ORIGINAL PAPER

Antimicrobial and antioxidant activity of ethanolic leaf extract derived from *Voacanga africana*

ABSTRACT

The antimicrobial activity of the ethanolic leaf extract of *Voacanga africana* was evaluated using standard methods. The phytochemical profile and antioxidant activity were also assessed using routine procedures. Dried and pulverized leaves of *V. africana* were extracted with ethanol. The result of the phytochemical screening revealed the presence of some bioactive compounds; tannins, flavonoids, phenols anthraquinones, saponins, phlobatanins, cardiac glycosides and terpenoids. The crude leaf extracts exhibited antimicrobial activity at concentrations of 250mg/ml-500mg/ml with a growth inhibitory zone of 1-6 mm for *Klebsiella* sp, 3-11mm for *Staphylococcus* sp, 9-13mm for *Escherichia coli* and 11-14mm for *Pseudomonas* sp. For the fungal isolates, the extract elicited a zone of inhibition of 6-11mm for *Penicillium* sp, 11-20 for *Aspergillus* sp. and 6-10 for *Fusarium* sp. The ethanolic leaf extract of *Voacanga africana* showed significant antioxidant activity using the DPPH test. These findings suggested that the ethanolic leaf extract of *V. africana* has potent antimicrobial and antioxidant activity.

KEY WORDS: antimicrobial, antioxidant activity, ethanolic leaf extract, phytochemical

INTRODUCTION

Finding healing powers in plants has been an ancient idea. Across the world, people have long applied poultices and imbibed infusions of hundreds, if not thousands, of indigenous plants (Cowan, 1999). However, since the discovery of antibiotics in the 20th century, the use of plant derivatives as antimicrobials has been virtually nonexistent. It has been reported that on average, two or three antibiotics derived from microorganisms are launched each year (Lampinen, 2005). Generally, antimicrobials are substances of natural, semi synthetic or synthetic origin that kills or inhibits the growth of microorganisms but causes little or no damage to the host (Khamaneh, 2016). After a downturn in that pace in recent decades, the pace is again quickening as scientists realize that the effective life span of any antibiotic is limited, leading to investigation and study of other sources of anti-infectives, especially of plant extracts (Talib,2011).

The use of plant extracts, as well as other alternative forms of medical treatments, is enjoying great popularity in the late 1990s (Cowan, 1999). Some of the phytochemicals which include; alkaloid,flavonoids, glycosides, phenols, phlobatanins, saponins, tannins had been found in crude extracts of some plant species, called medicinal plants (Okwu, 2001; Ano and Ubochi, 2007). Among these plants is a tropical shrub called *Voacanga Africana* which belongs to the family of Apocynaceae. It is an abundant, deciduous, mesophytic, sapwoody, perennial, aborescent shrub of the primary and secondary rain forest, within the tropical rain forest especially in Nigeria and the Guinea savannah wood belt.

The mature *V. africana* is known to grow up to a height of about 6m, not more than 10m, with low widely spreading crown, distributed mainly in West Africa from Senegal to the Sudan and South Angola (Iwu, 1993). It is known locally as kokiyar in hausa, pete-pete in igbo, Kirongasi in swahili and Ako- Dodo in yoruba. The leaves are opposite obovate and acuminate, dark green and glossy and usually stalkless. Flowers are white borne in axiliary or terminal loosely branch glabrous inflorescence.

Spherical, mottled green fruit occurs mainly in pairs, with seeds wrapped in yellow pulp. The plant is used to treat leprosy, diarrhea, generalized oedema, and convulsion in children and as infant tonic (Iwu 1993).

A decoction of the stem bark and root is used to treat mental disorders and the latex is applied to carious teeth. The decoction of the bark is considered analgesic, and is added to embrocating mixtures used as pastes during fracture repair. Root and bark decoctions are also used to treat cardiac spasms. The fruit decoction is used as a disinfectant and the leaf decoctions to treat asthma in children (Neuwinger, 2000). Seeds of *V. africana* contain medicinally useful phytochemicals, such as alkaloids, anthranoids, anthraquinones, cardiac glycosides, phenols, phlobatanins and tannins. These substances are antimicrobial and could be extracted for bacterial and fungal diseases management, pharmaceutical exploits, research in microbiology, biotechnology and general medicine (Duru and Onyedinike, 2010). The purpose of this study is to further investigate this plant, with a view to determining the antimicrobial and antioxidant activity of the leaf extracts in *in vitro* Culture.

MATERIALS AND METHODS

Plant Material Collection and Authentication

The leaves of *V. africana* were collected from the growing tree in a forest at Ikpoba Hill Benin City in the month of February, 2023. The plant was taken to the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City and were identified by Dr. J. O. Erhabor.

Test Organisms

Four clinical bacterial isolates; *Klebsiella* sp., *Staphylococcus* sp., *Escherichia coli*, *Pseudomonas* sp. and the test Fungi- *Penicillium* sp., *Aspergillus* sp. and *Fusarium* sp. were collected from University of Benin Teaching Hospital, Benin city, Edo state, Nigeria. They were separately sub-

cultured and the pure culture re-cultured on Nutrient Agar and Potato Dextrose Agar media, respectively and stored at 40^{o}C.

Preparation of extract

The fresh leaves were washed with distilled water, shade dried and pulverized. The leaves of *V. africana* were thoroughly washed with distilled water to remove debris and contaminants, they were then air-dried to crisp and then homogenized using a blender. The homogenized leaves were soaked in 95% ethanol for 48hrs, after which the leaf suspension was filtered. Concentration of the filtrate was done using a rotary evaporator set at the temperature of 40°C in order to separate the solvent and eventually to obtain the crude extract. The extract was filtered with Whatman No 1 filter paper. The crude extract obtained was stored at 2-8 °C until further use.

Phytochemical screening

The extracts were qualitatively analyzed for the presence of tannins, flavonoids, phenols, anthraquinones, saponins, phlobatannins, cardiac glycosides and terpenoids according to procedures previously described by Trease and Evans (1989).

Standardization of the test bacterial isolates

Prior to exposure of the test bacterial isolates to the several extract concentrates, the log phase or standardized culture of the test bacterial isolate was prepared using the procedure described by Idu *et al.* (2017) and Dunkwu-Okafor *et al.*, (2020).

Microbial Susceptibility Test

The agar well diffusion procedure as described by Radhika, *et al*. (2008), Asowata *et al.* (2013), Idu *et al.*, (2014) and Dunkwu-Okafor *et al.*, (2020) was utilized in determining the susceptibility of the test microbial cultures to differing extract concentrates. Five (5) wells, 8mm each were made on solidified Mueller Hinton agar (MHA) plates and Sabouraud dextrose agar (SDA) media plates, respectively with the aid of a sterile cork borer. Zero point two (0.2) ml of the log phase culture of the test microorganisms: *Klebsiella* sp. *Staphylococcus* sp. *Escherichia coli and Pseudomonas* sp. were seeded on the surface of the freshly prepared MHA agar plate while *Penicillium* sp. *Aspergillus* sp. and *Fusarium* sp. were seeded on the Potato Dextrose Agar (PDA) medium, using swab stick. The cut agar discs were removed with the aid of sterile forceps. Concentrations of 35.5g/ml, 75g/ml, 125g/ml, 250g/ml and 500g/ml of the extracts were separately introduced into separate cavities. Two (2) control holes were set up, one filled with gentamicin and the other filled with clotrimazole, to serve as positive control for the bacterial and fungal cultures respectively. The plates were incubated at 37^{o}C for 24 hours and 96 hours respectively for the bacterial and fungal isolates. The observed zones of inhibition were measured using transparent metric ruler.

Free radical scavenging activity

This was determined using the DPPH method (Ohinishi *et al*., 1994; Oke and Hamburger, 2002). Briefly, 0.1mM ethanol DPPH (Diphenyl-Picryl-hydraxyl) solution was added to different concentrations of the extract with gentle shaking. Triplicate measurements of the optical density (OD) change was conducted 10 minutes later with a spectronic-20 spectrophotometer at a wavelength of 517nm. The scavenging activity was measured as the decrease in absorbance of the samples versus DPPH standard solution.

RESULT

The crude leaf extracts exhibited antimicrobial actvivity at concentrations of 250mg/ml-500mg/ml with zone of inhibitions of 1-6mm for *Klebsiella* sp, 3-11mm for *Staphylococcus* sp, 9-13mm for *Escherichia coli*, 11-14mm for *Pseudomonas* sp, 6-11 for *Penicillium* sp, 11-20 for *Aspergillus* sp and 6-10 for *Fusarium* sp. At the highest concentration (250 µg/ml) of the extract of *V. africana*, the percentage decrease in DPPH absorbance was 61.33%. On the other hand, the extract produced a percentage decrease in DPPH absorbance of 25.3% at the lowest concentration used (50µg/ml).

Phytochemical screening

Table 1: Phytochemical Analysis of Ethanol Leaf Extracts of *Voacanga africana*

Parameters	Remark
Tannins	+
Flavenoids	+
Phenols	+
Anthraquinones	+
Saponins	+
Phlobatannins	+
Cardiac glycosides	+
Terpenoids	+

Key: + = present; - = absent

Microbial susceptibility test

Table 2: The zones of inhibition at 35.5mg/mL-500mg/mL, minimum inhibitory concentration and minimum bactericidal concentrations of bacterial and fungal isolates on ethanolic leaf extracts of *Voacanga africana* at 24hours and 96hours respectively.

Isolates	MIC	MBC	Zone of Inhibition				
			100%	50%	25%	12.5%	6.25%
			500mg/ml	250mg/ml	125mg/ml	75mg/ml	35.5mg/ml
Klebsiella sp.	250mg/ml	500mg/ml	6	1	-	-	-
Staphylococcus sp	125mg/ml	500mg/ml	11	6	3	-	-
Escherichia coli	250mg/ml	500mg/ml	13	9	-	-	-
Pseudomonas sp.	250mh/ml	500mg/ml	14	11	-	-	-
Penicillium sp	250mg/ml	500mg/ml	11	6	-	-	-
Aspergillus sp	250mg/ml	500mg/ml	20	11	-	-	-
Fusarium sp	250mg/ml	500mg/ml	10	6	-	-	-

Table 3: The zone of inhibition of the Bacterial and Fungal isolates on standard antibiotics gentamicin and clotrimazole respectively.

Isolates	Gentamicin	Clotrimazole
Bacteria		
Klebsiella sp.	22	-
Staphylococcus sp	19	-
Escherichia coli	23	-
Pseudomonas sp.	20	-
Fungi		
Penicillium sp		20
Aspergillus sp		27
Fusarium sp		21

Antioxidant activity

Table 4: The % Inhibition of DPPH against 50mg/ml -250mg/ml concentrations of ethanolic leaf extract of *Voacanga africana*

	Ethanolic Leaf Extract				
	50µg/ml	100µg/ml	150µg/ml	200µg/ml	250µg/ml
SRSA	25.56	39.84	42.85	46.60	48.86
HRSA	40.47	57.55	59.75	64.99	68.13
DPPH	44.51	53.12	56.16	57.84	61.33

Key:

SRSA: Superoxide radical scavenging activity

HRSA: Hydroxyl radical scavenging activity

DPPH: Dipheny-picryl-hydrazyl

DISCUSSION

It was observed that the leaf extracts contained some phytochemicals such as tannins, flavonoids, phenols, anthraquinone, saponins, phlobatanins, cardiac glycosides, and terpenoids. The extracts had antibacterial activity against *Escherichia coli, Pseudomonas* sp, *Klebsiella* sp. and *Staphylococcus* sp. It also demonstrated antifungal activity against *Penicillium* sp, *Aspergillus* sp and *Fusarium* sp as such suggesting that the leaf extracts of *V. africana* has a broad spectrum antimicrobial potency. The antibacterial and the antifungal potency may be due to the presence of the above listed phytochemicals. This result agrees with the report of (Duru and Onyedineke 2010).

The susceptibility test result showed that all the clinical isolates were susceptible to the ethanolic leaf extract of *Voacanga africana*. The susceptibility of the isolates to the leaf extract was less potent when compared with standard antibiotics gentamicin and clotrimazole for the bacterial and fungal isolates respectively agreeing with the report of Duru and Onyedineke (2010). Generally, the reduced efficacy of the extracts, relative to the standard antibiotics, used in the study may be due to the fact that they are still crude and require further purification (Duru and Onyedineke 2010).

All the selected clinical isolates had a minimum inhibitory concentration of 250mg/ml with the exception of *Staphylococcus* sp which had a minimum inhibitory concentration of 125mg/ml. The minimum bactericidal (MBC) and fungicidal (MFC) concentrations was 500mg/ml for all the bacteria and fungi isolates.

The results of the present study would suggest that the ethanolic extract of the leaves of *Voacanga africana* has significant antioxidant activity. The DPPH test provides information on the reactivity of test compounds with a stable free radical. Based on its odd electron, 2, 2- diphenyl-picryl-hydrazyl radical (DPPH) gives a strong absorption band at 517nm in visible spectroscopy (deep violet colour). As the electron becomes paired off in the presence of a free radical scavenger, the absorption varnishes, thus the resulting decolorization is stoichiometric with respect to the number of electrons taken up. The scavenging activity of the ethanol leaf extract of *Voacanga africana* may be due to the presence of phytochemical compounds such as flavonoids which are generally known to be good antioxidants according to the report by Olaleye *et al* (2004).

CONCLUSION

Voacanga africana leaves contained potentially medicinally useful phytochemicals such as tannins, flavonoids, phenols, anthraquinone, saponins, phlobatanins, cardiac glycosides, and terpenoids. These anti-nutrients could be extracted because of their antimicrobial and antioxidant activity

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