

# Comparative Antibiotic Activities of Ethanol Extracts of Leaves and Inflorescences of *Mitracarpus villosus* from two different Geographical Regions in Nigeria in Synergy and Antagonism with Conventional Antibiotics

Odima, C. A.<sup>1</sup>, Ubani, C. S.<sup>1</sup>, Eze, E. A.<sup>2</sup>, Oje, O. E.<sup>1</sup>, Agu, E. C.<sup>2</sup> & Joshua, P. E.<sup>1</sup>

1, Department of Biochemistry, University of Nigeria, Nsukka, Nigeria.

2, Department of Microbiology, University of Nigeria, Nsukka, Nigeria.

E-mail: charlesmariaaka@yahoo.com ; chike.ubani@unn.edu.ng

## Abstract

Ethanol extracts of leaves and inflorescences of *Mitracarpus villosus* from Ikom and Nsukka in the South-South and South-East geographical regions of Nigeria respectively, were analyzed for bioactivity using agar well diffusion method against four test organisms: *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus*. Results obtained revealed that the geographical location of the plant as well as its soil composition affects the quantity and quality of secondary metabolites produced by the plant. Results of quantitative phytochemical and soil analysis showed an inverse relationship between certain soil parameters and constituent phytochemicals. Ikom sample has higher nitrogen:  $0.160 \pm 0.001$ MEQ/100g compared with Nsukka sample with nitrogen content of  $0.065 \pm 0.007$ MEQ/100g. The constituent cyanide in Ikom sample:  $0.078 \pm 0.000$ mg/100g is lower than that of Nsukka sample which is  $0.127 \pm 0.000$ mg/100g. Also alkaloid of Ikom sample:  $1.620 \pm 0.004$ mg/100g is lower than Nsukka sample:  $2.107 \pm 0.003$ mg/100g. Soluble carbohydrate in Ikom sample,  $0.955 \pm 0.000$ mg/100g is lower than Nsukka sample containing  $1.915 \pm 0.000$ mg/100g. Inhibition zone diameters (IZDs) of Ikom extracts and Nsukka extract showed that Nsukka extract with IZD  $27.5 \pm 0.07$ mm has higher bioactivity than Ikom extract with IZD =  $14.5 \pm 0.07$ mm against *K. pneumoniae* at concentration of 100.000mg/ml. At concentration of 12.500mg/ml, Ikom extract had no inhibition on all test organisms, but Nsukka extract had IZDs of  $12.000 \pm 0.000$ mm and  $7.000 \pm 0.000$ mm against *E. coli* and *S. aureus* respectively. The MICs for Nsukka extract against *E. coli* = 12.500mg/ml and *S. aureus* = 12.500mg/ml are lower than that of Ikom extract: *E. coli* = 50.000mg/ml and *S. aureus* = 25.000mg/ml. These imply that Nsukka extract is more potent than Ikom extract against *E. coli* and *S. aureus*. In this *in vitro* study, synergism and antagonism have been consistent with the plant extracts against different test organisms. Nitrofurantoin 10 $\mu$ g, had synergy with both extracts against *E. coli*. Ceftriaxone 30 $\mu$ g had synergy with both extracts against *S. aureus* while Ciprofloxacin 10 $\mu$ g had synergy with both extracts against *K. pneumoniae* and *P. aeruginosa*. However, antibiotic antagonism was observed with different conventional antibiotics: Ceftriaxone 30 $\mu$ g showed antagonism with both extracts against *E. coli*, *K. pneumoniae* and *P. aeruginosa*. Gentamycin 10 $\mu$ g also showed antagonism with extracts against *S. aureus* and *P. aeruginosa*.

**Keywords:** Bioactivity, metabolites, phytochemicals, synergy, antagonism, inhibition.

## INTRODUCTION

Herbal medicine has been a long practice in Africa and is increasingly gaining grounds worldwide over orthodox medicine which is fast becoming unreliable as far as treatment of some diseases is concerned due to drug resistance and rapid mutations of the target organism. Man has always relied on the efficacy of herbs as antimicrobial, antiprotozoan, antihelminthic, muscle relaxant and vasodilatory agents.

Many animal model investigations or *in vitro* plant assays by herbalist and ethnopharmacologist reveal that there are many plants which are of immense medical importance and some of such plants are active even better than their conventional orthodox counterparts (Ernst, 2007). Plants have evolved the ability to synthesize chemical compounds that help them defend against attack from a wide variety of predators such as insects, fungi and herbivorous mammals (Tapsell, *et al.*, 2006). Some of these compounds whilst being toxic to plant predators turn out to have beneficial effects when used to treat human diseases. Such secondary metabolites are highly varied in structure, many are aromatic substances, most of which are phenols or their oxygen-substituted derivatives. Phytochemicals are naturally occurring, non-nutritive chemicals. They appear to work alone and in combination, and perhaps in conjunction, with vitamins and other nutrients in food to prevent, halt, or lessen disease. The functions of secondary metabolites are varied. For example, some secondary metabolites are toxins used to deter predation, and others are pheromones used to attract insects for pollination. Phytoalexins protect against bacterial and fungal attacks. Allelochemicals inhibit rival plants that are competing for soil and light.

Rise in the number of antibiotic-resistant pathogens has left clinicians with no option than to turn to multi-drug treatment to control infection (Levy and Marshall, 2004). The inhibitory effect of two drugs in combination can be larger or smaller than expected from their individual effects, corresponding to synergistic or

antagonistic interactions between the drugs respectively (Keith, *et al.*, 2005).

Synergistic interactions are usually thought of as advantageous since, for a given amount of drug, they more effectively inhibit the growth of drug-sensitive pathogens. However, *in vitro* studies have suggested that, for the same level of inhibition, more synergistic drug pairs may foster antibiotic resistance (Michel, *et al.*, 2008). Antagonistic drug combinations, on the other hand, are less effective at inhibiting drug-sensitive pathogens, but can reduce and even invert the selective advantage of single-drug resistant mutants, causing selection against resistance (Chait, *et al.*, 2007).

Most herbs used as antimicrobial agents are in most cases affected by the soil composition. Soils polluted by chemicals or sprayed with chemical weedkiller or fungicides are known to accumulate such chemicals which in turn affect the quality of phytochemicals produced by these plants.

*Mitracarpus villosus* is one of such plants believed to have some medicinal values. In Bokomo village, a traditional community in Ikom, central senatorial district of Cross River State, Nigeria; the plant was used externally after squeezing to rub on eczema inflamed skin. It causes serious skin lesion resulting in the peeling of the inflamed area and complete healing after 48-72hours of topical application.

## RESEARCH OBJECTIVES

- To determine the antibiotic susceptibility of Gram positive and Gram negative bacteria to ethanol extract of leaves and inflorescence of *Mitracarpus villosus* plant from South – South and South - East Geographical regions of Nigeria.
- To carry out a quantitative phytochemical analysis of the plant samples from the two regions.
- To carry out a preliminary quantitative soil analysis to check for the effect of soil on the phytochemicals from the two locations.
- To compare the effect of extract in synergy and/or antagonism with conventional antibiotics.

## MATERIALS AND METHODS

### COLLECTION OF PLANT MATERIALS/SAMPLES

The plant materials were harvested from two different geographical regions in Nigeria; Ikom and Nsukka in the South – South and South-East geographical regions of Nigeria respectively. Ikom lies in latitude 5° 58' 0"N of the equator and Longitude 8° 42' 0"E of the Greenwich meridian. Nsukka lies in latitude 6° 52' 0"N along the equator and longitude 7° 23' 0"E of the Greenwich meridian (Travelmat, 2011). Also soil sample was each collected at a depth of 40cm from the surface soil at the different locations where the plant materials were harvested and taken for quantitative analysis.

### IDENTIFICATION OF PLANT

The plant was identified in local Bokomo community by the village head, chief Joseph Aka Odima as “Riwa nkpufeb” in Nkome language and also in the plant anatomy section of Botany Department, University of Nigeria, Nsukka as *Mitracarpus villosus*.

Current name: *Mitracarpus villosus*  
Old name: *Mitracarpus scarba*  
Common name: Square shape  
Nkome(Ikom): Riwa nkpufeb  
Igbo(Nsukka): Ogwu ngwo

The test organisms were obtained from the Post Graduate Laboratory in Microbiology Department, University of Nigeria, Nsukka. They include: *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

### PREPARATION OF EXTRACT

Sigma Aldrich Absolute Ethanol was used in the extraction of the dried plant powder and the yields were:

Ikom Sample: 84g of dried plant powder = 5.0g of extract  
Nsukka Sample: 41g of dried plant powder = 2.1g of extract

Kermel Dimethylsulfoxide (DMSO) was used in the preparation of 2-fold dilution of plant extracts at 60%(v/v) with sterile distilled water.

Initial concentration: one gram of extract each and 10ml of DMSO(60%) (100mg/ml) was further diluted to yield other concentrations; 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 6.125mg/ml.

### PREPARATION OF MUELLER HINTON BROTH AND MUELLER HINTON AGAR

Mueller Hinton broth prepared in accordance with manufacturer's specifications was sterilized and dispensed into test tubes (sterile), inoculated and incubated to revive the microbial stock.

4.56g of M. H. agar was dissolved in 120ml of distilled water and then sterilized using autoclave at

121°C for 15 minutes. The sterile media was poured into sterile Petri dishes and allowed to solidify. The solid media was placed on the table and allowed for 24 hours to check for sterility.

The broth culture was standardized using 0.5 McFarland turbidity standard and then used to inoculate the plates. Agar wells were subsequently created on the plates using 6mm cork borer. Different dilutions of the extracts were separately introduced into the appropriately labeled agar wells. The plates were incubated (face up) at 37°C for 24 hours.

#### DILUTION OF EXTRACT FOR SYNERGISTIC OR ANTAGONISTIC INTERACTION

Evaluation of extracts for synergistic (or antagonistic) interaction with conventional antibiotics was carried out as earlier described (Nweze and Onyishi, 2011) with slight modification. Briefly, 2 ml of the chosen reconstituted extract was mixed with molten agar in such a way as to achieve the desired final concentration and allowed to gel together in the Petri dishes. The dishes containing the media were seeded differently with separate organisms. This was allowed to dry (5minutes), after which appropriate antibiotic disc with desired drug concentrations were placed on top of the culture plates and incubated for 24 hours at 37°C.

A quantitative phytochemical and soil analyses were carried out on the plant and soil samples respectively from Ikom and Nsukka. These chemical tests were carried out according to the methods described by Harbone (1973) as modified by Trease and Evans (1982). The relative concentration of each constituent was inferred from the intensity of the reaction.

#### RESULTS AND ANALYSIS

**Table 1: Quantitative phytochemical analysis of Ikom and Nsukka Samples**

|                               | SAMPLE | Quantity (Mean±SD) |
|-------------------------------|--------|--------------------|
| SOLUBLE CARBOHYDRATE(mg/100g) | IKOM   | 0.955±0.000        |
|                               | NSUKKA | 1.910±0.000        |
| CYANIDE(mg/100g)              | IKOM   | 0.078±0.000        |
|                               | NSUKKA | 0.128±0.000        |
| SAPONIN(mg/100g)              | IKOM   | 0.222±0.000        |
|                               | NSUKKA | 0.141±0.000        |
| TANNIN(mg/100g)               | IKOM   | 2.457±0.000        |
|                               | NSUKKA | 2.540±0.003        |
| FLAVONOID(mg/100g)            | IKOM   | 2.375±0.003        |
|                               | NSUKKA | 2.414±0.001        |
| GLYCOSIDE(mg/100g)            | IKOM   | 1.435±0.003        |
|                               | NSUKKA | 1.378±0.003        |
| ALKALOID(mg/100g)             | IKOM   | 1.626±0.004        |
|                               | NSUKKA | 2.107±0.003        |
| STERIOD(mg/100g)              | IKOM   | 2.079±0.000        |
|                               | NSUKKA | 2.136±0.000        |
| REDUCING SUGAR(mg/100g)       | IKOM   | 543.476±0.003      |
|                               | NSUKKA | 344.383±0.002      |
| TERPENOID(mg/100g)            | IKOM   | 0.234±0.004        |
|                               | NSUKKA | 0.264±0.004        |

**Table 2: Quantitative Soil Analysis of Soil samples from Ikom and Nsukka**

|                | Samples | Quantity (Mean±SD) |
|----------------|---------|--------------------|
| Clay           | IKOM    | 34.000±0.000       |
|                | NSUKKA  | 4.000±0.000        |
| Silt           | IKOM    | 29.000±0.000       |
|                | NSUKKA  | 2.000±0.000        |
| fine sand      | IKOM    | 13.500±0.707       |
|                | NSUKKA  | 41.000±0.000       |
| coarse sand    | IKOM    | 23.500±0.707       |
|                | NSUKKA  | 52.50±0.707        |
| Kcl            | IKOM    | 5.150±0.707        |
|                | NSUKKA  | 5.750±0.707        |
| carbon         | IKOM    | 0.985±0.707        |
|                | NSUKKA  | 0.755±0.707        |
| organic matter | IKOM    | 1.710±0.014        |
|                | NSUKKA  | 1.305±0.007        |
| Nitrogen       | IKOM    | 0.160±0.014        |
|                | NSUKKA  | 0.065±0.007        |
| Sodium         | IKOM    | 0.470±0.014        |
|                | NSUKKA  | 0.370±0.014        |
| potassium      | IKOM    | 0.165±0.007        |
|                | NSUKKA  | 0.175±0.007        |
| Calcium        | IKOM    | 2.300±0.141        |
|                | NSUKKA  | 1.000±0.000        |
| Magnesium      | IKOM    | 1.500±0.141        |
|                | NSUKKA  | 0.700±0.141        |
| CEC            | IKOM    | 30.400±0.000       |
|                | NSUKKA  | 12.400±0.000       |
| avail P        | IKOM    | 14.700±0.141       |
|                | NSUKKA  | 3.500±0.141        |

**Table 3a: Inhibition Zone Diameters of Different Concentrations of Ikom Extract**

| Organisms            | IZDs (mm)(Mean±SD) |           |           |             |             |              |
|----------------------|--------------------|-----------|-----------|-------------|-------------|--------------|
|                      | 100(mg/ml)         | 50(mg/ml) | 25(mg/ml) | 12.5(mg/ml) | 6.25(mg/ml) | 3.125(mg/ml) |
| <i>P. aeruginosa</i> | 18.5±0.07          | 15.5±0.07 | 12.5±0.07 | R           | R           | R            |
| <i>K. pneumonia</i>  | 14.5±0.07          | 11.5±0.07 | R         | R           | R           | R            |
| <i>E. coli</i>       | 16.5±0.07          | 16.5±0.21 | R         | R           | R           | R            |
| <i>S. aureus</i>     | 18.5±0.07          | 14.5±0.07 | 12±0.14   | R           | R           | R            |

Key: R= resistant

**Table 3b: Inhibition Zone Diameters of Different Concentrations of Nsukka Extract**

| Organisms            | IZDs (mm)(Mean±SD) |           |           |             |             |              |
|----------------------|--------------------|-----------|-----------|-------------|-------------|--------------|
|                      | 100(mg/ml)         | 50(mg/ml) | 25(mg/ml) | 12.5(mg/ml) | 6.25(mg/ml) | 3.125(mg/ml) |
| <i>P. aeruginosa</i> | 21.5±0.07          | 15.5±0.07 | R         | R           | R           | R            |
| <i>K. pneumoniae</i> | 27.5±0.07          | 22.5±0.07 | R         | R           | R           | R            |
| <i>E. coli</i>       | 26.5±0.07          | 24±0.14   | 18.5±0.07 | 12±0.00     | R           | R            |
| <i>S. aureus</i>     | 26.5±0.07          | 21.5±0.07 | 15.5±0.07 | 7±0.00      | R           | R            |

Key: R= resistant

From the results there is significant difference at  $p < 0.05$  in the mean IZDs between Ikom and Nsukka extracts against *E. coli* at concentrations of 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml. Also there is significant difference in the mean IZDs for *S. aureus* at concentrations of 100mg/ml, 50mg/ml and 25mg/ml likewise *K. pneumonia* at concentrations of 100mg/ml and 50mg/ml.

**Table 4: Inhibition Zone Diameters of Conventional Antibiotic disc on test organisms**

| Organisms            | IZDs (mm) of Antibiotics (µg) |       |        |       |       |      |       |       |      |
|----------------------|-------------------------------|-------|--------|-------|-------|------|-------|-------|------|
|                      | N100                          | CT 30 | CIP 10 | GN 10 | OF 10 | C 10 | PF 30 | CM 30 | AM30 |
| <i>P. aeruginosa</i> | R                             | 25    | R      | 18    | R     | 25   | R     | 23    | R    |
| <i>K. pneumoniae</i> | R                             | 25    | R      | R     | R     | 23   | R     | 22    | R    |
| <i>E. coli</i>       | R                             | 17    | R      | R     | R     | R    | R     | R     | R    |
| <i>S. aureus</i>     | R                             | R     | 28     | 27    | R     | R    | 23    | R     | R    |

Key: N100= Nitrofurantoin100µg; GN10= Gentamicin10µg; PF30=Pefloxacin30 µg; CT30=Cetriaxone30µg; CIP10=Ciprofloxacin10µg; C10=Chloramphenicol10µg; OF=Ofloxacin10µg; CM30=Clarithomycin30µg; AM30=Ampicilin30µg; R=Resistant.

**Table 5a: IZDs of Ikom Extract (6.25mg/ml) + Conventional Antibiotics**

| Organisms            | IZDs(mm) |      |        |       |       |      |       |      |      |
|----------------------|----------|------|--------|-------|-------|------|-------|------|------|
|                      | N100     | CT30 | CIP 10 | GN 10 | OF 10 | C 10 | PF 30 | CM30 | AM30 |
| <i>P. aeruginosa</i> | R        | 12   | 12     | 14    | R     | R    | R     | R    | R    |
| <i>K. pneumoniae</i> | R        | R    | 17     | R     | R     | R    | 14    | R    | R    |
| <i>E.coli</i>        | 16       | R    | R      | R     | R     | R    | R     | R    | R    |
| <i>S. aureus</i>     | R        | 16   | D      | D     | D     | R    | R     | R    | R    |

Key: N100= Nitrofurantoin100µg; GN10= Gentamicin10µg; PF30=Pefloxacin30 µg; CT30=Cetriaxone30µg; CIP10=Ciprofloxacin10µg; C10=Chloramphenicol10µg; OF=Ofloxacin10µg; CM30=Clarithomycin30µg; AM30=Ampicilin30µg; R=Resistant.  
 D= Delayed inhibition (after 48hours incubation)

**Table 5b: IZDs of Nsukka Extract (6.25mg/ml) + Conventional Antibiotics**

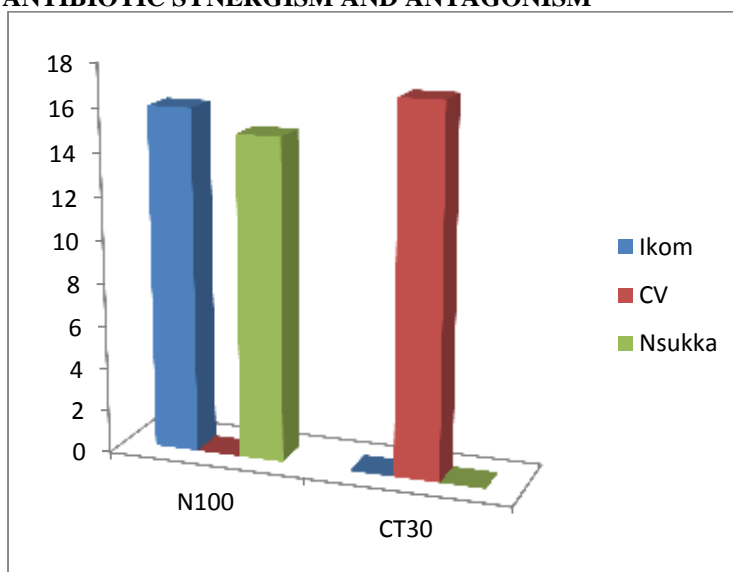
| Organisms            | IZDs(mm) |      |       |       |       |      |       |      |      |
|----------------------|----------|------|-------|-------|-------|------|-------|------|------|
|                      | N100     | CT30 | CIP10 | GN 10 | OF 10 | C 10 | PF 30 | CM30 | AM30 |
| <i>P. aeruginosa</i> | R        | 12   | 13    | 13    | R     | R    | R     | R    | R    |
| <i>K. pneumoniae</i> | R        | 14   | 15    | R     | R     | R    | R     | R    | R    |
| <i>E.coli</i>        | 15       | R    | R     | R     | R     | R    | R     | R    | R    |
| <i>S. aureus</i>     | R        | 21   | 20    | 21    | 14    | R    | R     | R    | R    |

Key: N100= Nitrofurantoin100µg; GN10= Gentamicin10µg; PF30=Pefloxacin30 µg; CT30=Cetriaxone30µg; CIP10=Ciprofloxacin10µg; C10=Chloramphenicol10µg; OF=Ofloxacin10µg; CM30=Clarithomycin30µg; AM30=Ampicilin30µg; R=Resistant.

**Table 6: Minimum Inhibitory concentrations (MICs) of different Extracts A & B on the test organisms**

| ORGANISMS            | MIC OF EXTRACT A (mg/ml) | MIC OF EXTRACT B (mg/ml) |
|----------------------|--------------------------|--------------------------|
| <i>E. coli</i>       | 50                       | 12.5                     |
| <i>P. aeruginosa</i> | 25                       | 50                       |
| <i>K. pneumoniae</i> | 50                       | 50                       |
| <i>S. aereus</i>     | 25                       | 12.5                     |

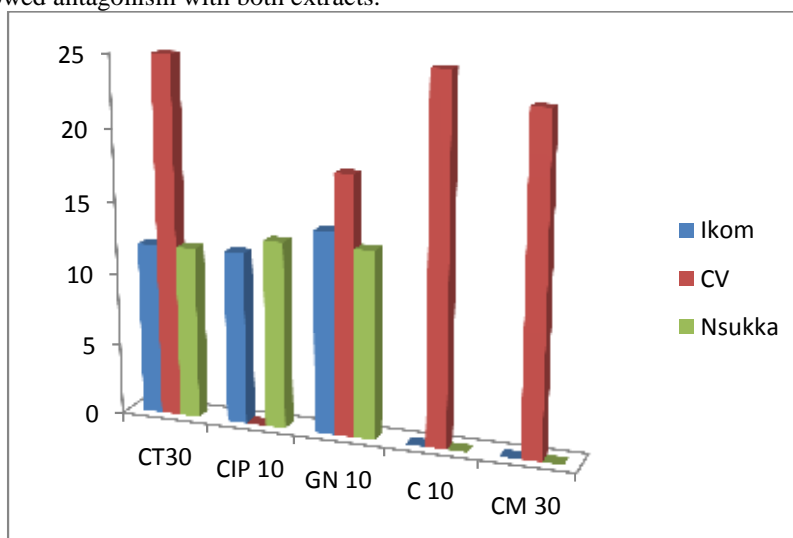
**EVALUATION OF ANTIBIOTIC SYNERGISM AND ANTAGONISM**



**Figure 1: IZD OF COMBINED EXTRACTS WITH CONVENTIONAL ANTIBIOTICS AGAINST *E. coli* SHOWING SYNERGISM AND ANTAGONISM**

Key: N100=Nitrofurantoin 100ug; CT30= Ceftriaxone 30ug; CV =conventional antibiotics

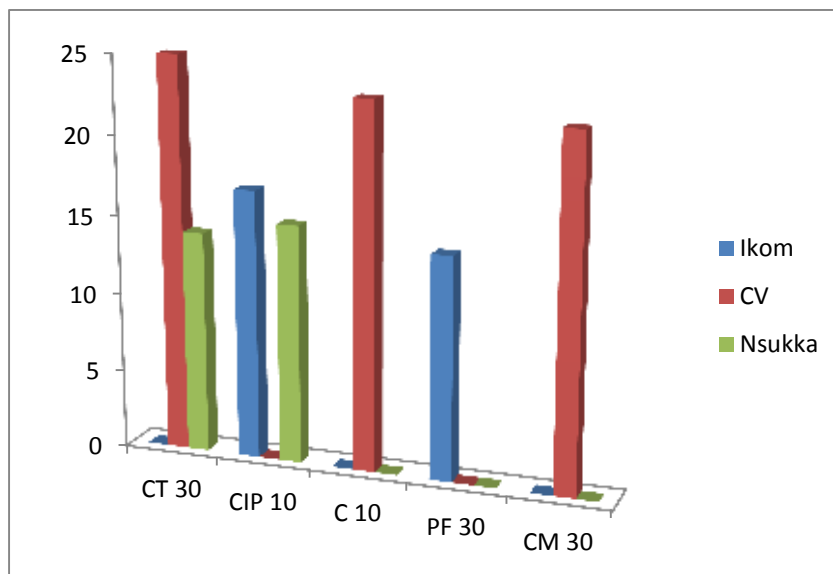
From figure 1 above it can be seen clearly that while N100 gave synergistic interaction with both extracts, CT30 showed antagonism with both extracts.



**Figure 2: IZD OF COMBINED EXTRACTS WITH CONVENTIONAL ANTIBIOTICS AGAINST *P. aeruginosa* SHOWING SYNERGISM AND ANTAGONISM**

Key: GN10= Gentamicin10µg; CT30=Ceftriaxone30µg; CIP10=Ciprofloxacin10µg; C10=Chloramphenicol10µg; CM30=Clarithomycin30µg; CV=conventional antibiotics

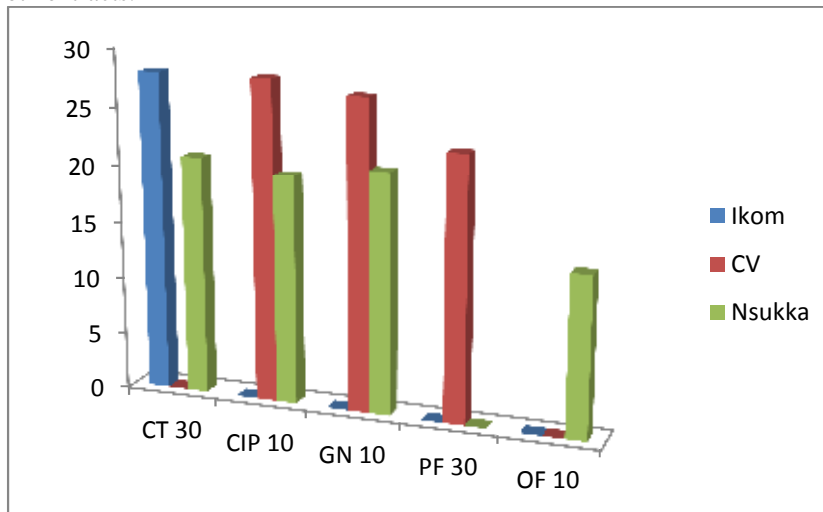
From figure 2 CIP10 had synergistic interaction with both extracts against *P. aeruginosa*, others CT30, GN10, C10 and CM30 respectively showed antagonism with both extracts against the test organism.



**Figure 3: IZD OF COMBINED EXTRACTS WITH CONVENTIONAL ANTIBIOTICS AGAINST *K. pneumoniae* SHOWING SYNERGISM AND ANTAGONISM**

Key: CT30=Ceftriaxone30µg; CIP10=Ciprofloxacin10µg; C10=Chloramphenicol10µg; Perfloracin 30µg; CM30=Clarithomycin30µg; CV=conventional antibiotics

From figure 3 above, with CT30, although both extracts showed antagonism, while Ikom extract was completely antagonized, Nsukka extract was only partially antagonized. CIP10 had synergism with both extracts. C10 showed complete antagonism with both extracts. PF30 combined with Ikom extract showed synergism but with Nsukka extract there was no interaction. CM30 had antagonistic interaction with both extracts.

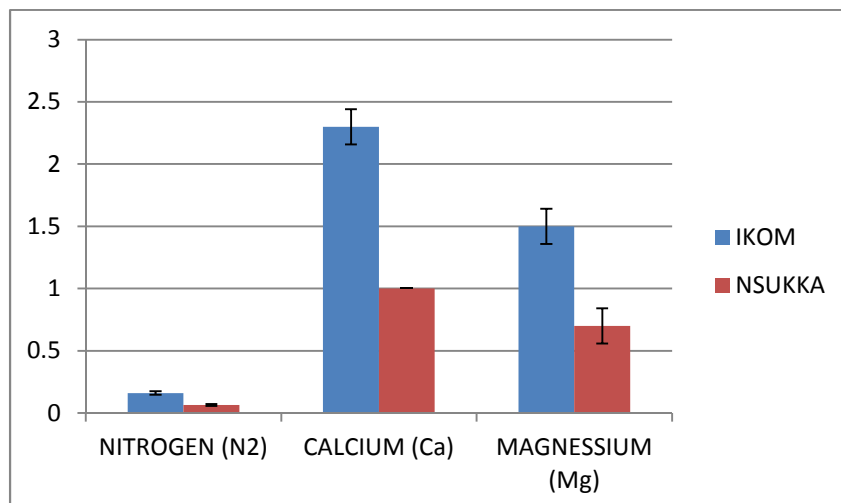


**Figure 4: IZD OF COMBINED EXTRACTS WITH CONVENTIONAL ANTIBIOTICS AGAINST *S. aureus* SHOWING SYNERGISM AND ANTAGONISM**

Key: CT30=Ceftriaxone30µg; CIP10=Ciprofloxacin10µg; Gentamycin10µg; Perfloracin 30µg; Ofloxacin10µg; CV=conventional antibiotics

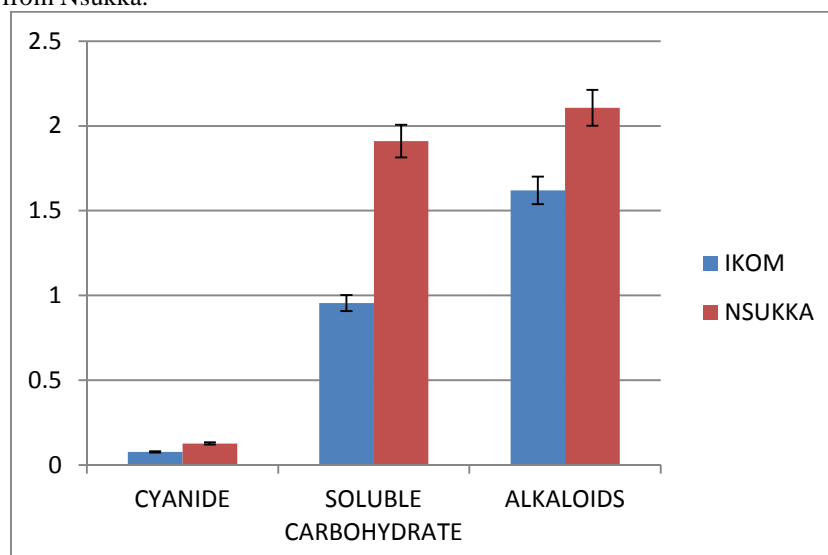
CT30 showed synergism with both extracts. CIP10 and GN10 showed complete antagonism with Ikom extract but only partial antagonism with Nsukka extract respectively. PF30 showed antagonism with both extracts. OF10 with Ikom extracts had no interaction but showed synergism with Nsukka extract.





**Figure 5:** VARIATION IN SOIL ELEMENT CONCENTRATION IN THE TWO SOIL SAMPLES

From figure 5 above, it follows that soil sample from Ikom had more nitrogen, calcium and magnesium than soil sample from Nsukka.



**Figure 6:** VARIATION IN SOME CONSTITUENT PHYTOCHEMICALS IN THE TWO PLANT SAMPLES

The figure 6 above shows that sample from Nsukka had greater amount of cyanide, soluble carbohydrate and alkaloids compared with sample from Ikom.

## DISCUSSIONS AND CONCLUSION

*Mitracarpus villosus* is one of the numerous plants with antibiotic activities against certain bacteria. Quantitative phytochemical and soil analysis of the plant and soil samples respectively from Ikom and Nsukka shows that the plant phytochemicals have an inverse relationship with some soil parameters especially nitrogen which is a constituent of cyanide and alkaloids. While Ikom soil sample has higher mineral elements like Nitrogen;  $0.160 \pm 0.001$  MEQ/100g resulting from use of NPK fertilizer frequently by farmers in Ikom to improve soil fertility, Nsukka plant sample has higher amount of constituent phytochemicals. Ikom sample Cyanide  $0.078 \pm 0.000$  mg/100g is lower than Nsukka sample  $0.128 \pm 0.000$  mg/100g and also alkaloids of Ikom plant sample;  $1.620 \pm 0.004$  mg/100g is lower than Nsukka sample:  $2.107 \pm 0.003$  mg/100g. Soluble carbohydrate in Ikom sample  $0.955 \pm 0.000$  mg/100g is also lower than Nsukka sample  $1.911 \pm 0.000$  mg/100g.

These results are comparable with phytochemical analysis of the same plant by Okoye and Kenechi (2009) in Anambra, South-East geographical region with alkaloids ( $5.090 \pm 0.080\%$ ) and cyanide ( $12.070 \pm 0.060\%$ ). These results show that the South-East geographical region with higher yield of bioactive compounds is a better source for the plant material for medical or industrial uses. These variations however, may result from



the fact that excess of nitrogen upsets the balance between organic and inorganic nitrogen compounds, ultimately leading to greater release of ammonium which is deposited in the soil. This in turn leads to soil acidification due to nitrification processes, and loss of minerals. So the indirect effects of nitrogen can also affect the life of all the soil microorganisms. Nitrogen in soil can exist in different forms. For instance, in its gas form ( $N_2$ ) which can be present in the air spaces of the soil, plants can't absorb it. The most useful forms are nitrates ( $NO_3^-$ ), nitrites ( $NO_2^-$ ) and ammonium ( $NH_3$ ). Soil carbon to nitrogen ratio (C:N ratio = 12:1) is reduced with excess nitrogen and this adversely affects the population of bacteria growing in such soils (Sachs, 1999).

Mean Inhibition zone diameters of Ikom extracts and Nsukka extract showed that Nsukka extract with IZD:  $27.50 \pm 0.070$ mm in table 3b has higher activity than Ikom extract IZD:  $14.50 \pm 0.070$ mm seen in table 3a against *K. pneumoniae* at concentration of 100mg/ml. Also while at concentration of 12.5mg/ml, Ikom extract had no inhibition on all test organisms, Nsukka extract had IZD;  $12.00 \pm 0.000$ mm and  $7.00 \pm 0.000$ mm against *E. coli* and *S. aureus* respectively. Results obtained on bioactivity of the same plant in Anambra state by Okoye and Kenechi (2009) showed IZDs (18.00mm) and (23.00mm) against *E. coli* and *S. aureus* respectively. Also in Niger state a savanna belt region, IZDs against different bacteria strains ranges from 8–23mm against *E. coli* and *S. aureus* (Irobi, and Daramola, 1994). These corresponds to results obtained from Nsukka showing that *Mitracarpus villosus* from the South-East region (savanna belt) has better bioactivity than Ikom in the South-South (tropical rain forest) against the test microorganisms. The MICs for Nsukka extract against *E. coli*= 12.5mg/ml and *S. aureus*= 12.5mg/ml are lower than MICs for Ikom extract: *E. coli*; 50mg/ml and *S. aureus*; 25mg/ml seen in table 6. These implies that Nsukka extract is preferable over Ikom extract against *E. coli* and *S. aureus*.

In this *in vitro* studies, synergism and antagonism have been consistent with the plant extracts against different test organisms. Against *E. coli*, Nitrofurantoin 10 $\mu$ g, had synergism with both extracts. Ceftriaxone 30 $\mu$ g had synergism with both extracts against *S. aureus*. While Cirpofloxacin 10 $\mu$ g had synergism with both extracts against *K. pneumoniae* and *P. aeruginosa*. However, antibiotic antagonism was also observed with different conventional antibiotics: Ceftriaxone 30 $\mu$ g showed antagonism with both extracts against *E. coli*, *K. pneumoniae* and *P. aeruginosa*. Gentamycin 10 $\mu$ g also showed antagonism with extracts against *S. aureus* and *P. aeruginosa*.

In conclusion, *Mitracarpus villosus* possess phytochemicals of medical importance which are affected by the mineral and organic composition of the soil on which they grow. However, further studies have to be carried out to isolate, characterize and elucidate the structures of the bioactive compounds from the plant for industrial drug formulation. Hence, from this research finding, South-East geographical regions (savanna belt) like Nsukka is preferable over South-South regions (tropical rain forest) like Ikom in the use of *Mitracarpus villosus* for treatment of bacterial infections and also for industrial drug formulation because of higher bioactivity of Nsukka plant sample compared with Ikom plant sample.

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