

ANTIOXIDANT AND ANTIMICROBIAL EFFECTS OF AQUEOUS AND ETHANOLIC EXTRACTS OF SELECTED MEDICINAL PLANTS FROM SOUTHERN NIGERIA

Abstract

Several indigenous and forgotten plants in the southern parts of Nigeria according to folklore possess many medicinal properties. Ethanol and aqueous extracts from six (6) of such plants namely *Boerhavia Diffusa*; *Datura Stramonium*; *Cassia Occidentalis*; *Scoparia Dulcis*; *Euphorbia Hirta* and *Baphia Nitida* from the southern Nigeria State of Akwa-Ibom were evaluated for both phytochemical composition and antimicrobial activities. The antioxidant activity of the extracts was determined by the DPPH inhibition method. Both the ethanolic and aqueous extracts of all the plants indicated the presence of flavonoids while the ethanolic extracts of all the plants indicated that tannins and alkaloids were present. *Cassia Occidentalis* and AOU, indicated relatively the highest DPPH scavenging activity at lowest concentrations (0.03125mg/ml) with AOU indicating 71.22mg/ml an equivalence of 87% performance with respect to ascorbic acid. Results showed that when tested against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Salmonella typhi* bacteria, the average inhibition zones ranged from 10.5-18.7mm, 10.5-18.3mm, 10-19mm respectively, with the ethanolic extracts, in general, showing the highest inhibition. Interestingly none of the plants showed inhibition against *Klebsiella pneumoniae*. With respect to fungus *Candida albican*, where there was activity, the inhibition zone was 10-15.3mm. Ethanolic extract of *Euphorbia Hirta* indicated the highest inhibition of 19mm against *Salmonella typhi*.

Keywords:-anti-oxidant, anti-microbial, inhibition, phytochemicals

1. Introduction

Knowledge of certain plants and herbs which possess a large collection of bioactive, chemically diverse compounds which can be applied towards the therapeutic regiment for the handling of diseases has gained favourable attention in recent years [1,2,3]. Furthermore, the prophylactic action coupled with the immune-enhancing effects of certain plants against several pathogens/microbial agents that plague humans and animals alike has prompted the need to screen for candidates termed as forgotten plants such that the exploitation of these does not constitute a threat to normal food production [4]. Moreover, to cope with the ever-increasing appeal for the use and consumption of natural food additives in everyday meals, multi-vitamin capsules and other health drinks, all of which are consumed in a bid to prevent diseases and boost immunity, it is therefore conceivable to examine the applicability and benefits of indigenous plant materials to mankind using modern scientific analysis methods

[5]. Phyto-medicine is a broadly accepted prophylactic and treatment option for an array of infections worldwide with certain identified bioactive compounds such as alkaloids, cardiac glycosides, flavonoids, glycoalkaloids, glycosides, phenols, tannins, terpenes and terpenoids being implicated in the therapeutic ability [6,7]. The function of these bioactive compounds, together with certain minerals within the plant are principally to avert the growth and proliferation of disease-causing pathogens, particularly some multi-drug resistant variants with the added benefit of producing marginal to no adverse side effects as well as essential nutritional supplements [8,9]. Studies show that the bioactive compounds of certain plant crude extracts such as *Waltheria indica* Linn work in symphony to stimulate anti-oxidative processes that hinders pathogen-induced free radicals [10,11]. Free radicals are metastable species which are generated as by-products of various biochemical reactions. Living organisms are equipped with a defense system (such as superoxide dismutase, catalases, glutathione peroxidase, glutathione reductase; or compounds such as glutathione, vitamins C and E, etc.) to neutralize these free radicals and other reactive oxygen species (ROS). If a balance exists between free radicals and the body's anti-oxidative defense system, the body is in healthy condition. However, depletion of antioxidant levels may lead to oxidative stress which can cause cellular and tissue damages, DNA mutation, cancer, neurodegenerative diseases (e.g. Parkinsonism, Alzheimer's disease), gastrointestinal ulcerogenesis, and even AIDS etc [11].

Several indigenous and forgotten plants in the southern parts of Nigeria possess many antimicrobial, anticancer, anti-oxidant, anti-cytotoxic, anti-malarial, anti-inflammatory, anti-viral, anti-allergic and anti-venom properties according to folklore. However, they are yet to receive the scientific validation required. Known only by the local names deep within the interior of their villages, this study aims at evaluating the antioxidant and antimicrobial effects of aqueous and ethanolic extracts of selected medicinal plants, herein referred to as:

Boerhavia Diffusa (Mfang Urukikot1= MU1), ***Datura Stramonium*** (Mfang Urukikot2= MU2), ***Cassia Occidentalis*** (Mfang Urukikot3= MU3), ***Scoparia Dulcis*** (Adung Urukikot leaves= AU1), ***Scoparia Dulcis*** (Adung Urukikot, stem = AU2), ***Euphorbia Hirta*** (AOU); and ***Baphia Nitida*** (Afuo Ubara = AFU)

2. MATERIALS AND METHODS

2.1 Plant Materials

The following Akwa Ibom native plants; *Boerhavia Diffusa* (MU1); *Datura Stramonium* (MU2); *Cassia Occidentalis* (MU3); *Scoparia Dulcis* (AU1(leaves), AU2 (stem); *Euphorbia Hirta* and *Baphia Nitida* were authenticated by Prof. Ndaeyo from the Department of Agriculture, University of Uyo. Fresh leaves/stem (AU2) were washed with distilled water and air-dried at room temperature in the dark for 72hrs. The dried leaves/stem of the plants were finely homogenized into a fine powder using a blender and kept in sealed plastic bags.

2.2 Extractions

2.2.1 Solvent: To 50g of each of the powdered plant was added to 200ml of ethanol and macerating for 48hrs. at room temperature. After filtration, the crude extracts were concentrated using a rotary evaporator under vacuum below 40°C and freeze-dried.

2.2.2 Aqueous: To 100g of each of the powdered plant was added 1L of distilled water and gently heated until it comes to boiling. The heat was turned off and the mixture was filtered after 30mins. After filtration, the crude extracts were concentrated using a rotary evaporator under vacuum below 40°C and freeze-dried.

2.3. Proximate Composition

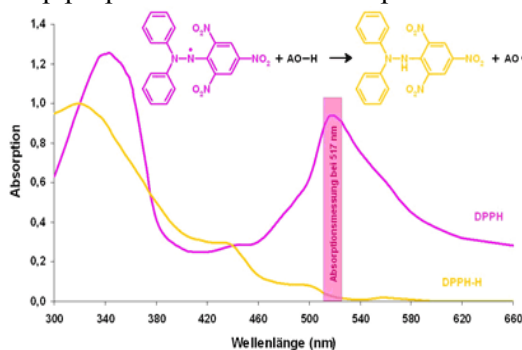
The proximate analysis (ash, moisture, crude fibre, crude lipid, crude protein, and carbohydrate) of the samples were determined using standard method [12]. All determinations were done in triplicate and reported in percentage.

2.4. Phytochemical screening

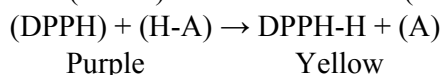
Preliminary qualitative phytochemical analysis was carried out to identify the secondary metabolites present in ethanolic and aqueous extracts of plants using standard methods [12].

2.5 Screening for antioxidant activity

The 2,2-Diphenyl-1- picrylhydrazyl (DPPH) free radical scavenging activity of the extracts were performed with some modifications using a UV/VIS spectrophotometer 1700 [13]. Absorbance of various concentrations (0.03125, 0.0625, 0.125, 0.25, 0.5mg/ml) of 1 ml of each plant extract were added to 3ml of 0.1mMol of DPPH methanol solution were measured. DPPH solution exhibit a deep purple color with an absorption maximum at 517nm.



The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as:



The extent of discoloration indicates the scavenging potentials of the antioxidant compounds. A lower absorbance value indicates the higher radical scavenging activity. Results were compared with the standard antioxidant (Ascorbic acid). The percentage inhibition was calculated as follows:

$$\text{Inhibition (\%)} = \frac{A_b - A_s}{A_b} \times 100$$

Where A_b = absorbance of the blank sample; A_s = absorbance of the extract

2.6 Antimicrobial activity

Bacterial infections are one of the leading causes of infectious diseases in developing countries. Drug resistant bacteria, particularly Staphylococci species, Klebsiella pneumoniae, and Pseudomonas species, are becoming commonplace in healthcare institutions and are possibly contributing to the occurrence of treatment failures [14], hence our evaluation of the various extracts. Recent trends in nanotechnology especially in the phyto-synthesis of metal nanoparticles have shown to have enhanced Antibacterial, Anticancer potential [15]. In our research, the agar well diffusion technique was used to determine the antimicrobial activity of the extracts on Mueller Hinton agar (MHA). The extracts were dissolved and diluted using 50%v/v dimethylsulphoxide (DMSO) to obtain different concentrations (250µg, 500µg, 750µg and 1000 µg) in 100µL. The microbial cultures used in this assay include bacteria (*Staphylococcus aureus*, *Streptococcus pyogenes*, *Klebsiella pneumoniae* and *Salmonella typhi*) and fungus (*Candida albicans*). 18–24hrs nutrient broth cultures of the organisms that has equaled the optical density of 0.5% McFarland solution were spread on MHA plates and labeled accordingly. Six wells, 8mm in diameter were made using sterile cork borer on each plate and the bases sealed with agar using micro pipette. 100µL of different concentrations of the extracts including the positive control (200µg of chloramphenicol and nystatin for bacteria and fungus respectively and the negative control (50% v/v DMSO) were introduced into the wells and incubated for 24 hrs. The experiment was done in triplicates for each of the extracts.

3 RESULTS

Results of the proximate composition indicating varying percentages of ash, moisture, fibre, lipid, protein and carbohydrate are shown in Table 1. The results for the phytochemical screening are shown in Table 2 with the presence of metabolites such as tannins, alkaloids, flavonoids, saponins, phenols, steroids, glycosides, Cardenolides, flobatanins, resins. Tables 3 and 4 show the results of the anti-oxidative activity and antimicrobial screening of the extracts respectively.

Table 1: Proximate Composition of the Samples

SAMPLES	% Ash	% Moisture	% Fibre	% Lipid	% Protein	% Carbohydrate
<i>Boerhavia Diffusa</i>	19.70	11.71	8.87	14.99	11.00	33.73
<i>Datura Stramonium</i>	16.05	11.04	17.09	18.16	27.71	9.95
<i>Cassia Occidentalis</i>	5.92	9.95	36.93	15.99	19.58	11.63
<i>Scoparia Dulcis(leaves)</i>	5.67	9.97	44.86	12.56	8.56	18.38
<i>Scoparia Dulcis(stem)</i>	2.75	9.03	41.02	10.93	19.15	17.12
<i>Euphorbia Hirta</i>	0.66	9.64	28.56	15.76	13.45	31.93
<i>Baphia Nitida</i>	4.13	10.27	22.06	13.61	18.85	31.08

156 Where *Boerhavia Diffusa* = (MU1), *Datura Stramonium* = (MU2), *Cassia Occidentalis* =
 157 (MU3), *Scoparia Dulcis*-leaves = (AU1), *Scoparia Dulcis*-stem = (AU2), *Euphorbia Hirta*
 158 =(AOU); and *Baphia Nitida* = (AFU)

159

160

161

162

163 **Table 2: Phytochemical Analysis of the Samples**

TEST	Ethanol Extracts							Aqueous Extracts						
	MU 1	MU 2	MU 3	AU 1	AU 2	AOU	AFU	MU 1	MU 2	MU 3	AU 1	AU 2	AOU	AFU
Tannins	+	+	+	+	+	+	+	-	-	-	+	+	+	+
Steroids	+	-	-	+	+	-	-	-	-	-	+	-	-	-
Saponins	+	-	+	-	-	+	-	-	-	-	-	-	+	+
Glycosides	-	+	-	-	+	-	-	+	+	-	+	-	-	-
Phenols	+	-	+	+	-	+	+	+	-	-	-	+	+	-
Alkaloids	+	+	+	+	+	+	+	-	-	+	+	-	-	+
Cardenolides	-	-	+	-	-	+	-	-	+	-	-	-	-	+
Terpenoids	+	+	+	+	-	+	+	+	+	+	+	+	-	-
Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phlobatanins	-	-	+	-	-	-	+	-	-	+	-	+	-	-
Resins	-	+	-	-	+	+	-	-	+	-	-	-	-	-

164 KEY: present = +, absent = -

165

166 **Table 3: Percentage Anti-oxidant activity at different concentrations (mg/ml) of the plant extract**
 167 **compared with ascorbic acid**

168

Sample	Concentration of plant extract (mg/ml)	Ethanol Extract % activity	Aqueous Extract % activity
<i>Boerhavia Diffusa</i> (MU 1)	0.5	65.78	53.76
	0.25	65.42	47.95
	0.125	58.61	41.82
	0.0625	52.57	43.42
	0.03125	37.94	36.65
<i>Datura Stramonium</i> (MU2)	0.5	27.27	49.61
	0.25	24.85	45.47
	0.125	09.81	38.88
	0.0625	-	31.75
	0.03125	23.74	25.99
<i>Cassia Occidentalis</i> (MU3)	0.5	33.69	47.45
	0.25	43.90	48.82
	0.125	37.53	54.76
	0.0625	25.20	62.93
	0.03125	53.58	67.22
<i>Scoparia Dulcis</i> (leaves)AU1	0.5	64.85	60.91
	0.25	64.46	58.74
	0.125	59.02	55.86
	0.0625	41.64	51.62
	0.03125	59.15	44.73
<i>Scoparia Dulcis</i> (stem)AU2	0.5	65.25	48.01
	0.25	47.48	57.16
	0.125	22.68	58.09
	0.0625	18.44	60.61
	0.03125	40.72	72.81
<i>Euphorbia Hirta</i> (AOU)	0.5	45.76	59.28
	0.25	51.86	45.89
	0.125	56.63	26.92
	0.0625	55.70	19.63
	0.03125	71.22	45.09
<i>Baphia Nitida</i> (AFU)	0.5	46.82	50.66
	0.25	32.89	40.05
	0.125	15.65	06.95
	0.0625	05.31	21.62

Ascorbic acid	0.03125	35.81	47.48
	0.5	79.03	
	0.25	76.82	
	0.125	79.40	
	0.0625	79.77	
	0.03125	81.24	

Table 4: Zone of inhibitory activity (mm) of different plant extracts against tested Microorganisms.

Plants	Conc. (µg)	Ethanollic Extract					Aqueous Extract				
		<i>S.a</i>	<i>S.p</i>	<i>K.p</i>	<i>S.t</i>	<i>C.a</i>	<i>S.a</i>	<i>S.p</i>	<i>K.p</i>	<i>S.t</i>	<i>C.a</i>
MU1	+ve	28.3	25.3	22.7	29.0	30.5	22.7	19.0	17.0	25.3	26.0
	250	15.3	13.0	-	-	-	-	-	-	-	-
	500	15.6	13.7	-	-	-	11.0	-	-	10.5	-
	750	17.5	15.0	-	11.5	-	12.7	11.3	-	11.0	-
	1000	18.7	15.3	-	13.0	-	13.7	13.7	-	12.0	-
MU2	+ve	28.7	21.7	21.3	28.3	30.3	25.3	20.0	18.7	24.3	25.7
	250	-	14.5	-	-	-	-	-	-	-	-
	500	-	14.7	-	10.0	-	10.5	-	-	-	-
	750	-	16.7	-	11.3	11.0	11.3	-	-	-	-
	1000	-	18.3	-	13.7	15.3	12.0	13.7	-	-	-
MU3	+ve	27.3	21.0	20.0	30.3	33.0	22.7	19.3	17.7	21.3	26.7
	250	10.5	-	-	-	-	-	-	-	-	-
	500	11.3	-	-	-	-	-	-	-	-	-
	750	13.0	-	-	-	-	-	-	-	-	-
	1000	14.7	-	-	-	-	-	-	-	-	-
AU1	+ve	28.7	23.3	20.7	30.3	29.0	23.3	23.3	18.7	20.0	26.0
	250	-	10.0	-	-	-	-	-	-	-	-
	500	10.0	11.0	-	-	-	10.0	-	-	-	-
	750	13.3	11.3	-	-	-	11.3	11.0	-	-	-
	1000	18.6	14.0	-	-	-	12.0	15.0	-	-	-
AU2	+ve	27.7	22.7	22.7	28.3	30.0	22.7	18.7	19.3	25.3	25.7
	250	-	-	-	-	-	10.0	-	-	-	-
	500	-	-	-	10.0	-	11.0	-	-	10.0	10.0
	750	11.0	-	-	10.7	-	12.7	12.0	-	11.3	10.7
	1000	14.0	-	-	13.0	-	13.7	13.0	-	12.0	13.3
AOU	+ve	29.3	22.3	21.3	29.7	29.7	22.7	19.0	17.7	26.7	26.0
	250	12.0	-	-	11.0	-	9.5	-	-	-	-
	500	11.5	12.0	-	14.0	-	10.67	10.0	-	-	-
	750	13.3	12.7	-	17.0	12.0	13.0	10.3	-	-	10.5
	1000	15.0	15.3	-	19.0	14.3	15.7	12.3	-	-	12.33
AFU	+ve	27.3	22.3	22.7	30.3	30.3	25.33	19.3	17.7	24.3	25.7
	250	-	-	-	-	-	-	-	-	-	-
	500	-	-	-	10.5	-	10.5	-	-	-	-
	750	11.0	-	-	12.7	-	12.0	10.5	-	10.0	-
	1000	15.3	-	-	14.0	-	14.7	12.3	-	11.0	-

S.a = *Staphylococcus aureus*; *S.p* = *Streptococcus pyogenes*; *K.p* = *Klebsiella pneumoniae*; *S.t* = *Salmonella typhi*; *C.a* = *Candida albicans*; Diameter of inhibition zone less than 6mm is represented as “-”.

4. DISCUSSION

4.1 Proximate analysis: of all the plant extracts revealed that extracts MU1 and MU2 had the highest overall ash (19.7, 16.05% respectively) and moisture (11.71, 11.04% respectively) contents (Table 1). The results also showed that the highest percentage content of fiber was found in extracts AU1 > AU2 > MU3 > AOU > AFU > MU2 > MU1 (44.86% - 8.87%). The varied distribution of Lipid and Protein amongst all extracts, for which both factors were highest in extract MU2 (18.16%, 27.71%) could be as a result of their distinctive biochemical diversities.

4.2 Phytochemical Screening: The preliminary qualitative phytochemical screening carried out showed that these plants contain vital secondary metabolites (Table 2). The bioactive compounds in medicinal plants have been reported to be the active principles responsible for the pharmacological potentials of medicinal plants [16]. The presence of these chemicals in the leaves and root of these plants justifies the local uses of these plants for the treatment of various ill conditions. Data obtained in this study revealed that flavonoids was present in all the samples while tannins was present in all but three aqueous (MU1, MU2, MU3) samples. This suggests that consumption of any of the studied plant extracts should aid in hepato-modulatory functions particularly in flushing out liver toxins, thwarting microbial growth and propagation, protect against inflammation, tumor and free radicals [17]. Saponins are natural glycosides that act as hypoglycemic, antifungal and serum cholesterol lowering agents in animals [18]. Saponins are essential elements in ensuring hormonal balance and synthesis of sex hormones [19]. Tannins are bitter polyphenolic compounds that hasten the healing of wounds. They also possess anti-diuretic and anti-diarrhea properties [20]. Terpenoids was present in all the extracts except ethanolic extract of AU2 and aqueous extracts of AOU and AFU. Terpenoids have been found to be useful in the prevention and therapy of several diseases, including cancer. Terpenoids are also known to possess antimicrobial, antifungal, anti-parasitic, antiviral, anti-allergenic, antispasmodic, anti-hyperglycemic, anti-inflammatory and immunomodulatory properties [21,22]. In all, aqueous extracts contained lesser metabolites.

4.3 Anti-oxidant activity:

The DPPH radical scavenging activity of Ascorbic acid in ethanolic solution regardless of concentration were virtually the same with an average of 79%. Figure 1 illustrates the inhibition trends observed when a range of concentrations (0.03125, 0.0625, 0.125, 0.25, 0.5mg/ml) of the ethanolic extracts are compared to ascorbic acid.

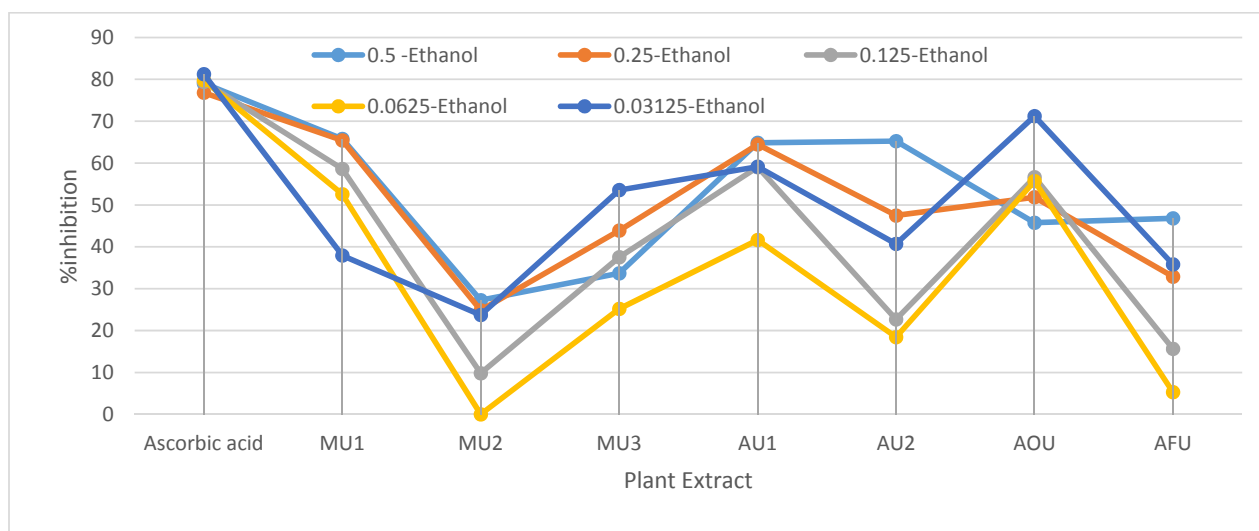


Figure 1: Inhibition trend of various concentrations of plant ethanolic extracts

Three ethanolic extracts (MU1, AU1 and AOU) all exhibited above 35% inhibition (equivalent to over 50% of ascorbic acid performance). Interestingly with respect to MU1, the % inhibition trends lower with increase concentration while with AOU the % inhibition increases with lower concentrations. The rest of the extracts had varying results and did not indicate a trend. With the exception of MU2, they had some concentrations that indicated inhibition above 35%. MU3 and AOU, indicated relatively the highest DPPH scavenging activity at lowest concentrations (0.03125mg/ml) with AOU indicating 71.22mg/ml an equivalence of 87% performance with respect to ascorbic acid. With respect to aqueous extract as indicated in figure 2, four of the plants indicated activity above 35% (MU1, MU3, AU1 and AU2). AU1 had least changes over the concentration ranges.

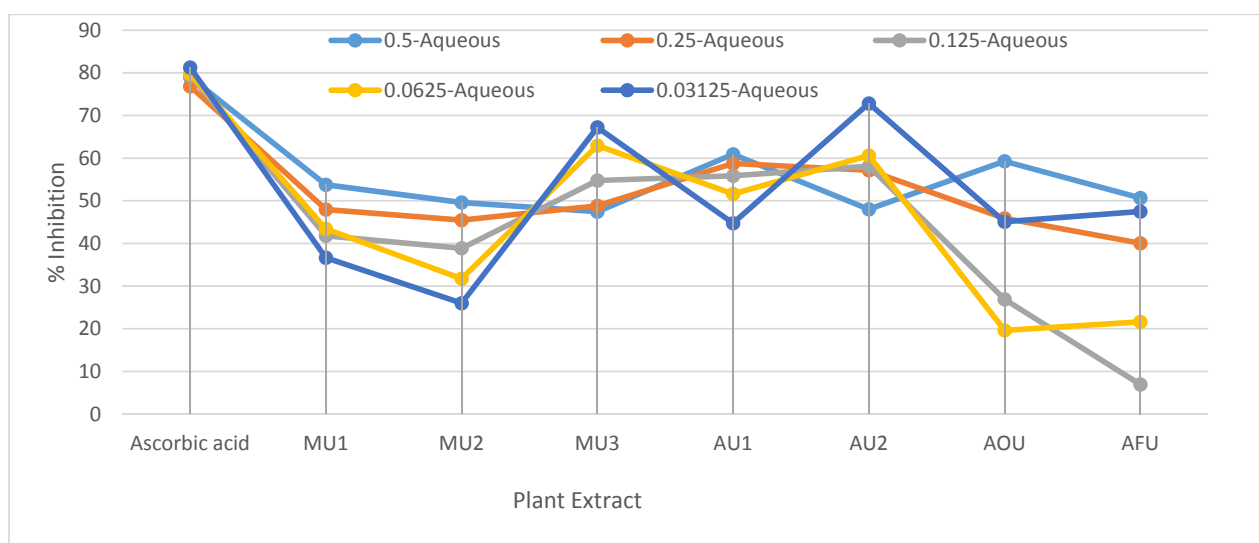


Figure 2: Inhibition trend of various concentrations of plant aqueous extracts

As seen in figure 2, the inhibition is highest for AU2 (72.81mg/ml) at lowest concentration followed by MU3 (67.22mg/ml). With aqueous extract of MU1, MU2, the activity increases with increase in concentration. Comparing both ethanolic and aqueous extracts at the lowest concentrations, Figure 3, in general it was observed that the aqueous extracts had relatively higher % inhibition compared to the ethanol samples. MU3, AU2 and AOU indicated higher inhibition. Interestingly MU1 and MU2 had minimal to no changes regardless of the medium of extraction.

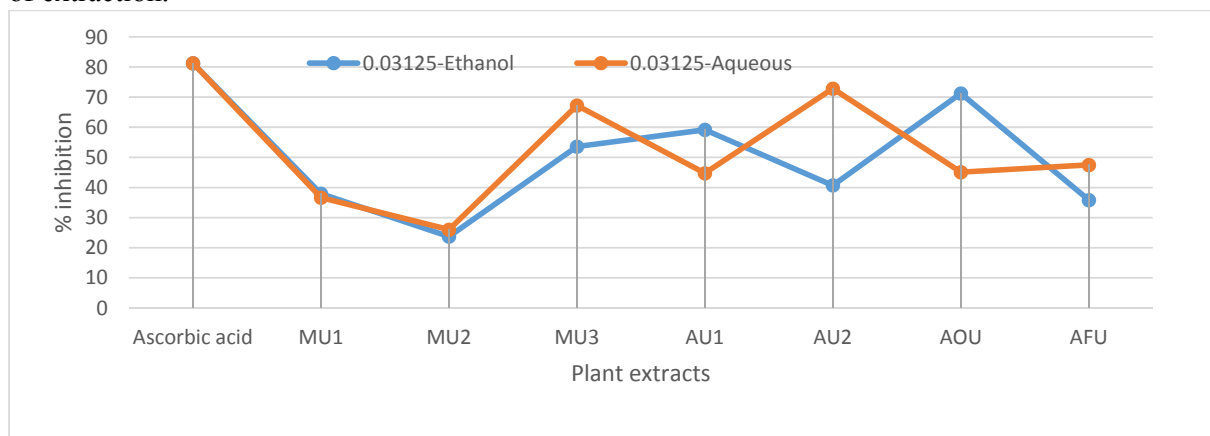


Figure 3: Comparison of inhibition trends at the lowest concentration levels of the extracts.

The results showed the anti-oxidant activity to be higher with ethanolic extracts than the aqueous of the samples. Nonetheless, the activities compared relatively well with the ascorbic acid used as a standard reference. This might be due to the presence of higher flavonoids and tannins, the most required bio-compounds for scavenging activity in these extracts. Flavonoids and tannins which are phenolic compounds found in plants act majorly as free radical scavengers. The results suggest that the antioxidant activities of these plants may contribute to their claimed antimicrobial properties.

4.4 Anti-microbial activity: The agar disc diffusion method was used to evaluate the antimicrobial activity by measuring the inhibition zone against the test microorganisms. 100µL of different concentrations of the extracts including the positive control (200µg of chloramphenicol and nystatin for bacteria and fungus respectively and the negative control (50% v/v DMSO) were introduced into the wells and incubated for 24 hrs.

4.4.1 *Staphylococcus aureus* (S.a)- Inhibition-Gram negative bacteria

The ethanolic extract of MU2 and the aqueous extract of MU3 did not show any inhibition zone above 6mm. However, the extracts of all plants in either ethanol or water indicated some degree of activity with inhibition zone ranging from 9.5mm up to 18.7mm. Interestingly the ethanolic extract of MU1, MU3, AOU and the aqueous extract of AU2, AOU show inhibition at all concentration levels. The others indicated inhibition only at concentrations above 500µg(Table 5)

Table 5: Inhibition zone (mm) of *Staphylococcus aureus*(s.a)

S.a	Ethanol Extract							Aqueous Extract						
	MU1	MU2	MU3	AU1	AU2	AOU	AFU	MU1	MU2	MU3	AU1	AU2	AOU	AFU
+ve	28.3	28.7	27.3	28.7	27.7	29.3	27.3	22.7	25.3	22.7	23.3	22.7	22.7	25.33
250	15.3	-	10.5	-	-	12	-	-	-	-	-	10	9.5	-
500	15.6	-	11.3	10	-	11.5	-	11	10.5	-	10	11	10.67	10.5
750	17.5	-	13	13.3	11	13.3	11	12.7	11.3	-	11.3	12.7	13	12
1000	18.7	-	14.7	18.6	14	15	15.3	13.7	12	-	12	13.7	15.7	14.7

4.4.2 *Streptococcus pyogenes* Inhibition-Gram negative bacteria

The ethanolic extract of the following plants MU3, AU2, AFU and the aqueous extract of MU3 did not indicate any activity. Interestingly only ethanolic extracts of MU1, MU2, AU1 show activity at all concentrations. The rest only indicated activity at concentration of 500µg and above.

Table 6: Inhibition zone (mm) of *Streptococcus pyogenes*(s.p)

S.p	Ethanol Extract							Aqueous Extract						
	MU1	MU2	MU3	AU1	AU2	AOU	AFU	MU1	MU2	MU3	AU1	AU2	AOU	AFU
+ve	25.3	21.7	21	23.3	22.7	22.3	22.3	19	20	19.3	23.3	18.7	19	19.3
250	13	14.5	-	10	-	-	-	-	-	-	-	-	-	-
500	13.7	14.7	-	11	-	12	-	-	-	-	-	-	10	-
750	15	16.7	-	11.3	-	12.7	-	11.3	-	-	11	12	10.3	10.5
1000	15.3	18.3	-	14	-	15.3	-	13.7	13.7	-	15	13	12.3	12.3

4.4.3 *Klebsiella pneumoniae* inhibition- Gram negative bacteria

None of the extracts indicated activity against this bacteria at the concentrations evaluated. Table 7

Table 7: Inhibition zone (mm) of *Klebsiella pneumoniae* (k.p)

K.p	Ethanol Extract							Aqueous Extract						
	MU1	MU2	MU3	AU1	AU2	AOU	AFU	MU1	MU2	MU3	AU1	AU2	AOU	AFU
+ve	22.7	21.3	20	20.7	22.7	21.3	22.7	17	18.7	17.7	18.7	19.3	17.7	17.7
250	-	-	-	-	-	-	-	-	-	-	-	-	-	-
500	-	-	-	-	-	-	-	-	-	-	-	-	-	-
750	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1000	-	-	-	-	-	-	-	-	-	-	-	-	-	-

4.4.4 *Salmonella typhi* inhibition- Gram negative bacteria

The ethanolic extract of MU3, AU1 and the aqueous extract of MU2, MU3, AU1 and AOU did not indicate any activity. The other remaining extracts exhibited inhibition at concentrations of 500µg and above except ethanolic AOU that showed activity at all concentration levels (Table 8).

Table 8: Inhibition zone (mm) of *Salmonella typhi* (s.t)

S.t	Ethanol Extract							Aqueous Extract						
	MU1	MU2	MU3	AU1	AU2	AOU	AFU	MU1	MU2	MU3	AU1	AU2	AOU	AFU
+ve	29	28.3	30.3	30.3	28.3	29.7	30.3	25.3	24.3	21.3	20	25.3	26.7	24.3
250	-	-	-	-	-	11	-	-	-	-	-	-	-	-
500	-	10	-	-	10	14	10.5	10.5	-	-	-	10	-	-
750	11.5	11.3	-	-	10.7	17	12.7	11	-	-	-	11.3	-	10
1000	13	13.7	-	-	13	19	14	12	-	-	-	12	-	11

4.4.5 *Candida albican*-Yeast-Fungus

Only of the extracts indicated activity at concentrations above 500µg, ethanolic extractions of MU2, AOU and the aqueous extractions of AU2 and AOU. The rest of the extracts evaluated indicated no activity. Table 9

Table 9: Inhibition zone (mm) of *Candida albican*

C.a	Ethanol Extract							Aqueous Extract						
	MU1	MU2	MU3	AU1	AU2	AOU	AFU	MU1	MU2	MU3	AU1	AU2	AOU	AFU
+ve	30.5	30.3	33	29	30	29.7	30.3	26	25.7	26.7	26	25.7	26	25.7
250	-	-	-	-	-	-	-	-	-	-	-	-	-	-
500	-	-	-	-	-	-	-	-	-	-	-	10	-	-
750	-	11	-	-	-	12	-	-	-	-	-	10.7	10.5	-
1000	-	15.3	-	-	-	14.3	-	-	-	-	-	13.3	12.33	-

Conclusion

Results reported here contribute to the knowledge of antibacterial and therapeutic activities of seven Nigerian medicinal plants extracts from both organic and aqueous solvents. These plants are mainly used by indigenes for anti-venom and anti-bee sting purposes. The findings that these medicinal plants possess antioxidant and antimicrobial properties strongly approve of their use as herbal medicine.

Ethical approval and consent: NA

References

1. Zampini I. C., Cuello S., Alberto M. R., Ordonez R. M., Almeida R. D., Solorzano E. and Isla M.I. Antimicrobial activity of selected plant species from the Argentine *Puna* against sensitive and multi-resistant bacteria. *Journal of Ethanopharmacology*. 2009;124:499-505.
2. Bhalodia N. R. and Shukla V. J. Antibacterial and antifungal activities from leaf extracts of *Cassia fistula* L. An ethnomedicinal plant. *Journal of Advanced Pharmaceutical Technology and Research*. 2011;2: 104-109.

3. Vashney S., Vashney P., Dash S. K., Gupta M. K., Kumar A., Singh B., and Sharma A. Antibacterial activity of fruits of *Termineliachebula* and *Terminaliabelerica* against mastitis field isolates. *Medicinal Plants*. 2012;4:167-169.
4. Emmanuel S. A., Olajide O., Abubakar S., Akiode S. O., and Etuk-Udo G. Chemical Evaluation, Free Radical Scavenging Activities and Antimicrobial Evaluation of the Methanolic Extracts of Corn Silk (*Zea mays*). *Journal of Advances in Medical and Pharmaceutical Sciences*. 2016; 9:1-8.
5. Abdel R. S., Abd-Ellatif S., Deraz S., and Khalil A. Antibacterial activity of some wild medicinal plants collected from western Mediterranean coast, Egypt: Natural alternatives for infectious disease treatment. *African Journal of Biotechnology*. 2011;10:10733-10743.
6. Okwute S. K., Fatokun A. O., and Orishadipe A. T., Phytochemical Screening and in Vitro Antimicrobial Activity of *WaltheriaIndica* Linn Leaf Extracts. *Biomedical Sciences*. 2017; 3: 86-93.
7. Bupesh G, Vijayakumar T S, Manivannan S, Beerammal M, Manikadan M, et al. Identification of Secondary Metabolites, Antimicrobial and Antioxidant Activity of Grape Fruit (*Vitis vinifera*) Skin Extract. *Dia Obe Int Jo* 2016; 1(1): DOIJ-MS-ID-000102
8. Uttu A. J., Sallau M. S., Hamisu I., Mubarak B. D. and Abdullahi Y. I. Phytochemical and antimicrobial screening of stem bark extracts from *Glossonemaboveanum* (Decne). *Journal of Pharmacy and Phytochemistry*, 2015;4:86-88.
9. Adebisi A., Effa E. B., Ayo R., Bello I., Habila J., and Ishaku G. A. Antimycobacterial, Antimicrobial and Phytochemical Evaluation of *Pulicariacrispa* and *Scopariadulcis* Plant Extracts. *Journal of Advances in Medical and Pharmaceutical Sciences*. 2016;7:1-11.
10. Dahiya P., and Purkayastha S. Phytochemical screening and antimicrobial activity of some medicinal plants against multi-drug resistant bacteria from clinical isolates. *Indian Journal of Pharmaceutical Science*. 2012;74: 443–450.
11. Okwute S. K., Olajide O. O., Etuk-Udo G., and Orishadipe A.T. Phytochemical Screening, *In-vitro* Antimicrobial Activity and Antioxidant Characteristics of *Tetrapleuratetraptera* Extracts. *European Journal of Medicinal Plants*. 2016; 17:1-10.
12. Useh, M.U., Adebisi, A. B. and Dauda, M. S. Proximate Composition, Phytoconstituents and Mineral Contents of Soybean (*Glycine Max*) Flour Grown and Processed in Northern Nigeria. *Advances in Applied Sciences*, 2017;2(4): 48-53.
13. Olajide O, Orishadipe A, Fatokun O, akande T, Iliya I. The chemical composition of essential oil from the root of *Cissampelo sowariensis* (P.beauv) and free radical scavenging of its extract. *African Journal of Pure and Applied Chemistry*. 2013;7:225-230
14. Mabhiza D., Chitemerere T., and Mukanganyama S. Antibacterial Properties of Alkaloid Extracts from *Callistemon citrinus* and *Vernoniaadoensis* against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *International Journal of Medicinal Chemistry*, 2016;1-7.
15. Bupesh G, Manikandan E, Thanigaiarul K, Magesh S, Senthilkumar V, et al. Enhanced Antibacterial, Anticancer Activity from *Terminalia chebula*. *Medicinal*

388 Plant Rapid Extract by Phytosynthesis of Silver Nanoparticles Core-shell
389 Structures. J Nanomed Nanotechnol 2016; 7: 355. doi:10.4172/2157-7439.

390 16. Edeoga HO, Okwu DE, Mbaebie O. Nutritional values of some known conventional
391 leafy vegetables of Nigeria. African Journal of Biotechnology. 2005;4: 685-688.

392 17. Okwu, D.E. Phytochemical and Vitamin Content of Indigenous Species of South-
393 Eastern Nigeria. Journal of Sustainable Agriculture and the Environment. 2004; 6:30-
394 37.

395 18. Sapna D. Desai, Dhruv G. Desai, Harmeet Kaur. Saponins and their Biological
396 Activities. Pharma Times. 2009; Vol 41 - No. 3

397 19. Okwu, D. E. Evaluation of the Chemical Composition of Indigeneous Spices and
398 Flavouring Agents. Journal of Science. 2004; 7: 455-459

399 20. Okwu D.E. Phytochemicals, Vitamins and Mineral contents of two Nigeria Medicinal
400 plants. Int. J. Mol. Med. Adv. Sci. 2005; 1(4): 375-381.

401 21. Shah BA, Qazi GN, Taneja SC. Nat Prod Rep. 2009; 26(1):72-89.

402 22. Rabi T, Bishayee A. Breast Cancer Res Treat. 2009; 115(2):223-39.

403

404

405