

# INVESTIGATION OF ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF THE METHANOLIC EXTRACT OF THE LEAVES OF VOACANGA AFRICANA STAPE. (APOCYNACEAE) AND PSYDRAX SUBCORDATA (DC.) BRIDSON (RUBIACEAE)

Bamisaye Olaofe Oyawaluja<sup>\*1</sup>, Josephine Aribiba Williams<sup>1</sup>, Herbert A. B. Coker<sup>1</sup>

<sup>1</sup>Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Lagos

\*Corresponding Author: bamoyawa01@gmail.com, +2348033999408, +2348023534167

## ABSTRACT

### Background

The importance of medicinal plants in traditional and modern health care practices and in providing clues to new areas of research is now well recognised. Bacterial resistance has been increasingly reported worldwide and is one of the major causes of failure in the treatment of infectious diseases. Plant derived antioxidant could be useful as food additives to prevent food deterioration and also to impart human health and prevent oxidative stress associated diseases. Natural-based products, including plant secondary metabolites (phytochemicals), can be exploited to ameliorate the problem of microbial resistance and oxidative stress. The choice of plants (Voacanga africana and Psydrax subcordata) for this study was based on folkloric use and literature search to authenticate the tradition claims.

### Methods

The leaves methanolic extract of Voacanga africana and Psydrax subcordata were investigated for antimicrobial and antioxidant activity. The antimicrobial activities were also evaluated using the agar well diffusion method while the antioxidant activities of the plants were evaluated using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, nitric oxide scavenging assay, lipid peroxidation scavenging assay, total antioxidant capacity assay, total phenolic content, total flavonoid content and ferric reducing power assay.

### Results

The extracts of Voacanga africana and Psydrax subcordata significantly inhibited DPPH radical with an IC<sub>50</sub> value of 69.2±11.6µg/ml and 106.9±5.3µg/ml

respectively), inhibited lipid peroxidation (IC<sub>50</sub> value of 65.7±13.5µg/ml and 75.4±11.6 µg/ml respectively) and also inhibited the accumulation of nitrite in vitro (IC<sub>50</sub> value of 75.1±11.7µg/ml and 80.1±12.9µg/ml respectively). The plant extracts yielded 47.8±0.07 and 65.2±0.04mg Gallic acid equivalent/100g phenolic content respectively, 2.8±0.05mg and 28.2±0.05mg quercetin equivalents/100g flavonoid content respectively, total antioxidant capacity of 160.6±0.05 and 110.7±0.05mg ascorbic equivalent/100g and reducing power of 0.2±0.07 and 0.2±0.05µg/ml, respectively. The antimicrobial assay showed that Voacanga africana has activity against gram positive and gram negative bacteria organisms, which include Staphylococcus aureus (34.0±1.2), Bacillus subtilis (37.5±0.0) and Pseudomonas aeruginosa (35.5±0.9) at 50µg/ml. Psydrax subcordata was only active against Staphylococcus aureus (20.8±0.5). However, no antifungal activity was observed for both plants.

### Conclusion

Voacanga africana and Psydrax subcordata possess antioxidant and antimicrobial activities and these results therefore provide evidence to support their traditional uses. The observed antioxidant potentials and phenolic content of the extract suggest that the methanolic leaves extract of Voacanga africana and Psydrax subcordata is a potential source of natural antioxidants.

**Keywords:** antimicrobial, antioxidant, phytochemical, Voacanga africana, Psydrax subcordata

## INTRODUCTION

The medicinal properties of some plants have been implicated and explored in both traditional and orthodox medicine. The potency of these plants has been traced to some active principles; alkaloids, flavonoids, glycosides, etc. referred to as secondary metabolites. These active ingredients are contained in leaves, stem bark, roots, seed, of higher plants. Phytochemical screening of these organs helped to advance the course of science and medicine<sup>1</sup>. Medicinal plants are plants containing inherent active ingredients tending or used to cure disease or relieve pain. Plants represent a huge storehouse of drugs: they produce more than 10,000 different compounds to protect themselves from predators. These compounds could be potential drugs<sup>2,3</sup>. Historically, plant medicines were discovered by trial and error. Just as people learnt to exploit plants for food, so they learnt to use plants as medicine<sup>4</sup>. For example, our ancestors noticed that aches and pains went away when they drank tea made from the bark of a willow tree, *Salix* sp. Later, scientists discovered that willow bark contains salicylic acid, the active ingredient in aspirin.

Studies in the use of plant extracts for control of diseases have shown the importance of natural chemicals as possible sources of

non-phytotoxic and easily biodegradable alternative fungicides and antibiotics<sup>5</sup>. Virtually all native plant species are used for the treatment of one ailment or another. These involve the traditional medicinal use for despoil, preventive, curative and magical purposes<sup>6</sup>. Some chemical substances in the plant tissues brought about the medicinal value of such drug plants.

Antimicrobial was derived from the Greek words; anti (against), mikros (little) and bios (life) and refers to all agents that act against microbial organisms- bacteria (antibacterial), fungal (antifungal), etc. An antimicrobial is any substance of natural, synthetic or semisynthetic origin that kills or inhibits the growth of microorganisms, but cause little or no damage to the host. Antimicrobial resistance is one of the most serious public health threats that results mostly from the selective pressure exerted by antibiotic use and abuse<sup>7,8</sup>. Due to this increasing resistance, many antimicrobial agents are losing their efficacy<sup>9,10,11</sup>. Consequently, the therapeutic options for the treatment of infections have become limited or even unavailable. According to the World Health Organization (WHO), infectious diseases are these second causes of death around the world<sup>12</sup>. Therefore, it is necessary to search and develop new alternative compounds to

ameliorate the problem of microbial resistance.

Free radical production occurs continuously in all cells as part of normal cellular function. However, excess free radical production originating from endogenous or exogenous sources might play a role in many diseases. Antioxidants prevent free radical induced tissue damage by preventing the formation of radicals, scavenging them, or by promoting their decomposition.

An antioxidant can be defined as: "any substance that, when present in low concentrations compared to that of an ox-disable substrate, significantly delays or inhibits the oxidation of that substrate"<sup>13</sup>. The physiological role of antioxidants, as this definition suggests, is to prevent damage to cellular components arising as a consequence of chemical reactions involving free radicals. In recent years, a substantial body of evidence has developed supporting a key role for free radicals in many fundamental cellular reactions and suggesting that oxidative stress might be important in the pathophysiology of common diseases including atherosclerosis, chronic renal failure, and diabetes mellitus.

The most important free radicals in many disease states are oxygen derivatives, particularly superoxide and the hydroxyl radical. Radical

formation in the body occurs by several mechanisms, involving both endogenous and environmental factors. Although free radical production occurs as a consequence of the endogenous reactions and plays an important role in normal cellular function, it is important to remember that exogenous environmental factors can also promote radical formation, such as; ultraviolet light will lead to the formation of singlet oxygen and other reactive oxygen species in the skin<sup>14</sup>, atmospheric pollutants such as ozone and nitrogen dioxide lead to radical formation and antioxidant depletion in the Broncho alveolar lining fluid, and this may exacerbate respiratory disease<sup>15,16</sup>.<sup>17</sup> and cigarette smoke contains millimolar amounts of free radicals, along with other toxins<sup>18</sup>.

*Voacanga africana* Stapf. Commonly known as Pepe pete (Igbo, Nigeria) and Ako dodo (Yoruba, Nigeria), is a small flowering plant in the dogbane family, Apocynaceae, which grows to 6m in height. It is native to tropical Africa. The small tree has leaves that are up to 30 cm in length, and the tree produces yellow or white flowers. It is propagated by seed. Tea made from the leaf is said to be a strengthening potion that relieves fatigue and shortness of breath. It is also used to prevent premature childbirth and to treat painful hernias and menstruation. It is

used in many areas of Africa to treat heart troubles<sup>19</sup>.

*Psydrax subcordata* (DC.) Bridson of Rubiaceae (also known as *Canthium subcordatum*) is a fruit bearing plant, commonly housing colonies of ants and considerably varying in size according to the area in which it grows. More commonly, only a small tree up to 15metres tall, in Sierra Leone specimens up to 30metres tall. . The tree is harvested from the wild for its timber which is locally used. It is naturally found in swamp forest at elevations around 1,500meters, often on open sites such as old farmlands<sup>20</sup>. It is propagated by seed. *Psydrax subcordata* has been used for the management of haemorrhoids, stomach ulcer, piles, abdominal pains, dyspepsia, enteritis, stomach aches, gastritis, heartburns and intestinal complaints<sup>21</sup>.

This present study aims to investigate the antioxidant and antimicrobial activity of the methanolic extracts of the dried leaves of *Voacanga africana* and *Psydrax subcordata*.

## MATERIALS AND METHODS

### Collection and Identification of plants

*Voacanga africana* plant was collected from the wild in Osun state and *Psydrax subcordata* was

collected from University of Lagos, Akoka Campus. Plants were identified at the herbarium of the Department of Botany, Faculty of Science, University of Lagos. Voucher numbers for the plants given were; LUH 8162 and LUH 7561, for *V. africana* and *P. subcordata* leaves respectively.

### Processing of plants material

The leaves of *V. africana* and *P. subcordata* were handpicked and air dried for weeks. Drying was completed in the oven at 40°C. The leaves were grounded using a grinding machine.

### Extraction of plant material

*V. africana* powdered leaves 1,886g and 250g of *P. subcordata* powdered leaves were macerated with absolute methanol separately, with occasional mixing and changing of solvent every 4days for 3weeks. The extracts were filtered using Whatman no.1 and concentrated using rotary evaporator. Further drying was done in the oven at 40°C and dried extracts were kept in a cool dry place at room temperature.

### PHYTOCHEMICAL SCREENING

The phytochemical screenings were carried out on the methanol extracts, using standard procedures to identify the following secondary metabolites; alkaloids, flavonoids, tannins,

cardiac glycosides, saponins, anthraquinones and reducing sugars as described by Trease and Evans<sup>22</sup>.

### ANTIMICROBIAL ACTIVITY

The antimicrobial activity of *V. africana* and *P. subcordata* was evaluated using the agar well diffusion assay to determine the susceptibility of the selected microorganisms to the extracts of the aforementioned plants. Gram positive organisms (*Bacillus subtilis* and *Staphylococcus aureus*), Gram negative organisms (*Pseudomonas aeruginosa* and *Escherichia coli*) and fungi (*Aspergillus niger*, *Aspergillus flavus*, *Candida albican* and *Penicillium spp*) were used in this assay. Levofloxacin, a broad spectrum antibiotic that is effective against both gram negative and gram positive bacterial was used as the reference drug at a concentration of 50µg/ml, 25µg/ml, 12.5µg/ml and 6.25µg/ml. Bifonazole was also used as the reference drug for the fungi, of the same concentration as levofloxacin.

#### Preparation of test organism

The organisms used were bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus*, and fungi (*Aspergillus niger*, *Candida albican*, *Candida pseudotropicalis* and *Trichophytum rubum*) obtained from previously subcultured plates of various assay organisms in

pharmaceutical microbiology laboratory of the Department of Pharmaceutical Technology and Pharmaceutics, College of Medicine, Idi-araba, Lagos state. For bacteria: The assay organisms include gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) and gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*). From previously sub-cultured plates of various assay organisms, the different organisms were calibrated using sterile normal saline (15ml). Mc-Farland 0.5 standard was used as the turbidity standard.

For fungi: Spores from the fungi (*Candida albican*, *Aspergillus niger*, *Aspergillus flavus* and *Penicillium spp.*) were used to prepare spore suspension in a tween-saline mixture. The medium is made up of 0.05% of tween 80 in normal saline (USP). The spore load was adjusted to 10<sup>8</sup> spore forming unit (SFU) per ml, using a serial dilution technique.

#### Determination of antimicrobial activity of the extracts

The antimicrobial activities of the different extracts were evaluated by the agar well-diffusion method<sup>23</sup>. Mueller-Hinton and Sabouraud Dextrose plates were inoculated by streaking the swab over the entire agar surface using bacterial suspensions and fungi suspensions. The plates were allowed to dry at room

temperature. Using a sterile cork borer, 10mm diameter wells were bored in the agar. The antimicrobial activity of the extracts was checked by introducing 300, 150 and 75mg/ml concentrations into triplicate wells. An additional well in each plate was filled with the solvent 5% methanol as a control. Concentrations of 50, 25, 12.5 and 6.25µg/ml were prepared for the standard reference drugs: levofloxacin for bacteria and bifonazole for fungi, used as positive controls. The culture plates were allowed to stand on the bench for 30 min at room temperature and were incubated at 35°C for 24hours. After 24hours, the antimicrobial activity of the extracts and the antibiotics were determined. Zones of the inhibition around each of the extracts and the antibiotics were measured to the nearest millimeter<sup>24, 25</sup>.

### DETERMINATION OF ANTIOXIDANT ACTIVITY

#### Preparation of stock solution

Extracts of 0.1g of each were dissolved respectively in 20ml of absolute methanol. 1ml of the resulting stock solution was transferred to a test tube and diluted to 10mls with distilled water.

#### Lipid peroxidation assay

Exactly, 0.1ml of the diluted stock

solution was transferred into a test tube, 0.1ml of liver homogenate was added and then 1.6ml of Tris-HCl buffer was also added. To the resulting solution, 0.5ml of 10% Trichloroacetic acid (TCA) was added, 0.5ml of 0.75% thiobarbituric acid (TBA) was also added to make the total volume up to 2.8mls. The solution was then boiled for 1hour at 90°C. It was then cooled in ice and centrifuged for 15 minutes at 3000rpm. Absorbance of the supernatant was read against the blank at 582nm. Ascorbic acid was used as standard<sup>26</sup>. The scavenging effect was calculated using the expression

$$\% \text{ inhibition} = [A_0 - A_1] \times 100 / A_0$$

Where  $A_0$  is the absorption of the blank sample and  $A_1$  is the absorption of the extract

### Nitric oxide scavenging activity

A 4ml sample of the plant extract and standard solution of different concentration (25, 50, 75 and 100µg/ml) were taken and transferred to separate test tubes respectively. 2mls of 1% sodium nitroprusside (5mM in phosphate buffer). The resulting solution was left to incubate for 2 hours and 30 minutes at 30°C to complete the reaction. After incubation, 0.5ml of grease reagent (1% Sulphanilamide, 0.1% naphthylethylene diamine dihydrochloride in 2%  $H_3PO_4$ ) was then added. It was then incubated again for 30 minutes and

absorbance was read at 540nm<sup>27</sup>.

Ascorbic acid was used as standard. The percentage (%) inhibition activity was calculated from the following equation:  
 $\% \text{ inhibition} = [(A_0 - A_1) / A_0] \times 100$ .  
 Where,  $A_0$  is the absorbance of the Control and  $A_1$  is the absorbance of the extract or standard.

### DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity

An aliquot of 0.5ml of extract in ethanol (95%) at different concentration (25, 50, 75 and 100µg/ml) was mixed with 0.2ml of reagent solution (0.004g of DPPH reagent in 100ml methanol). The control contained only of DPPH while methanol was used as blank. The mixture was vigorously shaken and left to stand at room temperature for 30 mins. The absorbance was read at 517 nm<sup>28,29</sup>. The scavenging effect was calculated using the expression;  
 $\% \text{ inhibition} = [A_0 - A_1] \times 100 / A_0$

Where  $A_0$  is the absorption of the blank sample and  $A_1$  is the absorption of the extract

### Estimation of total phenolic compound

1ml of the diluted stock was transferred into a test tube, and 0.4ml of Folin- Ciocalteu reagent added. The resulting solution was incubated for 2 hours at 30°C to complete the reaction. After this, 4ml of sodium carbonate solution

(7.5% w/v) was added and made up to 10ml, further incubated for 90 mins at room temperature. The absorbance of the solution was measured at 565nm. The concentration of total phenol was expressed as Gallic acid equivalent (GAE) (mg/g of dry mass) which is a commonly used reference value<sup>30</sup>.

### Estimation of total flavonoid content

2ml of the diluted stock was transferred into a test tube, 2ml of 2% Aluminium chloride in ethanol was added. The resulting mixture was incubated at room temperature for 1hour and the absorbance of the reaction mixture was measured at 510nm. The calibration curve was obtained using different concentrations of quercetin in methanol<sup>31</sup>.

## RESULTS

### Phytochemical Screening

Phytochemical screening revealed the presence of alkaloids, tannins, saponins, reducing sugar, flavonoid and cardiac glycoside in Voacanga africana extract and cardiac glycoside, saponin and flavonoid in Psydrax subcordata extract.

Table 1: Summary of results obtained from phytochemical screening of the Plant extracts.

## Keys

+ = present - = Absent

*Pseudomonas aeruginosa*. *Psydrax subcordata* showed activity only against *Staphylococcus aureus*. The extracts demonstrated no antifungal activity against *Aspergillus niger*, *Aspergillus flavus*, *Candida albican* and *Penicillium spp.*

and cardiac glycoside in *Voacanga africana* extract and cardiac glycoside, saponin and flavonoid in *Psydrax subcordata* extract. The extracts; *V. africana* and *P. subcordata* (at doses of 300mg/mL, 150mg/ml and 75mg/ml, each) showed considerable growth inhibition against tested organisms. Levofloxacin at all concentrations showed significant inhibition against all the tested bacterial except *Escherichia coli* while Bifonazole only showed considerable growth inhibition on the fungus, *Aspergillus niger*. Among the extracts, *P. subcordata* had the highest percent growth inhibition ( $27.5 \pm 1.4$ ) against *Staphylococcus aureus* at 300mg/ml, ( $23.5 \pm 1.6$ ) at 150mg/ml and ( $19.0 \pm 0.6$ ) at 75mg/ml. *V. africana* was active against *Pseudomonas aeruginosa* and *Bacillus subtilis* organisms while *P. subcordata* showed inhibition only against *Staphylococcus aureus*. The sensitivity test result showed that the extracts, were less potent than the standard drugs; Levofloxacin and Bifonazole, used in the study. Generally, the less potency of the extracts, relative to the standard drugs used in the study may be due to the fact that, they are still crude and require further purification. The leaves extract of *V. africana* had broad spectrum antibacterial activity against gram negative (*Pseudomonas aeruginosa*) and gram positive (*Bacillus subtilis* and

S/N	Test	<i>Voacanga africana</i> (leaves)	<i>Psydrax subcordata</i> (leaves)
1	<b>Test for sugars</b>		
	Molisch test	+	-
	Fehling's test	-	-
2	<b>Test for alkaloids</b>		
	Dragendoff test	+	-
	Mayer test	+	-
3	<b>Test for flavonoid</b>		
	Lead acetate	+	+
	Ferric chloride	+	+
4	<b>Test for tannins</b>		
	condensed tannin	+	-
	hydrolysable tannin	+	-
5	<b>Test for saponin</b>		
	frothing test	+	+
6	<b>Test for cardiac glycoside</b>		
	Keller -Killiani test	+	+
	Lieberman's test	+	+
	Salkowski test	+	+
7	<b>Test for anthraquinone</b>		
	Free anthraquinone	-	
	Combined anthraquinone	-	

## Antimicrobial activity

*Voacanga africana* had activity against *Bacillus subtilis*, *Staphylococcus aureus* and

## DISCUSSION

Phytochemical screening revealed the presence of alkaloids, tannins, saponins, reducing sugar, flavonoid

**Table 2: Zone of inhibition of levofloxacin reference standard against tested bacteria**

Organisms	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml
Staphylococcus aureus	34.0 ± 1.2mm	29.0 ± 0.7mm	28.7 ± 0.5mm	22.0 ± 1.0mm
Bacillus subtilis	37.5 ± 0.3mm	35.3 ± 2.1mm	34.0 ± 2.5mm	29.7 ± 1.4mm
Pseudomonas aeruginosa	35.5 ± 0.9mm	31.3 ± 2.1mm	30.0 ± 1.4mm	20.0 ± 2.3mm
Escherichia coli	-	-	-	-

**Table 3: Zone of inhibition of the methanolic extract of V. africana against tested bacteria**

Organisms	300mg/ml	150mg/ml	75mg/ml	Solvent (5% methanol)
Staphylococcus aureus	20.8 ± 0.5mm	20.5 ± 0.5mm	-	-
Bacillus subtilis	20.5 ± 0.3mm	18.5 ± 0.9mm	17.5 ± 1.0mm	-
Pseudomonas aeruginosa	21.0 ± 0.6mm	20.0 ± 0mm	18.5 ± 0.9mm	-
Escherichia coli	-	-	-	-

**Table 4: Zone of inhibition of the methanolic extract of P. subcordata against tested bacteria**

Organism	300mg/ml	150mg/ml	75mg/ml	Solvent (5% methanol)
Staphylococcus aureus	27.5 ± 1.4mm	23.5 ± 1.6mm	19.0 ± 0.6mm	-
Bacillus subtilis	-	-	-	-
Pseudomonas aeruginosa	-	-	-	-
Escherichia coli	-	-	-	-

**Table 5: Zone of inhibition of bifonazole reference standard against tested fungi**

Organisms	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml
Aspergillus niger	29.0 ± 2.0mm	19.8 ± 0.3mm	19.5 ± 0.5mm	15.5 ± 0.5mm
Aspergillus flavus	-	-	-	-
Candida albican	-	-	-	-
Penicillium spp.	-	-	-	-

N/B: There was no inhibition for *P. subcordata* and *V. africana* extracts against the fungi organisms.

**Antioxidant activity**

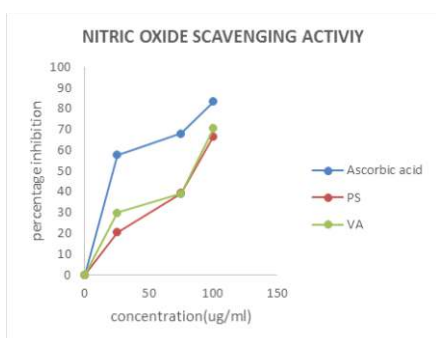
All extracts had significant DPPH radical scavenging activity, lipid peroxidation and nitric oxide scavenging activity. But, *Voacanga africana* showed more free radical scavenging activity than *Psydrax subcordata*. While *Psdrax subcordata* had higher phenolic and flavonoid values than *Voacanga africana* leaves extract.

**Table 6: Total flavonoids and phenolic**

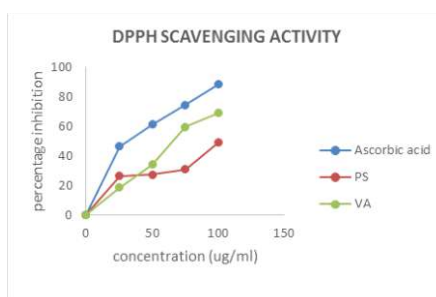
Plants	Total flavonoid (mg/100g)	Total phenolic (mg/100g)
<i>P. subcordata</i>	28.2 ± 0.05	65.2 ± 0.04
<i>V. africana</i>	2.8 ± 0.05	47.8 ± 0.07

**Table 7: Scavenging activity of *V. Africana*, *P. subcordata* and Ascorbic acid**

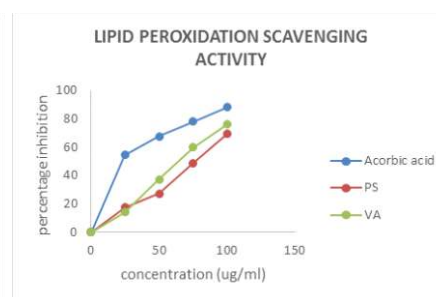
Samples	DPPH I <sub>c50</sub> (µg/ml)±SEM	NITRIC OXIDE I <sub>c50</sub> (µg/ml)±SEM	LIPID PEROXIDATION I <sub>c50</sub> (µg/ml)±SEM
Ascorbic acid	50 ± 8.1	46.8 ± 7.0	40.4 ± 7.2
<i>P. subcordata</i>	106.9 ± 5.3	80.1 ± 12.9	75.4 ± 11.6
<i>V. africana</i>	69.2 ± 11.6	75.1 ± 11.7	65.7 ± 13.5



**Fig.1: Nitric oxide scavenging activity of ascorbic acid and methanol extract**



**Fig.2: DPPH scavenging activity of ascorbic acid and methanol extract**



**Fig.3: Lipid scavenging activity of ascorbic acid and methanol extracts**

PS – *Psydrax subcordata*  
 VA – *Voacanga africana*



Staphylococcus aureus) organism. *P. subcordata* leaves extract was only active against *Staphylococcus aureus*. *Escherichia coli* were resistance to both the standard drug and extract samples. The extracts demonstrated no antifungal activity against *Aspergillus niger*, *Aspergillus flavus*, *Candida albican* and *Penicillium* spp. The antibacterial potency may be due to the presence of some active principles, like Phenols, Tannins Alkaloids, Flavonoid and Cardiac glycosides<sup>32,33</sup>. Plant phenolic constitutes one of the major groups of compounds acting as primary antioxidants or free terminators (Agarwal, 1989). Phenolics are able to scavenge reactive oxygen species due to their electron donating properties. The phenolic compounds such a phenolic acid and flavonoids are most important antioxidant food source. The quantitative analysis of phenolic acids and flavonoids by measurement UV absorption is well known<sup>34</sup>. In the present study, the total phenolic and flavonoids content of extract *Voacanga africana* and *Psydrax subcordata* leaves were analyzed. The total phenolic content was determined using Folin-Ciocalteu method, reported as Gallic acid equivalent. The total phenolic content was 47.8±0.07 and 65.2±0.04mg/100g of extracts respectively. Whereby the flavonoids content was 2.8±0.05 and 28.2±0.05mg of quercetin equivalence/100g of extracts respectively.

The extracts showed significant DPPH radical scavenging activity. The methanolic leaves extract showed concentration-dependent scavenging activities in a similar manner as ascorbic acid in the DPPH radical scavenging assay. Basically, a higher DPPH radical-scavenging activity is associated with a lower IC<sub>50</sub> value. From the result obtained, *V. africana* exhibited a higher radical scavenging activity than *P. subcordata*, when compared with the ascorbic acid standard. The IC<sub>50</sub> values (µg/mL) of *V. africana* and *P. subcordata* were 69.2±11.6 and 106.9±5.3 respectively and the reference drug ascorbic acid (50±8.1) suggest that the extracts have lesser ability to scavenge free radicals as compared to ascorbic acid.

Also, *V. africana* and *P. subcordata* exhibited good nitric oxide scavenging activity leading to the reduction of the nitrite concentration in the assay medium. The nitric oxide scavenging capacity was concentration dependent. *V. africana* and *P. subcordata* has a potent nitric oxide scavenging activity, when compared with the ascorbic standard, with IC<sub>50</sub> values of 75.1±11.7, 80.1±12.9 and 46.8±7.0 respectively. *V. africana* showed a more potent nitric oxide scavenging activity than *P. subcordata*.

The extracts *V. africana* and *P. subcordata* showed a very good

concentration-dependent inhibition of lipid peroxidation (IC<sub>50</sub> = 65.8±13.5µg/ml and 75.4±11.6 µg/ml) compared to the standard antioxidant ascorbic acid (IC<sub>50</sub> = 40.4±7.2µg/ml). This corroborates the observed potent hydroxyl radical (OH) radical scavenging activity of the extract and suggests that the extract may afford a protective effect against the damaging effects of free radical. *V. africana* produced a more protective effect than *P. subcordata*. It can be deduced that *V. africana* gave a better scavenging activity than *P. subcordata*.

## CONCLUSION

The present study has demonstrated that the crude extracts of *Voacanga africana* and *Psydrax subcordata* have considerable antioxidant and antimicrobial activities. The results of the bioassays on the selected medicinal plants therefore give scientific credence to their folkloric use. Based on the findings of this study, *Voacanga africana* has a higher antimicrobial and antioxidant effect than *Psydrax subcordata*.

The presence of plant secondary metabolites such as tannins, flavonoids, alkaloids, glycosides, saponins and phenols, accounts for the significant antioxidant and antimicrobial activities of the plants. Future work will attempt to isolate and characterize pure compounds responsible for their

activity.

## REFERENCES

1. Urquiag I and Leighton F (2000). Plant Polyphenol Antioxidants and Oxidative Stress. *Biological Research*; 33: 55 – 64.
2. King SR and Moran K (1996). Biological diversity, indigenous knowledge, drug discovery and intellectual property rights: creating reciprocity and maintaining relationships. *Journal of Ethnopharmacology*; 51 (1-3): 45-57.
3. Okigbo RN, Eme UE and Ogbogu S (2008). Biodiversity and Conservation of Medicinal and Aromatic Plants in Africa. *Biotechnology and Molecular Biology Reviews*; 3(6): 127-134.
4. Jamshidi-Kia F, Lorigooini Z and Amini-Khoei H.(2018). Medicinal Plants: Past History and Future Perspectives. *Journal of Herbmec Pharmacology*; 7 (1): 1-7.
5. Okigbo RN and Nmeka, IA (2005). Control of yam tuber rot with leaf extracts of *Xylopia aethiopica* and *Zingiber officinale*. *African Journal of Biotechnology*; 4(8): 804-807.
6. Osemeobo GJ. and Ujor G (1999). Non Wood Forest Products in Nigeria. Data collection and analysis for sustainable forest management in ACP countries- Linking national and international efforts. EC-FAO partnership programme. Federal Department of Forestry, Abuja, Nigeria: p 42.
7. Berdy J (2012). Thoughts and facts about antibiotics: Where we are now and where we are heading. *Journal of Antibiotic*. 65: 385–395.
8. French GL (2010). The Continuing Crisis in Antibiotic Resistance. *International Antimicrobial Agents*. 36: S3–S7.
9. Pitout JD and Laupland KB (2008). Extended-spectrum  $\beta$ -lactamase-producing Enterobacteriaceae: An Emerging Public-health Concern. *Lancet Infection Disease*; 8: 159–166.
10. Berger J, Diab-Elschahawi M and Blacky AA (2010). Matched Prospective Cohort Study on Staphylococcus Aureus and Escherichia Coli Bloodstream Infections: Extended Perspectives Beyond Resistance. *American Journal of Infection Contaminant*; 38: 839–845.
11. David MZ and Daum RS (2010). Community-associated methicillin-resistant Staphylococcus aureus: Epidemiology and clinical consequences of an emerging epidemic. *Clinical Microbiology Review*; 23: 616–687.
12. Mathers C, Fat DM and Boerma JT (2008). The Global Burden of Disease: 2014 Update. World Health Organization, Geneva, Switzerland, Scientific Research Publishing: 1-2
13. Halliwell B and Gutteridge JC (1995). The Definition and Measurement of Antioxidants in Biological Systems. *Free Radical Biological Medicine*; 18: 125–126.
14. McCaughan JS (1999). Photodynamic therapy—a review. *Drugs Aging*; 15: 49–68.
15. Cross CE, Vander Vliet A and O'Neill C (1994). Oxidants, Antioxidants, and Respiratory Tract Lining Fluids. *Environ Health Perspective*; 102:185–191.
16. Kelly FJ, Mudway I and Krishna MT (1995). The Free Radical Basis of Air Pollution Focus on Ozone. *Respiratory Medicine*; 89: 647–656.
17. Kelly FJ and Tetley TD (1997). Nitrogen dioxide Depletes Uric Acid and Ascorbic acid but not Glutathione from Lung Lining Fluid. *Biochemical Journal*; 325: 95–99.
18. Pourcelot S, Faure H and Firoozi F (1999). Urinary 8-oxo-7, 8-dihydro-2'-deoxyguanosine and 5- (hydroxymethyl) uracil in smokers. *Free Radical Research*; 30:173–180.
19. Graeme B. (2010). Garden of Eden: The Shamanic Use of Psychoactive Flora and Fauna and the Study of Consciousness (Book Review). *Australian Journal of Medical Herbalism*; 22(3): 107.
20. Burkill HM (1985). The Useful Plants of West Tropical Africa, vols. 1-3. 2<sup>nd</sup> edn. Royal Botanic Gardens.
21. Agyare C, Asase A, Lechtenberg M, Niehues M, Deters A and Hensel A (2009). An Ethnopharmacological Survey and In-vitro Confirmation of Ethnopharmacological Use of Medicinal Plants Used for Wound Healing in Bosomtwi-Atwima-Kwanwoma area, Ghana. *Journal of ethnopharmacology*; 125(3): 393-403.

22. Trease GE and Evans WC (1989). *Pharmacognosy*, 13th edition. ELBS/Bailliere Tindall, London: 345-346, 535-536, 772-773.
23. Murray PR, Baron EJ, Pfaller MA, Tenover FC and Tenover FC (1995). *Manual of Clinical Microbiology*. 6th ed. Washington DC: ASM Press. pp. 15-18
24. Lino A and Deogracios O (2006). The In-vitro Antibacterial Activity of *Annona senegalensis*, *Securidacca longipendiculata* and *Steanotaenia araliacea*- Ugandan Medicinal plants. *African Health Science*; 6(1): 31-35.
25. Wendakoon C, Calderon P and Gagnon D (2012). Evaluation of Selected Medicinal Plants Extracted in Different Ethanol Concentrations for Antibacterial Activity against Human Pathogens. *Journal of Medicinal and Aromatic Plants*; 1: 60-68.
26. Buege J and Aust DS (1978). Microsomal lipid peroxidation. In: Fleisscher S, Packer L, eds. *Methods in enzymology*. New York: Academic Press. 52: 302-311.
27. Alisi CS and Onyeze GOC (2008). Nitric Oxide Scavenging Sability of Ethyl Acetate Fraction of Methanolic Leaf Extracts of *Chromolaena odorata* (Linn.). *African Journal of Biochemistry Research*; 2: 145-150.
28. Cuendet M, Hostettmann K and Potterat O (1997). Iridoid Glucosides with Free Radical Scavenging Properties from *Fagraea blumei*. *Helvetica Chirurgica Acta* 80: 73-83.
29. Burits M and Bucar F (2000). Antioxidant Activity of *Nigella sativa* essential oil", *Phytotherapy Research*; 14: 323-328.
30. McDonald S, Prenzler PD, Antolovich M and Robards K (2001). Phenolic Content and Antioxidant Activity of Olive Extracts. *Food Chemistry*; 73: 73-84.
31. Chang C, Yang M and Wen H (2002). Estimation of Total Flavonoid Content in Propolis by Two Complementary Colorimetric Methods. *Journal of Food and Drug Analysis*; 10:178-182.
32. Ebena RUB, Madunagu BE, Ekpe ED and Otung IN (1991). Microbiological Exploitation of Cardiac Glycosides and Alkaloids from *Garcinia kola*, *Boreria ocymoides*, *Kola nitida* and *Citrus aurantifolia*. *Journal of Applied Bacteriology*; 71: 398-401.
33. Banso A and Adeyemo SO (2007). Phytochemical and Antimicrobial Evaluation of Ethanolic Extracts *Draclena Mannii* Bark. *Nigeria Journal of Biotechnology*; 18: 27-32.
34. Jurd L and Geissmao TA (1956). Absorption Spectra of Metal Complexes of Flavonoid Compounds. *Journal of Organic Chemistry*; 21: 1395-1401.