combat these pathogens which are causative agents of serious and life threatening infections.

Conclusion

The results of this study suggest that the methanol leaf extract of *V. glaberrima* contains bioactive principles that have good antibacterial and antifungal activity, validating the antimicrobial use of the plant in traditional medicine. Further work aimed at the isolation of the bioactive principles is being pursued.

Conflicts of interest

The authors have none to declare.

REFERENCES

Abdullahi MI, Uba A, Yaro AH, Maxwell O, Yusuf AJ, Kabir S, Alhasan AM, Umar A, Bello SS, Nasir I (2015). Phytochemical Screening, Acute Toxicity study and Evaluation of Antidiabetic properties of the methanolic leaf extract of *Vernonia glaberrima* (Asteraceae). J. Pharm. Chem. Biol. Sci. 3(2):169-177.

Ahmed I, Beg AZ (2001). Antimicrobial and Phytochemical studies on 45 Indian medicinal plants against multi-drug resistant *Stapylococcus aureus*. J. Ethnopharmacol. 74:113-123.

Baorto EP, Baorto D, Windle ML, Lutwick LI (1994). *Staphylococcus aureus* infection**.** Available at: http://emedicine.medscape.com/article/971358-overview.

Burkill HM (1997). The useful plants of West Tropical Africa. Royal Botanical Gardens, Kew. 4:275.

Bush LM (1989). Best alternative to Vancomycin for serious Methicillin-Resistant *Staphylococcus aureus* infections; Let's just say it. Clinical Infectious Diseases*.* Available at: http://cid.oxfordjournals.org/content/53/9/965.full**.**

Centers for Disease Control and Prevention (CDC) (2013). Antibiotic/antimicrobial resistance: Threat Report 2013. Available at: http://www.cdc.gov/drugresistance/threat-report-2013/

Centers for Disease Control and Prevention (CDCP) (2015). Methicillin-resistant *Staphylococcus aureus*. Available at: http://www.cdc.gov/mrsa/.

Gershman MD, Kennedy DJ, Noble-Wang J, Kim C, Gullion J, Kacica M, Jensen B, Pascoe N, Saiman L, McHale J, Wilkins M, Schoonmaker-Bopp D, Clayton J, Arduino M, Srinivasan A (2008). "Multistate outbreak of *Pseudomonas fluorescens* bloodstream infection after exposure to contaminated heparinized saline flush prepared by a compounding pharmacy". Clin. Infect. Dis. 47(11): 1372–1379.

Goosens H, Ferech M, Stichele VR, Elseviers M (2005). Outpatient antibiotic use in Europe and association with resistance: a crossnational database study. Lancet 365(9459):579-587.

Kosunen TU, Seppala K, Sarna S, Aromaa A, Knekt P, Virtamo J, Salomaa-Rasanen A, Rautelin H (2005). Association of Helicobacter pylori IgA antibodies with the risk of peptic ulcer disease and gastric cancer. World J. Gastroenterol. 11(43):6871-6874.

Kusters JG, van Vliet AH, Kuipers EJ (2006). Pathogenesis of *Helicobacter pylori* Infection. Clin. Microbiol. Rev. 19(3):449-90.

Ibrahim T, Ajala L, Adetuyi F, Jude OB (2008). Assessment of the antibacterial activity of V. amygdalina and O. gratissimum leaves on selected food borne pathogens. Int. J. Third World Med. 8:2.

Mathew AG, Cissell R, Liamthong S (2007). Antibiotic resistance in bacteria associated with food animals: a United States perspective of livestock production. Foodborne Pathog. Dis. 4(2):115-133.

Newman DJ, Cragg GM (2007). Natural Products as Sources of New Drugs over the Last 25 years. J. Nat. Prod. 70:461-477.

Orzech KM, Nichter M (2008). From Resilience to Resistance: Political Ecological Lessons from Antibiotic and Pesticide Resistance. Annu. Rev. Anthropol. 37:267-282

Sardari SA, Gholameraza RG, Daneshtalab M (1998). Phytopharma-ceuticals. Part 1: antifungal Activity of Selected Iranian and Canadian plants. Pharm. Biol. 36:180-188.

Silver LL, Bostian KA (1993). Discovery and development of new antibiotics: the problem of antibiotic resistance. Antimicrob. Agents Chemother. 37:377-383.

Sung JJ, Chung SC, Ling TK, Yung MY, Leung VK, Ng EK, Li MK, Cheng AF, Li AK (1995). Antibacterial treatment of gastric ulcers associated with Helicobacter pylori. N. Engl. J. Med. 332(3):139-42.

Tania MAA, Axréia FS, Mitzi B, Telma SMG, Elza AS, Artur SJ, Carlos LZ (2000). Biological Screening of Brazilian Medicinal Plants. Mem. Inst. Oswaldo Cruz, Rio de Janeiro 95(3):367-373.

U.S. National Library of Medicine, NLM (2014). Helicobacter Pylori Infections. *Medline Plus*. Available at: http://www.nlm.nih.gov/medlineplus/helicobacterpyloriinfections.html

Volekobia A, Kostalova D, Sochorova R (2001). Isoquinoline alkaloid from *Mahonia aquifolium* stem bark is active against *Malsseizia* species. Folia Microbiol. 46:107-111.

World Health Organization, WHO. (2012). The evolving threat of antimicrobial resistance: Options for action. Available at: http://apps.who.int/medicinedocs/en.

World Health Organization, WHO (2014). Antimicrobial resistance: global report on surveillance. Available at: http://www.who.int/drugresistance/document/surveillance/en/.