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Antimicrobial Potential of *Vernonia Amygdalina* on Gram Positive Isolates from Renal Disease Patients in Umuahia, Abia State

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Abstract **Original Research Article**

This research study aimed at evaluating the antimicrobial potential of ethanolic and aqueous extracts of *Vernonia amygdalina (*Bitter leaf) on gram positive isolates from renal disease patients in Umuahia Abia State. Using Ilodibia *et al.* method with modification where the ethanolic and aqueous extracts of the plants were prepared by adding the powdered sample of *V. amygdalina* in 250 ml of distilled water. The concentration of the extract was determined by adding 50g, 75g, 100g and 150g, each in 250ml of distilled water respectively. The experimental set-up was left for 48 hours at room temperature and thereafter filtered using Whatman filter paper No.1. The extract was then concentrated by heating on water bath at 70°C to 50ml volume of the extract. From this research ethanolic extracts showed higher zones of inhibition than the aqueous extract, this is attributed to the fact that ethanol extracted more of the bioactive component of the plant when compared to aqueous. *V. amygdalina* exhibited high antibacterial activity on gram positive organisms.The most sensitive Gram positive organism to the ethanolic extract of *V. amygdalina* is *Staph epidermidis* (17.85), *followed by Strep pneumonia* (12.67) then *Staph aureus* (12.29). The control (Ciprofloxacin) has higher antimicrobial activity in all the isolates.

Keywords*: antimicrobial potential, vernonia amygdalina, gram positive isolates, renal disease patients*.

INTRODUCTION

Kidney disease is receiving increased global public health attention because of a significant increase in the prevalence of the disease, the enormous cost of treatment, and the appreciation of its role as a risk factor for cardiovascular disease [1]. The National Kidney Foundation Disease Outcomes Quality Initiative guidelines [17] define Chronic Kidney Disease as kidney damage or a glomerular filtration rate of less than 60 ml/min per 1.73 m^2 for at least 3 months.

The World Health Organization and Global Burden of Disease (GBD) project report published in 2006 showed that diseases of the kidney and urinary tract contribute to the GBDs – with approximately 850,000 deaths every year and 15,010,167 disability-adjusted lives.

The 2010 global ranking of premature causes of death show that kidney diseases moved up from position 32 in 1990 to position 24 in 2010. These findings refer only to the more advanced stages of kidney disease especially end stage renal disease (ESRD). The proportion of patients with milder kidney dysfunction is definitely larger and contributes significantly to cardiovascular mortality [2].

CKD affects an estimated 14% of adults in sub-Saharan Africa, but very little research has been done on the cause, progression, and prevention of CKD [3,4]. Several hospital based studies in Nigeria have put the prevalence of CKD between 1.6 - 12.4% [5]. Okwuonu *et al.*, [6] in his study conducted in Olokoro, a semi-urban community in Umuahia South Local Government Area of Abia State Nigeria placed the prevalence of CKD at 7.8%. A deterioration in renal function correlates with disturbances of various specific and nonspecific host defense reactions.

The increased use of antibiotics in medicine has contributed largely to the antibiotic-resistant microorganisms also the frequency of toxic side effects associated with antibiotic therapy is an impetus for the substitution of safer and equally efficacious alternatives, particularly in the setting of renal impairment urging the search for new and effective drugs [7].

The aim of this research is to evaluate the antimicrobial potential of *Vernonia amygdalina (*Bitter leaf) on gram positive isolates from renal disease patients in Umuahia Abia State.

MATERIALS AND METHODS

MATERIALS

Study Area

The study was conducted at the two tertiary health care facilities in Umuahia comprising of Federal Medical Centre and Abia Specialist hospital.

CHEMICALS

All chemicals and biochemicals used were of analytical grade, purchased from a reputable company and used without further purification.

PLANT MATERIAL

Vernonia amygdalina commonly known as bitter leaf was collected as whole plant from Okechukwu Dike's farm in Nkwogwu Okaiga Ohuhu in Abia State. The leaves of *V amygdalina* were dried at room temperature separately. They were cut into small pieces and ground into fine powder using a blender. The powder was properly packaged and stored in the refrigerated at 4°C for further use.

Extraction of *Vernonia amygdalina*

The aqueous and ethanolic extraction of *Vernonia amygdalina* was done using Ilodibia *et al.* [8] method with modification.

Aqueous extraction

The aqueous extracts of the plant was prepared by adding the powdered sample of *Vernonia amygdalina* in 250 ml of distilled water. The concentration of the extract was determined by adding 50g, 75g, 100g and 150g, each in 250ml of distilled water respectively. The experimental set-up was left for 48 hours at room temperature and thereafter filtered using Whatman filter paper No.1. The extract was then concentrated by heating on water bath at 70°C to 50ml volume of the extract.

Ethanol extraction

The ethanolic extract of the *Vernonia amygdalina* plant was prepared by soaking the powdered sample of the leaf and stem in 250 ml of ethanol. The concentration of the extract was determined by adding 50g, 75g, 100g and 150g, each in 250ml of ethanol respectively. The experimental set-up was left for 48 hour at room temperature and thereafter filtered using Whatman filter paper No.1, the extract was then concentrated by heating on water bath at 60°C to 50 ml. The extracts stocks were stored in an air tight container in a refrigerator at 4°C. Using sterile 5ml plain tubes aliquots were taken for daily use to avoid contamination.

CULTURE MEDIA

Solid media used was commercially purchased and includes:

Nutrient agar

Nutrient agar medium was prepared according to manufacturer's instruction by dissolving 28g of the powder in one liter of distilled water by boiling, sterilized by autoclaving at 121°C for 15 minutes, cooled to about 50°C and distributed in 15 ml amount per Petri-dish. The poured plate was left to solidify at room temperature on leveled surface and residual moisture was dried using hot air oven at reduced temperature.

Blood agar

Blood agar medium was prepared according to manufacturer's instruction by dissolving 40 g in one liter of distilled water by boiling, and sterilized by autoclaving at 121°C for 15 minutes. Then cooled to about 50°C, defibrinated blood was added aseptically to give final concentration 10%, and mixed gently. 15ml of complete medium was poured into each sterile Petri dish. They were allowed to solidify at room temperature on flat surface.

MacConkey agar

MacConkey agar was prepared according to manufacturer's instructions by dissolving 52 g of MacConkey agar in 1 liter of distilled water, and boiled to dissolve the ingredients completely. The medium was sterilized by autoclaving at 121°C for 15 minutes and poured into sterile Petri dishes in 15 ml amount. The poured plates were left to solidify at room temperature on the flat surface.

Urea Agar

The medium was prepared according to manufacturer's instructions by dissolving 2.4 g of powder in 95 ml distilled water by boiling and sterilized by autoclaving at 121°C for 15 minutes. The medium was cooled to 50 -55 °C and 5 ml of sterile (20%) urea solution was added aseptically. The medium was distributed in 10 ml amounts in sterile test tubes and allowed to set in inclined position.

Simmon´s citrate agar

Twenty three grams of Simmon´s citrate agar was suspended in 1 liter of distilled water, dissolved by boiling and sterilized by autoclaving at 121°C for 15 minutes. The medium was poured aseptically into sterile McCartney bottles and allowed to solidify in inclined position.

Mannitol salt agar

One hundred and eleven grams (111g) dehydrated medium was suspended in a liter of distilled water, steamed to dissolve and the pH adjusted to 7.5,

then autoclaved at 121°C for 15 minutes, cooled and poured into Petri-dishes. The poured plates was allowed to solidify at room temperature on flat surface

SEMI SOLID MEDIA

Motility medium

Motility medium was prepared as described by Barrow and Feltham (1993). It consisted of 10 g peptone, 3 g meat extracts, 5g sodium chloride, 4g agar, 80 g gelatin and 1 liter distilled water. First gelatin was soaked in water for 30 minutes then the other ingredients were added. The pH adjusted to 7.4. This medium was dispensed in 5 ml volume into 20 ml test tubes containing the appropriate Cragie tubes, and then the medium was sterilized by autoclaving at 121°C for 15 minutes.

Liquid Media

Nutrient broth:

This medium contained, beef extract, peptone and sodium chloride. Thirteen grams of nutrient broth was added to 1 liter of distilled water according to Barrow and Feltham [9], and mixed well. Then the pH adjusted to 7.2-7.4 and the medium was distributed in 3 ml amount into clean test tubes, and then sterilized by autoclaving at 121°C for 15 minutes.

Peptone water

This medium contained peptone and sodium chloride. It was prepared according to Barrow and Feltham [9] by dissolving 10 g of peptone and 5 g of sodium chloride in 1 L of distilled water and mixed well. Then the pH adjusted to 7.2 - 7.4 and the medium was distributed in 3 ml amount into clean test tubes and sterilized by autoclaving at 121°C.

Peptone water sugar

Peptone water sugar medium was prepared as described by Barrow and Feltham (1993). It contained peptone water 900 ml, phenol red indicator 10 ml, sugar 10 g and distilled water 90 ml. The pH of peptone water was adjusted to 7.1-7.3 before the addition of phenol red indicator and was homogenized. The complete medium was then distributed into portions of 3 ml into sterile test tubes containing inverted Durham's tubes, loosely covered with caps and sterilized by autoclaving at 115°C (101b/inch²) for 10 minutes. The sterilized medium was then tightly covered and kept at 4°C until used.

Glucose phosphate (MR – VP) medium

This medium was prepared according to Barrrow and Feltham [9]. Peptone 5g and 5g of phosphate buffer (K2HO4) were added to one liter of distilled water, steamed till dissolved, filtered and pH was adjusted to 7.5. Then 5 g of glucose were added, mixed well, distributed into clean test tubes and sterilized by autoclaving at 115 °C for 15 min.

Sample Collection

One hundred and eleven (111) samples was collected from confirmed renal failure/kidney disease patients, who attends Tertiary Health Facilities in Umuahia, they were instructed on how to collect mid stream specimens of urine in sterile universal bottles. Urine from catheter was aseptically collected from in-patients suffering from oliguria. The samples were stored in thermos flasks containing icepacks and transported to the Diagnostic laboratory unit of University Health Services department for immediate processing and culturing.

Microscopic examination of urine

Ten ml of urine from each sample was centrifuged at 1500 rpm for 15 minutes, the supernatant was decanted and the deposit used for preparation of wet mounts. A drop of urine deposit was placed onto a clean grease free slide, covered with a cover slip and examined under the microscope for the presence of pus cell, RBC, yeast cell and cast cell per field using the dry high power objective lens(x40). The presence of 5-7 or more cell of pus and RBC will be considered a significant pyuria and significant RBC in urine.

Isolation and Purification of isolates

The collected urine samples were inoculated onto CLED, EMB, 10% defibrinated sheep blood agar and MacConkey agar. The inoculated plates were then incubated aerobically at 37°C for 24 hours as described by Barrow and Feltham [9]. Further incubation was continued for another 24 hours for those plates without growth after 24hrs, plates without significant growth after 48hours were discarded.

Colonial characteristics of those with significant growth were observed, smears were made from each type of colony stained for Grams technique.

All bacterial isolates were purified by several sub-culturing from single well-separated colony on nutrient agar plates. The purity will be checked by examining Gram stained smear. The pure culture was then used for studying cultural and biochemical characteristics and antibiogram of the isolates on conventional and plant extracts.

IDENTIFICATION OF ISOLATES

Microscopic Examination

Smears was made from purified colonies, fixed by heating and stained by Gram stain method of Barrow and Feltham [9]. Then examined microscopically for cell morphology and arrangement, and staining reaction. Gram stain was also used in checking the purity.

Gram Stain

Smear-Preparation

Using a sterile wire loop a colony was picked and smeared on a clean slide with a drop of saline and was passed through flame to heat fix.

Gram Staining Procedure/Protocol

- The air-dried, heat-fixed smear of cells was flooded for 1 minute with crystal violet staining reagent.
- Slide was washed in a gentle and indirect stream of tap water for 2 seconds.
- Flooded with the mordant: Gram's iodine and allowed for 1 minute.
- The slide was washed in a gentle and indirect stream of tap water for 2 seconds.
- It was flooded with decolorizing agent (acetone) and washed off immediately under running tap water.
- The slide was flooded with counter-stain, safranin for 30 seconds to 1 minute.
- Slide was washed in a gentle and indirect stream of tap water until no color appears in the effluent and allowed to air dry.
- The stained slide was viewed under oil immersion using a Brightfield microscope.

Gram staining result: Gram-positive cell is purple and the gram-negative cell is pink to red.

Cultural Characteristics

All cultures on solid media were examined with naked eye for growth and colonial morphology and any changes in medium. Blood agar was used as an enriched, non-inhibiting medium for primary isolation of bacteria and for determination of colonial morphology and hemolytic activity.

The liquid media was also examined with naked eye for turbidity, color change, formation of sediments and accumulation of gas in the Durham's tube in case of carbohydrates media.

Biochemical Tests

Sugar fermentation test

The test was carried out as described by Cheesbrough [10]. The peptone water sugar was inoculated with isolated organism, incubated at 37°C and then examined within 24 hours. Acid production was indicated by appearance of yellowish color, while gas production was indicated by presence of an empty space in the inverted Durham's tubes.

Oxidase test

The method of Cheesbrough [10] was followed. Strips of filter paper was soaked in 1% solution of tetramethyl-phenylenediamine dihydrochloride and dried in hot air oven and then placed on clean glass slide by sterile forceps. A fresh young test culture on nutrient agar was picked off with sterile glass rod and rubbed on the filter paper strip. Development of purple color within 5-10 seconds indicates a positive reaction.

Catalase test

The test was carried out as described by Cheesbrough [10]. A drop of 3% aqueous solution of hydrogen peroxide was placed on a clean glass slide. A colony of test culture on nutrient agar was picked off and put on the drop of hydrogen peroxide. Evolution of gas and appearance of bubbles indicated positive test.

Coagulase test

The test was performed as described by Cheesbrough [10]. To 0.5 ml of 1:10 dilution of human plasma in saline, 0.1 ml of 18-24 h old broth culture of test organism was added, then incubated at 37°C and examined after 6-24 h for coagulation. Definite clot formation indicated positive result.

The test was also performed as described by Cheesbrough [10] on slide. Two colonies of tested culture were placed on a clean glass slide, emulsified in a drop of normal saline and then a loopful of human plasma was added to the bacterial suspension. Appearance of coarse visible clump was recorded as positive result.

Indole production test

Indole production test was carried out as described by Chesbrough [10]. The test organism was inoculated into tryptone peptone water and incubated at 37°C for 48 hours. One milliliter of the Kovac´s reagent was run down along side of test tube. Appearance of red color within a minute indicated positive reaction.

Methyl red (MR) test

Methyl red test was carried out as described by Cowan and Steel [11]. The test organism was inoculated into glucose phosphate medium, incubated at 37°C for 48 hours. Two drops of methyl red reagent were added, shaken well and examined. Appearance of red color indicated positive reaction, whereas orange or yellow color indicated negative reaction.

Voges-Proskauer (VP) test

The test was performed as described by Cowan and Steel [11]. The test culture was inoculated into glucose phosphate medium and incubated at 37°C for 48 h. After completion of MR test, 0.6ml of 5% α-naphthol solution and 0.2ml of 40% potassium hydroxide (KOH) were added. When red color developed within 30 minutes, the reaction was regarded as positive.

Urease activity tests

The test was carried out as described by Cheesbrough [10]. The test organism was inoculated heavily on urea broth medium and incubated at 37°C for 12-24hrs. Appearance of pink color indicated positive reaction.

Citrate utilization

The test was performed as described by Cowan and Steel [11]. The test culture was inoculated as a single streak over the surface of slope of Simon's citrate medium and incubated for 48hours then examined. Growth of the organism and change of color to blue indicated positive test.

Motility test

The Craigi tube in semi-solid nutrient agar prepared as described by Cruckshank *et al*. [12] was inoculated. A small piece of the colony of the bacterium under test was picked by the end of the straight wire and stabbed in the center of semi solid agar in the Craigi tube and then incubated at 37°C overnight. The organism was considered motile as the medium became turbid.

ANTIMICROBIAL SUSCEPTIBILITY TEST

The zone of inhibition of the extracts was determined using agar well diffusion method as described by ICMSF, (1998a, b). Bacteria isolates were inoculated onto solidified nutrient agar using streaking method. Wells were bored into the agar medium using a sterile 6 mm cork borer. The wells were then filled up with 0.02 ml (20µl) of the extract and care was taken not to allow the solution to spill on the surface of the medium. The plates were allowed to stand on the laboratory bench for 1-2 hours for proper absorption of the solution into the medium, the plates were inverted and the wells properly labeled with a marker. The plates were incubated aerobically at 37°C for 12-24 hours. Sensitivity of the organisms to the extract was recorded by measuring the zone of inhibition. The extent of inhibition was expressed in terms of the diameter of the inhibition zone as measured with a transparent meter rule in milli-meter. The effects of the extracts on the isolated pathogens were compared with those of the standard antibiotic ciprofloxacin as control.

STATISTICAL ANALYSIS

The data from the study was subjected to statistical analysis by IBM SPSS version 25 using Analysis of variance (ANOVA). The group mean zone of inhibition of the various extracts on the isolated organisms were compared to the control (ciprofloxacin), All analyses were carried out at 5% level of significance ($P<0.05$). Data were represented as mean ± standard error.

RESULTS

One hundred and eleven (111) samples were collected from renal disease patients in Umuahia for this research work, after the various analysis the following microorganisms were isolated; *Staphylococcus epidermidis, Staphylococcus aureus, Streptococcus pneumonia, E.coli, Klebsiella pneumonia, Pseudomonas aeruginosa, and Proteus. E.coli*-17 (15.32%), was seen as the highest occurring bacteria followed by *Staph aureus* 14 (12.61%), *Staph epidermidis* 11 (9.91%), *Streptococcus pneumonia* 10 (9.0%), *Klebsiella pneumonia* 10 (9.0%), *Pseudomonas aeruginosa 7 (*6.31%) and *Proteus* 2 (1.80). 40 (36.0%) samples had no significant growth thus termed nil growth as seen in table 1 and 2 below.

Table 3 is the antimicrobial activity of different concentration of ethanolic and aqueous extracts of *Vernonia amygdalina* (mean zone of inhibition in milli-meter) on gram positive isolates while

Table-1: Bacterial isolates from Renal Disease Patients in Umuahia

Week	No. of samples	Organism Isolated	Frequency	Nil growth
Week 1	22	*Staph epidermidis*	3	4
		Staph aureus	4	
		Streptococcus	2	
		E.coli	3	
		Klebsiella	4	
		Pseudomonas	1	
		Proteus	1	
Week 2	17	*Staph epidermidis*	2	1
		Staph aureus	4	
		Streptococcus	4	
		E.coli	4	
		Klebsiella	2	
Week 3	15	*Staph epidermidis*	1	5
		Streptococcus	1	
		E.coli	2	
		Klebsiella	2	
		Pseudomonas	3	
		Proteus	1	
Week 4	27	*Staph epidermidis*	2	16
		Staph aureus	2	
		Streptococcus	1	
		E.coli	4	
		Klebsiella	1	
		Pseudomonas	1	

Week 5	20	*Staph epidermidis* *Staph aureus* *Streptococcus* *E.coli* *Pseudomonas*	1 2 1 3 2	11
Week 6	10	*Staph epidermidis* *Staph aureus* *Streptococcus* *E.coli* *Klebsiella*	2 2 1 1 1	3

Table-2: Total Frequency of Bacterial Isolates

Total No. of Samples	Organism Isolated	Frequency	Percentage
111	*Staph epidermidis*	11	9.91
	Staph aureus	14	12.61
	Streptococcus	10	9.0
	E.coli	17	15.32
	Klebsiella	10	9.0
	Pseudomonas	7	6.31
	Proteus	2	1.80
	Nil Growth	40	36.0
Total		**111**	**100**

In table 3 the mean zones of inhibition in milli-meter of the Vernonia extract against the gram positive isolates shows that in general the ethanolic extracts have more antimicrobial activity than the aqueous extract. The extract has more inhibitory effect on *Staph epidermidis* with the highest mean zone of 17.85 at 150mg/ml, followed by *Streptococcus pneumonia* with mean zone of 12.67 while S*taphylococcus aureus* has 12.29 as its highest mean zone of inhibition. When compared with the mean zone of the control antibiotic (Ciprofloxacin) the following inhibition zones were observed *Staph epidermidis* (21.85), *Streptococcus pneumonia* (20.67) and *Staphylococcus aureus* (23.57). This shows that the conventional Ciprofloxacin (control) has higher antimicrobial activity than ethanolic extract of *Vernonia amygdalina*. At 150mg/ml the ethanolic extract has 17.85mm against *Staph epidermidis* which makes it sensitive for its treatment. Across the various groups (50, 75, 100, 150 mg/ml) there is no statistical difference since P>0.05 (greater than 0.05).

In the aqueous extract insignificant mean zone of inhibition in milli meter was observed, in all the gram positive isolate the mean zones of inhibition was less than five milli-meter(<5mm). In comparism with the control antibiotic (Ciprofloxacin) the control has significant mean difference with the following values in milli-meter, *Staph epidermidis*-21.85, *Staphylococcus aureus*-23.57 and *Streptococcus pneumonia*-20.67.

Table-3: Antimicrobial activity of different concentration of ethanolic and aqueous extracts of *Vernonia amygdalina* (mean zone of inhibition) on gram positive isolates

Concentration	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Streptococcus Pneumonia*	P-value
Mean inhibition zone of Ethanolic extract in milli-meter				
E50mg/ml	9.14 ±1.48	10.92±1.27	9.56±1.94	0.669
E75mg/ml	11.57 ±2.21	17.38 ±0.94	11.56 ±2.66	0.063
E100mg/ml	12.14±2.34	17.23±1.10	12.44±2.62	0.148
E150mg/ml	12.29±2.34	17.85 ±1.21	12.67 ±2.58	0.110
CONTROL(CIPRO) 0.01mg/ml	23.57±1.26	21.85±2.17	20.67±1.29	0.499
Mean inhibition zone of Aqueous extract in milli-meter				
A50mg/ml	0.71±0.71	2.77±1.21	0.89±0.89	0.252
A75mg/ml	1.29±0.88	2.92±1.27	1.00±0.88	0.398
A100mg/ml	1.93±1.23	3.85±1.42	1.78±1.35	0.484
A150mg/ml	2.71±1.42	4.77±1.46	1.89±1.34	0.373
CONTROL(CIPRO) 0.01mg/ml	23.57±1.26	21.85±2.17	20.67±1.29	0.499

Antibiogram chart for Ciprofloxacin: Zone of ≤15mm means Resistant, >15mm to 18mm means intermediately sensitive, >18mm means sensitive.
The P-value above is for the various isolates within the specific concentration.
Cipro=Ciprofloxacin
P-value <0.05 is significant (less than 0.05)
P-value>0.05 is not significant (above 0.05)

DISCUSSION

The antibacterial activity of *Vernonia amygdalina* was found to be dependent on the nature of solvent used for the extraction and the concentration of the bioactive component of the plant. In overall view, the ethanolic extracts showed higher zones of inhibition than the aqueous extract, this is attributed to the fact that ethanol extracted more of the bioactive component of the plant when compared to aqueous. This finding is in agreement with Udochukwu *et al*. [13] who found that ethanolic extract has more concentration of the bioactive component than the aqueous extract.

In this research study, *Vernonia amygdalina* exhibited more antibacterial activity on gram positive organisms. This finding is in agreement with research of Cos *et al.* [14] who says that *Vernonia amygdalina* was more sensitive toward gram positive bacteria.

The ethanolic extract of *Vernonia amygdalina* has more antimicrobial activity against the bacterial isolates than the aqueous extract as shown in the zones of inhibition (mm), this is attributed to the higher concentration of bioactive components in the ethanolic extract. The higher the concentration of bioactive component (phytochemical) the greater the antimicrobial zone of inhibition. The concentration of bioactive component in ethanol is higher than aqueous which is in line with findings of Udochukwu *et al*. [13] who found that ethanolic extract has more bioactive component than the aqueous extract.

Bioactive component of plant exhibits different modes of action against microorganisms, which ranges from disturbance of the cytoplasmic membrane, disruption of proton motive force, disruption of active transport mechanism and coagulation of cell composition [15].

The most sensitive Gram positive organism to the ethanolic extract of *Vernonia amygdalina* is *Staphylococcus epidermidis* (17.85), *followed by Streptococcus pneumonia* (12.67) while *Staph aureus* (12.29) was the least sensitive, ethanolic extract of *Vernonia amygdalina* was found to possess inhibitory activities against Gram positive bacteria. This finding is in agreement with earlier work by Udochukwu *et al*. [13] and Evbuomwan *et al*. [16] who reported on the antimicrobial activity of *Vernonia amygdalina.*

CONCLUSION

Gram positive isolates were sensitive to the extracts especially *Vernonia amygdalina* having greater inhibition zones.

From this research study it is observed that for Gram positive organisms there is generally no significant difference between the inhibition zones of 75mg/ml, 100mg/ml and 150mg/ml concentrations. In comparison with the Ciprofloxacin (control), the inhibition of the microbes is significantly higher in the Ciprofloxacin (control) than in the plant extracts (both aqueous and ethanol), only ethanolic extract of *Vernonia amygdalina* was intermediately sensitive to *Staph epidermidis.*

Vernonia amygdalina is primarily used as spices and vegetables in our traditional meals due to

their health benefits, these vegetables are also rich in antioxidants and have antibacterial, nephron-protective potency they should be incorporated into daily meals of renal disease patients. We recommend purification of *Vernonia amygdalina* to isolate the particular bioactive component that possesses the antimicrobial properties.

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