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Research Article

Comparative Bacteriocidal Effect of Utazi (*Gongronema Latifolium*) and Onugbu (*Vernonia Amygdalina*) on Gram Negative Bacteria Isolated From Clinical Specimens at Specialist Hospital Owerri, Nigeria

Uchegbu, UN[1], Uche-Uchegbu, N[2*], Dike-Ndudim, J[3], Onyenweaku, FC[4] and Ndukwe, CK[5]

[1]Department of Medical Laboratory Services Federal Medical Centre, Owerri, Imo State, Nigeria
[2]Deprtment of Internal Medicine, Federal Medical Centre, Owerri, Imo State, Nigeria
[3]Department of Medical Laboratory Science, Imo State University, Owerri, Imo State Nigeria
[4]Research Student Imo State University, Owerri. Imo State, Nigeria
[5]Research Assistant

***Corresponding author:**
Uche-Uchegbu, N

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Abstract: The lowered immunity of patients with chronic kidney diseases and the increased bacterial resistance to a control antibiotic have given rise to the need to research and offer reliable evidences for the therapeutic values of the leafy vegetables like *Vernonia amygdalina* (Bitter leaf) and *Gongronema latifolium* (Utazi) in treating urinary tract infections caused by Gram negative bacteria. One hundred and twenty-seven (127) midstream urine samples were aseptically collected from patients suspected to develop renal diseases who attend the Specialist Hospital, Owerri into sterile, screw-capped universal containers and were inoculated onto CLED, EMB, Blood agar and MacConkey agar plates. These plates were then incubated at 37°C for 24 hours to obtain pure colonies. Out of the one hundred and twenty-seven (127) urine samples, forty-three (33.86%) samples yielded no significant growth whereas eighty-four (66.14%) yielded significant bacterial growth. The predominant Gram negative isolate from the samples was *Escherichia coli* 35(41.67%), while the least isolated Gram negative organism was *Proteus mirabilis*9(10.71%). Other Gram negative isolates include *Klebsiella pneumoniae* 24(28.57%) and *Pseudomonas aeruginosa* 16(19.05%). Among the Gram negative isolates, *Klebsiella pneumoniae* had the highest mean zone of inhibition; (8.28), with *Escherichia coli* having the least mean zone of inhibition; (4.45). *Pseudomonas aeruginosa* and *Proteus mirabilis;* (6.75), respectively. Ciprofloxacin which was the control antibiotic had statistical significant difference ($P<0.05$) on the Gram negative isolates when compared with the aqueous and ethanolic extracts of the plants. *Pseudomonas aeruginosa* and *Proteus mirabilis* were completely resistant to the antimicrobial activity of *Vernonia amygdalina* aqueous extract. At 50mg/mL, *Klebsiella pneumoniae* was found to be resistant while at 75-150 mg/mL concentrations, an insignificant mean zone of inhibition was noted. Generally, the ethanolic extracts showed higher zones of inhibition than the aqueous extracts at all concentrations.

Keywords: Gram negative isolates, *Vernonia amygdalina, Gongronema latifolium,* antimicrobial activity.

INTRODUCTION

In typical clinical conditions, there are changes that may occur in excreted urine samples. These changes may include, but not limited to findings obtained during episodes of oligouria, anuria, albuminuria, haematuria, etc. The resultant changes in urinary pH, osmolarity and urea have their own effects in urinary tract infections (UTIs). The accumulation of

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various uremic toxins inhibits the antimicrobial activity of granulocytes, macrophages and other defense mechanisms. These conditions may support the development of UTIs in patients with renal disease (Ojiaku, O.A., & Nwanjo, H.U. 2006). The increased use of antibiotics in medicine has contributed largely to the increase in number of antibiotic-resistant microorganisms (Okwuonu, C. G. *et al*., 2015). Also, the frequency of toxic side effects associated with antibiotic therapy is a major reason for the substitution of chemotherapy for safer and equally efficacious alternatives, particularly in the setting of renal impairment urging the search for new and effective plant products (drugs) with therapeutic pharmacognosy (Cheesbrough, M. 2004). Traditional healers have long used plants to prevent or cure infectious conditions as these plants have been shown to be rich in secondary metabolites such as terpenoids, alkaloids and flavonoids which have been demonstrated to possess antimicrobial properties in vitro (Osuagwu, A. N. *et al*., 2013).

The immunological suppression fund in patients with chronic kidney diseases is associated with a vast array of both innate and acquired host defense reactions (ICMSF. 1998) and conventional antibiotics have become increasingly ineffective in combating the arrays of infections that plague these patients hence, the need to research and offer evidences for the use of the leafy vegetables; *Vernonia amygdalina* (Bitter leaf) and *Gongronema latifolium* (Utazi) arises.

Despite the well-known antimicrobial and nephro-protective potential of *Vernonia amygdalina* (Bitter leaf) and *Gongronema latifolium* (Utazi), their efficacy against bacterial isolates from the urine samples of patients with chronic kidney disease have not been fully evaluated (Darwish, R. M. *et al*., 2010). As leafy vegetables used in food preparation, it is believed to be less toxic when used in therapeutic treatment of bacterial infections in patients with kidney disease. This study is aimed at offering evidence that these vegetables can be used as nephro-protective dietary options for patients with kidney disease (Minari, J. B. 2012).

This study focuses on determining the antibacterial quality of both *Vernonia amygdalina* and *Gongronema latifolium* on the isolated bacteria. Also, to correlate the efficacy of a conventional antibiotic agent (Ciprofloxacin) and that of *Vernonia amygdalina* and *Gongronema latifolium* on the isolated bacteria from the urine of patients with chronic kidney disease in Umuahia.

MATERIALS AND METHODS

Study Area

Owerri is the capital city of Imo State in Southeastern Nigeria. It has a population of 359,230 people (Minari, J. B. 2012). Owerri covers a land area of about 245 km² and lies on latitude 5°32' N and Longitude 7°29'E with an elevated altitude of 152 meters. The average temperature is 26.2°C.The residents of Owerri are mostly civil servants and technical workers. There are also traders and farmers but they are in the minority. Self-medication is a common practice in Owerri and the city has few markets to serve the fresh food demand of the city. This study was conducted at the Specialist Hospital, Owerri. All Ethical considerations were fully taken into account.

Preparation of Plant Materials/Extracts

Gongronema latifolium locally known in Eastern Nigeria as Utazi and *Vernonia amygdalina* commonly known as bitter leaf, were identified by the Botany Department of Imo State University, Owerri after they were bought from the Eke-Ukwu Owere market. The leaves of *Gongronema latifolium* and *Vernonia amygdalina* were dried separately at room temperature before being grounded. The powder was packaged in disinfectant-free containers and subjected to refrigeration at 4°C.

The aqueous and ethanol extracts of the leaves were prepared using a modified procedure of the method described by (Barrow, G.H., & Feltham, R.K. 1993), thus;

The powdered leave samples were dissolved in 250mL of distilled water to constitute the aqueous extract. The extract was further constituted into different concentrations by adding 50g, 75g, 100g and 150g of the grounded leaves, respectivelyin 250mL of distilled water and allowed to stand at room temperature for 48 hours before filtering off with a Whatman filter paper No. 1. The extracts were then concentrated by heating in a water bath at 60°C to 50mL volume of the extracts.

The ethanol extract of the leaves was prepared by submerging the powdered leaves in 250mL of ethanol. The concentrations of the extract were constituted by adding 50g, 75g, 100g and 150g each of the leaves in 250mL of ethanol, respectively and left for 48 hours at room temperature before filtering using Whatman filter paper No. 1. The extracts were then concentrated by heating in a water bath at 60°C to 50mL volume of the extracts.

The extract stocks were then stored in air-tight containers in a refrigerator at 4°C. Aliquots of exactly 5mL were taken in sterile plain tubes for daily use to avoid contamination of the stock (extracts).

Sample Collection and Processing

One hundred and eleven midstream urine samples were aseptically collected from confirmed renal failure/ chronic kidney disease (CKD) patients who attend the Specialist Hospital into sterile, screw-capped universal containers. Urine samples from catheters werealso aseptically collected from in-patients

suffering from oligouria. The samples were stored in thermoflasks containing ice packs and transported to the diagnostic laboratory unit for immediate processing and culture.

CLED, MacConkey and Blood agar plates were used to culture the urine samples. The inoculated plates were then incubated at 37°C for 24 hours as described by (Cheesbrough, M. 2004) to obtain pure colonies. Incubation was continued for another 24 hours for plates without growth after the first 24 hours. Pure colonies were isolated for the morphological identification and studyingof their antibiogram, and a comparison was made between the control antibiotic(Ciprofloxacin) and the plant extracts.

The zone of inhibition of the extracts was determined using agar well diffusion method as described by (Evbuomwan, L. *et al.*, 2018). Bacterial isolates were inoculated onto solidified nutrient agar streaking across the surface. Wells were bored into the media and the wells filled up with 0.02 mL of the extract. The plates were allowed to stand for 1-2 hours for proper absorption of the solution into the media and then incubated aerobically at 37°C for 12-24 hours. The sensitivity of the organisms to the extracts was recorded by measuring the zones of inhibition. The effects of the extracts on the isolated pathogens were compared with that of the control antibiotic; Ciprofloxacin.

IDENTIFICATION OF ISOLATES

Cultural Features and Gram Reaction

The isolates were identified based on their cultural features and Gram reaction, as described by (Cheesbrough, M. 2004).

ESCHERICHIA COLI:

- Cultural features - haemolytic and mucoid on blood agar. Lactose fermenting on MacConkey agar.
- Gram reaction – red rod and indole positive.

PSEUDOMONAS AERUGINOSA:

- Cultural features – flat, haemolytic and pigment-producing on blood agar. Non-lactose fermenting on MacConkey agar.
- Gram reaction – gram negative rod and oxidase positive.

PROTEUS SPECIES:

- Cultural features – characteristic fishy odour and swarming tendency on blood agar. Non-lactose fermenting on MacConkey agar.
- Gram reaction –pleomorphic rod and urease positive.

KLEBSIELLA PNEUMONIA:

- Cultural features – large lactose fermenting on MacConkey agar and large mucoid colonies on blood agar.
- Gram reaction – Gram negative capsulated rods

Representative colonies were picked using a heat sterilized wireloop and smeared on a clean, grease-free glass slide, air dried, heated and stained as described by (Cheesbrough, M. 2004).

Microscopy

The isolates were identified after staining using the Gram staining procedure under the oil immersion (100x) objective lens of a binocular microscope based on their Gram reaction.

Result: Gram negative bacteria retained the secondary stain and appeared red-pink.

Statistical Analysis

Data from the study were statistically analyzed by IBM SPSS version 25 using Analysis of Variance (ANOVA). The group mean zone of inhibition of the various extracts on the isolates were correlated with 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 twenty-seven (127) samples were collected from renal disease patients in Umuahia. Out of the 127 urine samples, 43(33.86%) samples yielded no significant growth whereas 84(66.14%) yielded significant growth (typical of bacteriuria). The predominant Gram negative isolate from the samples was *Escherichia coli* with a prevalence of 35(41.67%), while the least isolated Gram negative organism was *Proteus mirabilis* with a percentage distribution of 9(10.71%). Other Gram negative isolates include *Klebsiella pneumoniae* 24(28.57%) and *Pseudomonas aeruginosa* 16(19.05%).

Among the Gram negative isolates, *Klebsiella pneumoniae* had the highest mean zone of inhibition; 8.28, followed by *Proteus mirabilis*; (6.75) and *Pseudomonas aeruginosa*; (6.75) and *Escherichia coli;* (4.45). The control antibiotic had statistical significant difference ($P<0.05$) on the Gram negative isolates when compared with the aqueous and ethanolextracts. *Pseudomonas aeruginosa* and *Proteus mirabilis* were completely resistant to the antimicrobial activity of *Vernonia amygdalina* aqueous extract. At 50mg/mL, *Klebsiella pneumoniae* was found to be resistant while at 75-150 mg/mL concentrations, an insignificant mean zone of inhibition was noted. From the study, *Vernonia amygdalina* had a notable antimicrobial activity on Gram negative bacterial isolates. It is observed that there is no significant difference in the mean zones of inhibition of the Gram negative isolates in both the ethanolic and aqueous extracts of *Gongronema latifolium*. However, between the *Gongronema latifolium* extracts and the control; Ciprofloxacin, there is a significant difference. When the ethanolic and aqueous extracts of *Gongronema latifolium* and *Vernonia amygdalina* are compared with the control

antibiotic (Ciprofloxacin), a significant difference is seen with the control antibiotic performing far better than the extracts.

Table 1: Frequency Distribution of Gram Negative Bacterial Isolates from the urine samples collected from the Specialist Hospital, Owerri

Organism Isolated	Frequency	Percentage (%)
Escherichia coli	35	27.56
Klebsiella pneumoniae	24	12.60
Pseudomonas aeruginosa	16	18.90
Proteus mirabilis	9	7.09
Nil Growth	43	33.86
Total	**127**	**100**

Table 2: Antimicrobial activity of different concentrations of ethanol and aqueous extracts of *Vernonia amygdalina* (mean zone of inhibition) on the Gram negative bacterial isolates

Mean Zone of Inhibition in the Ethanolic Extracts (mm)	Concentration (mg/mL)	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Proteus mirabilis*
	50	1.78±0.8	2.0±0.8	2.0±0.0	3.0±0.0
	75	2.89±1.2	2.82±1.0	5.0±0.0	3.0±0.0
	100	3.0±1.1	2.64±1.0	5.0±0.0	3.0±0.0
	150	3.11±1.1	2.91±1.0	5.5±0.5	3.0±0.0
	Cipro (Control)	4.67±1.1	6.73±0.6	9.0±0.0	10.0±0.0
Mean Zone of Inhibition in the Aqueous Extracts (mm)	50	0.22±0.2	0	0	0
	75	0.22±0.2	0.09±0.1	0	0
	100	0.33±0.2	0.27±0.3	0	0
	150	0.33±0.2	0.27±0.3	0	0
	Cipro	4.67±1.1	6.73±0.6	9.0±0.0	10.0±0.0

Table 3: Antimicrobial activity of the different concentrations of the ethanol and aqueous extracts of *Gongronema latifolium* (mean zone of inhibition) on the Gram negative bacterial isolates

Mean Zone of Inhibition in the Ethanolic Extracts (mm)	Concentration (mg/mL)	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Proteus mirabilis*
	50	1.22±0.5	1.73±0.5	0.0	4.0±0.0
	75	1.44±0.6	2.09±0.5	1.0±0.0	4.0±0.0
	100	2.0±0.7	2.27±0.5	1.0±0.0	4.0±0.0
	150	2.11±0.8	2.45±0.6	1.0±0.5	4.0±0.0
	Cipro (Control)	4.67±1.0	6.73±0.6	9.0±0.0	10.0±0.0
Mean Zone of Inhibition in Aqueous Extracts (mm)	50	0.0	0	0	0
	75	0.0	0.0	0	0
	100	1.22±0.5	1.27±0.4	0	0
	150	1.78±0.7	1.91±0.6	0	0
	Cipro	4.67±1.0	6.73±0.6	9.0±0.0	10.0±0.0

Table 4: Antimicrobial activity of the different concentrations of the ethanol and aqueous extracts of *Vernonia amygdalina* and *Gongronema latifolium* (mean zone of inhibition) on the Gram negative bacterial isolates

Mean Zone of Inhibition in the Ethanolic Extracts (mm)	Concentration (mg/mL)	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Proteus mirabilis*
	50	0.89±0.4	1.36±0.4	2.0±0.0	2.0±0.0
	75	1.56±0.7	1.82±0.5	2.0±0.0	2.0±0.0
	100	2.22±0.6	2.09±0.5	2.0±0.0	2.0±0.0
	150	2.44±0.6	2.36±0.5	2.0±0.0	2.0±0.0
	Cipro (Control)	4.67±1.0	6.73±0.6	9.0±0.0	10.0±0.0
Mean Zone of Inhibition in the Aqueous Extract (mm)	50	0.11±0.1	0.27±0.2	0	0
	75	0.22±0.1	0.55±0.4	0	0
	100	0.44±0.2	0.82±0.5	0	0
	150	0.56±0.3	1.09±0.5	0	0
	Cipro	4.67±1.1	6.7±0.6	9.0±0.0	10.0±0.0

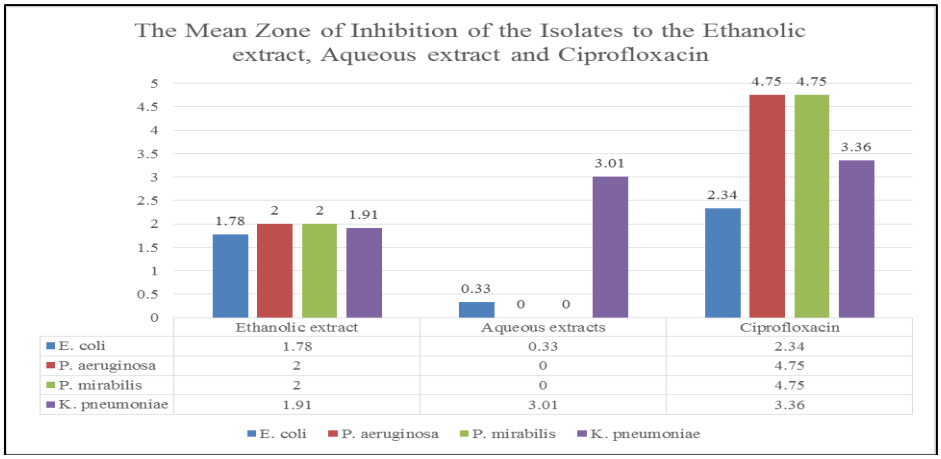

Figure 1: Chart showing the mean zones of inhibition of the different Gram Negative Bacterial Isolates to the different extracts and the Control antibiotic; Ciprofloxacin

DISCUSSION

This study reveals that the antibacterial quality of *Vernonia amygdalina* and *Gongronema latifolium* was found to depend on the nature of the solvents used for the extraction as well as the concentration of the bioactive components of the plants it can extract. In overall view, the ethanol extracts showed higher zones of inhibition than the aqueous extracts. This is attributed to the fact that ethanol extracted more of the bioactive components of the plants when compared to the aqueous extracts of the same concentrations. This finding is in agreement with a study by (National Population Census. 2006), which found out that ethanol extracts have more concentrations of the bioactive components than the aqueous extracts of the plants understudied.

From this study, it was discovered that *Vernonia amygdalina* has greater zone of inhibition than *Gongronema latifolium* which is in agreement with (Darwish, R. M. *et al*., 2010; Adetunji, C. O. *et al*., 2013; ICMSF. 1998). In this study, *Vernonia amygdalina* exhibited notable antibacterial activity on the Gram negative bacterial organisms. The resistance and low sensitivity seen in the Gram negative isolates may be due to the nature of their bacterial cell wall in addition to theirpeculiar cytoplasmic membrane. Most Gram negative bacterial organisms have a second thin phospholipid bilateral external to the peptidoglycan (outer membrane) which poses a barrier to the easy penetration of certain antibiotics. This finding is in agreement with the research conducted by (Barrow, G.H., & Feltham, R.K. 1993), which agreed that *Vernonia amygdalina* is very sensitive against Gram negative bacteria.

The most sensitive Gram negative organism to the ethanolic extracts of *Vernonia amygdalina* was*Klebsiella pneumoniae* (8.28), while*Escherichia coli* (4.45), *Pseudomonas aeruginosa* and *Proteus mirabilis* (6.75), respectivelywere found to be sensitive in the aqueous extracts with insignificant mean inhibition zone values. *Pseudomonas* aeruginosa and *Proteusmirabilis* were resistant. This is in concordance with the findings of (Adetunji, C. O. *et al*., 2013), which demonstrated that the ethanolic extracts of *Vernonia amygdalina* has the highest zones of inhibition on *Pseudomonas aeruginosa* while it is least sensitive to the aqueous extracts.

There was no significant difference from the zones of inhibition between ethanolic and aqueous extracts of *Gongronema latifolium*on the Gram negative isolates. However, in comparison with the control antibiotic, there is a significant difference in the zones of inhibition with the control performing better than the extracts.

It was also observed that the antimicrobial activity of the mixture of equal volumes of *Vernonia amygdalina* and *Gongronema latifolium* on the respective Gram negative isolates were greater than the inhibition zones obtained from only *Gongronema latifolium* while it was reduced in the inhibition zones obtained from only *Vernonia amygdalina*. This may be attributed to the fact that the solution had higher concentrations of the bioactive components found in *Vernonia amygdalina*.

These plants which are commonly used as vegetables and spices in local cuisines, have in recent studies and this very present one reported to possess antibacterial and nephro-protective properties. Therefore, should be incorporated regularly in the daily meals of patients with risk factor to develop renal diseases, if possible. Suggestively, if considered to serve antimicrobial purposes, they should not be combined but instead used separately so that compliance to dietary therapy can be evaluated properly.

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