# Assessment Of The Antibacterial Activity Of Vernonia Amygdalina And Occimum Gratissimum Leaves On Selected Food Borne Pathogens

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**Citation**

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**Abstract**

The bioactive compounds activities of leaves extracts of two Nigerian edible vegetables were carried out by agar well diffusion method on selected food borne pathogens of medical significance. Both aqueous and ethanolic extracts of these plants leaves were tested against E.coli, Staphylococcus aureus, Bacillus cereus Shigella dysentriae and
Salmonella typhimurium with the later showing better and significant anitibacterial activity on all the test isolates. The minimum inhibitory concentration (MIC) of both extracts ranged between 7.5mg/ml -25mg/ml for aqueous extract and 12.5mg/ml -15mg/ml for ethanolic extract. The extracts of these plants may be used to treat gastroenteritis at the various concentration used for this work.

## INTRODUCTION

The search for newer sources of antibiotics is a global challenge preoccupying research institution, pharmaceutical companies and academia, since many infection agents are becoming resistance to synthetic drugs(Latha and Kannabiran,2006).Plants have the major advantage of still being the most effective and cheaper alternative sources of drugs. The local use of natural plants as primary health remedies due to their pharmacological properties is quite common in Asia, Latin America and Africa (Bibitha et al,2002)Many plants are consumed as food without in-depth knowledge of their exact chemical composition and contribution to health, although their utilization has passed through several ancestral generations who probably realized from experience that those plant food materials are beneficial (Ghani et al, 1989).

Traditional therapy involves the use of plant exacts or their active principles which may serve as a source of modern drugs and source of intermediate compounds for synthesizing analog drugs with more desirable properties (Akerele, 1993). The development of medicinal chemistry, as a major route for the discovery of novel and more active therapeutic agents, further investigation into the chemical and biological activities of the plants needed to be carried out (Rao & Roja, 2002). The work is to evaluate the antibacterial activity of these two Nigerian edible vegetables on selected food borne pathogens of medical significance as the results will increase the knowledge on the tradomedical use of these plants.

## MATERIALS AND METHODS

COLLECTION OF PLANT MATERIALS: Fresh, pesticide free leaves of both plants were collected from University of Agriculture, Abeokuta Ogun State.

TEST ORGANISMS: The test organisms used for the study were medical isolates collected from the microbiology unit of University of Lagos, Teaching Hospital, Idiaraba, Lagos. Their identification was re-affirmed using standard methods of Cheesebroug (1991).

EXTRACTION :( Aqueous and Ethanolic Extraction): The extracts were prepared as described by Madunagu et al (2001). The leaves samples were thoroughly rinsed with sterile distilled water and sodium hypochlorite for surface sterilization. 20g of ground pulp of each plant's leaves were added to 100mlof sterile distilled water and ethanol (95%) for 72hours. The extracts were filtered using what man no.1 filter paper and membrane filter for sterilization. The extracts were concentrated with rotary evaporator before been stored in the refrigerator at 40c prior to use.

PHYTOCHEMICAL SCREENING: The extracts were re-screened for bioactive agent according to the methods described by Jigna et al (2006). Components screened for were saponin, tannins, alkaloids, polyphenols, flavonoids, glycoside, coumarine and reducing compounds.

ANTIMICROBIAL SUSCEPTIBILITY TESTING: Five different concentrations of the extracts were prepared 10mg/ml, 20mg/ml, 30mg/ml, 40mg/ml, and 50mg/ml and tested against the tests organism by agar-well diffusion method of Aida et al ;(2001) A combination of equal volumes and concentration of extracts of the two plants were used to repeat the evaluation of the antibacterial activity of the extracts using the methods described earlier.

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION: The lowest concentration of the extracts that will inhibit the growth of test organisms (MIC) was determined by the methods of Atlas, (1995).

## RESULTS AND DISCUSSION

Table 1 indicated the result of the phytochemical screening of the two plants. None of the extracts contained steroid. Flavonoids and glycosides are more significant in the extracts of both plants. Table 2 showed the result of the antibacterial activity of the plants extracts at various concentrations .It was found out that the higher the concentration of the extract, the higher the diameter of zones of the inhibition. Table 3 showed the minimum inhibitory concentration (MIC) of the extracts. The MIC were determined to be 25mg/ml for aqueous extracts of the two plants on Shigella dysentriae and Salmonella typhimurium, 15mg/ml for Bacillus cereus and 7.5mg/ml for E. coli and Staphylococcus aureus. It was found out to be 12.5mg/ml for ethanolic extract of V. amygdalina for E. coli, Staph. aureus, B. cereus and 15mg/ml for Shigella dysenteriae and Salmonella typhimurium. The ethanolic extract of O. gratissimum was found out to have the MIC of 12.5mg/ml for all the test isolates. Table 4 showed the results of combining equal volumes and concentration of both plants extracts. The results when compared to that of table 2 showed that these medicinal plants have wider antibacterial activities when used singly than when combined in a mixture.

Medicinal plants are used by a large proportion of developing nation. The reason for this may be a true improvement of disease conditions after herbal treatment. In these countries, the search for new drugs is centered upon the investigation of medicinal plants. The present research has tested the crude extracts of V amygdalina and O. gratissimum on bacterial strains capable of causing gastroenteritis. The two plants were found to be rich in tannin alkaloids, saponin, glycosides, flavonoids and reducing compounds. It is possible that the antibacterial activities of these plants may reside in these active principles as noted by Famorobi (2003). The antibacterial susceptibility test showed that the ethanolic extracts of both plants has higher inhibition on all the test isolates giving a zone of inhibition with diameter range of 1.0±0.71 to 9.3±0.63mm as compared to the aqueous extract with low inhibition activity of 1.0±0.71 to 5.7±1.2mm. The high activity of the ethanolic extracts verifies the use of the ethanolic extraction method by local herbalists (Allero and Afolayan, 2006). The combine extracts showed lower activity than individual plants. The reduced inhibitory effects of the combine plant can be ascribed to antagonism among the component parts which corroborates the work of Akpulu etal; (1994). The common practice of combining plants parts in traditional medicine is probably not the best as shown by this study and also shown by Mintesnot and Mogessie, (2004).

## CONCLUSION

The information provides by this study on MIC of the extracts will make it easier for dosage determination and chemotherapeutic index of the extract if they were to be processed into drugs. In the antibacterial susceptibility testing using the agar well diffusion method, absence of any clear zones of inhibition by the extract could be due to low concentration of diffusable compounds, time of collection of the plant material and climate which might in turn affect the amount of constituents of the plants. Thus treating gastroenteritis as a result of food intoxication with various crude preparations may not be effective because once the pathogens elucidate a sufficient amount of toxin in the food, it is the toxin and not the pathogen that cause the disease after ingestion of the contaminated food.In many cases, extracts of active constituents that are effective in vitro experiments may not show the same effectiveness when applied in-vivo. Therefore the fact that the extracts of these medicinal Plants inhibited some medically important bacteria proves that these plants might have some potential as an alternative source of antibacterial substances.

### Figure 1

TABLE 1: PHYTOCHEMICAL SCREENING OF THE CRUDE EXTRACT OF and

| Secondary metabolic | Plant | Ethanolic extract | Aqueous Extract |
|---|---|---|---|
| Alkaloids | *V. Amygdalina* | ++ | + |
| | *O. gratissimum* | ++ | + |
| Tannins | *V. Amygdalina* | +++ | ++ |
| | *O. gratissimum* | +++ | ++ |
| Steroids | *V. Amygdalina* | - | - |
| | *O. gratissimum* | - | - |
| Saponin | *V. Amygdalina* | ++ | ++ |
| | *O. gratissimum* | +++ | ++ |
| Reducing compound | *V. Amygdalina* | +++ | ++ |
| | *O. gratissimum* | +++ | + |
| Glycosides | *V. Amygdalina* | +++ | ++ |
| | *O. gratissimum* | ++ | + |
| Flavonoids | *V. Amygdalina* | ++ | ++ |
| | *O. gratissimum* | ++ | ++ |
| coumarins | *V. Amygdalina* | ++ | ++ |
| | *O. gratissimum* | ++ | ++ |

KEY
Absent -- Present ++
Fairly Present + Highly Present +++

### Figure 2

Table 2: Antibacterial Activity of Ethanolic and Aqueous Extract of and at varying concentrations (mg/ml) on test organisms with zoness of inhibition in millimeters (mm))

| Plant extracts | *E. coli* | | *Staph. aureus* | | *Bacillus cereus* | | *Salmonella typhi* | | *Shigella dysenteriae* | |
|---|---|---|---|---|---|---|---|---|---|---|
| Conc. (mg/ml) | VA | OG | VA | OG | VA | OG | VA | OG | VA | OG |
| | | | | | Aqueous Extract | | | | | |
| 10 | 1.1±003 | 1.5±010 | 1.0±0.33 | 1.0±0.51 | 0.0 ±0.00 | 0.0± 0.00 | 00. ±0.00 | 0.0± 0.00 | 0.0 ±0.00 | 0.0±0.00 |
| 15 | 2.0± 000 | 2.0±001 | 1.5±0.11 | 2.0±0.33 | 1.0± 0.36 | 1.0±0.01 | 0.0± 0.00 | 0.0±0.60 | 0.0±0.17 | 0.0±0.00 |
| 20 | 2.5±1.00 | 3.5±002 | 2.5±0.86 | 3.5±0.15 | 2.0±0.22 | 2.0±0.16 | 0.5±0.10 | 1.0±0.10 | 0.0±0.00 | 0.0±0.00 |
| 25 | 3.0±0.20 | 3.9±005 | 3.5±0.33 | 3.8±0.22 | 2.2±0.31 | 2.4±0.05 | 1.5±0.14 | 2.0±0.36 | 0.5±0.00 | 1.0±0.01 |
| 30 | 3.2±0.05 | 4.2±1.00 | 3.7±0.19 | 4.0±011 | 2.5±0.83 | 2.6±0. 22 | 1.7±0.72 | 2.1±0.14 | 0.9±0.14 | 1.2±0.05 |
| 35 | 3.5±0.00 | 4.5±00 2 | 3.9±0.39 | 4.3±0.86 | 2.6±0.09 | 2.8±0.17 | 1.9±0.33 | 2.4±0.32 | 1.2±0.16 | 1.5±0.08 |
| 40 | 3.9±0.00 | 4.7±0.20 | 4.1±0.15 | 4.5±0.63 | 2.8±0.16 | 3.1±0.31 | 2.1 ±0.54 | 2.6±0.62 | 1.4±0.14 | 1.7±0.11 |
| 45 | 4.2±0.02 | 5.1±0.05 | 4.3±0.16 | 4.7±0.14 | 3.0±0.39 | 3.3±0.11 | 2.3±0.62 | 2.8±0.18 | 1.8±0.16 | 1.9±0.16 |
| 50 | 4.3±1.12 | 5.3±0.14 | 4.5±0.51 | 4.9±0.16 | 3.1±0.16 | 3.6±0.50 | 2.6±0.71 | 3.1±0.04 | 2.1±0.12 | 2.1±0.30 |
| 55 | 4.5±0.88 | 5.7±0.39 | 4.6±0.67 | 5.2±0.09 | 3.3±0.18 | 3.9±0.01 | 2.9±0.10 | 3.4±0.34 | 2.4±0.16 | 2.4±0.10 |
| | | | | | Ethanolic Extract | | | | | |
| 10 | 1.5±0.22 | 2.0±001 | 1.0±0.12 | 2-0±0.03 | 0.0±0.01 | 1.5±0.01 | 0.0±0.00 | 1.0±0.01 | 0.0±0.00 | 1.0±0.31 |
| 15 | 20±0.12 | 2.5±010 | 2.0±0.04 | 2.5±0.14 | 1.5±.050 | 2.0±0.22 | 0.0±0.00 | 1.5±0.20 | 0.0±0.00 | 1.5±0.14 |
| 20 | 3.0±0.01 | 3.5±0.09 | 3.0±0.06 | 3.5±0.32 | 3.0±0.17 | 2.5±0.31 | 1.0±0.03 | 2.0±0.17 | 1.0±0.01 | 2.0±0.23 |
| 25 | 3.6±0.34 | 4.2±0.14 | 4.0±0.16 | 4.6±0.12 | 3.5±0.02 | 3.0±0.16 | 1.5±0.20 | 2.5±0.24 | 1.5±0.31 | 2.5±0.30 |
| 30 | 4.0±0.52 | 5.0±0.51 | 4.8±0.52 | 5.6±0.10 | 3.9±0.36 | 3.4±0.10 | 2.0±1.14 | 2.9±0.30 | 2.0±0.33 | 2.9±0.16 |
| 35 | 4.4±0.90 | 5.8±0.51 | 5.3±0.33 | 6.7±0.04 | 4.3±0.14 | 4.0±0.14 | 2.2±0.88 | 3.4±0.01 | 2.2±0.00 | 3.3±0.90 |
| 40 | 4.9±0.11 | 6.6±0.00 | 5.9±0.11 | 7.4±0.34 | 4.7±0.16 | 4.3±0.62 | 2.5±0.39 | 3.7±0.11 | 2.6±0.67 | 3.7±0.30 |
| 45 | 5.3±0.10 | 7.6±0.01 | 6.4±0.12 | 8.4±0.18 | 5.1±0.09 | 4.7±0.18 | 2.9±0.33 | 4.0±0.16 | 2.9±0.16 | 4.0±0.11 |
| 50 | 5.0±0.09 | 8.4±0.37 | 6.9±0.15 | 9.5±0.17 | 5.9±0.51 | 6.0±0.40 | 3.2±0.51 | 4.2±0.61 | 3.2±0.25 | 4.2±0.33 |
| 55 | 6.3±0.36 | 9.3±0.54 | 7.3±0.01 | 9.6±0.14 | 6.4±0.22 | 5.2±0.15 | 3.7±0.22 | 4.4±0.14 | 3.7±0.54 | 4.4±0.88 |

### Figure 3

TABLE 3: MINIMUM INHIBITORY CONCENTRATION (MIC) OF CRUDE EXTRACT OF and

| TEST ORGANISM | VA VE 12.5mg/ml | VA VE 0.25mg/ml | VA VE 3.125mg/ml | VA VE 1.562mg/ml | OA OE 12.5mg/ml | OA VE 6.25mg/ml | OA VE 3.125mg/ml | OA VE 1.562 mg/ml |
|---|---|---|---|---|---|---|---|---|
| *E. Coli* | 1.0 1.6 | Nil Nil | Nil Nil | Nil Nil | 1.2 1.90 | Nil Nil | Nil Nil | Nil Nil |
| *Staph. Aureus* | 1.0 1.3 | Nil Nil | Nil Nil | Nil Nil | 1.10 1.70 | Nil Nil | Nil Nil | Nil Nil |
| *Bacillus cereus* | 0.4 1.0 | Nil Nil | Nil Nil | Nil Nil | 0.6 1.1 | Nil Nil | Nil Nil | Nil Nil |
| *Shigella dysenteriae* | Nil Nil | Nil Nil | Nil Nil | Nil Nil | Nil 1.0 | Nil Nil | Nil Nil | Nil Nil |
| *Salmonella typhimurium* | Nil Nil | Nil Nil | Nil Nil | Nil Nil | Nil 1.0 | Nil Nil | Nil Nil | Nil Nil |

VA= Aqueous Extracts of *V. amygdalina* VE = Ethanolic extract of *V. amygdalina*
OA = Aqueous Extracts of *O. gratissimum* s OE = Ethanolic Extracts of *O.gratissimum*

### Figure 4

TABLE 4: ANTIBACTERIAL activity of equal volumes(5ml)and concentration(40mg/ml) of both extract on test organism

| PLANTS EXTRACT | TEST ORGANISM | Concentrations/zones of inhibition 10mg/ml | 20mg/ml | 40mg/ml | 60mg/ml | 80mg/ml |
|---|---|---|---|---|---|---|
| VA + OA | *E. Coli.* | 2.6±0.10 | 3.5±0.22 | 2.9±0.38 | 2.0±0.16 | 2.5±0.11 |
| | *Staph. aureus* | 1.0±0.33 | 4.1±0.01 | 3.4±0.01 | 2.7±0.23 | 3.0±0.14 |
| | *Bacillus cereus* | 0.8 ±0.04 | 2.7±0.24 | 2.1±0.11 | 1.9±0.31 | 2.1±0.17 |
| | *Shigella dysenteriae* | 0.8 ±0.52 | 2.0±0.14 | 1.7±0.71 | 1.2±0.13 | 1.0±0.32 |
| | *Samonella typhimurium* | 0.7 ±0.66 | 1.4±0.37 | 1.0±0.42 | 0.8 ±0.19 | 1.0±0.56 |
| VE + OE | *E. Coli.* | 2.0±0.17 | 3.9±0.14 | 3.2±0.33 | 3.0±0.00 | 3.0±0.19 |
| | *Staph. aureus* | 1.8±0.14 | 4.3±0.17 | 3.7±0.62 | 3.2±0.22 | 3.2±0.22 |
| | *Bacillus cereus* | 1.2±0.12 | 2.9±0.27 | 2.4±0.14 | 2.1±0.19 | 2.4±0.86 |
| | *Shigella dysenteriae* | 1.1±0.00 | 2.3±0.11 | 2.1±0.02 | 1.8±0.00 | 1.3±0.03 |
| | *Samonella typhimurium* | 1.0±0.10 | 1.6±0.10 | 1.3±0.00 | 1.0±0.36 | 1.2±0.50 |

VA = Aqueous extract of *Vernonia amygdalina*, VE = Ethanolic extract of *Vernonia amygdalina*
OA = Aqueous extract of *Ocimum gratissimum*, OE = Ethanolic extract of *Ocimum gratissimum*

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