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Antibacterial Activity of *Ocimum gratissimum* (Nchu-Anwu) and *Vernonia amygdalina* (Bitter-Leaf) Antibacterial Activity of *Ocimum gratissimum* (Nchu-Anwu) and *Vernonia amygdalina* (Bitter-Leaf)

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Authors' contributions

This work was carried out in collaboration between all authors. Author AUO originated the concept, designed experiments and collected data. Authors RCE and JNDN performed critical reviews of the manuscript. Authors CEO and JKN performed the data analysis and wrote the first draft. All authors approved of the final manuscript.

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ABSTRACT

This study was carried out to evaluate the antibacterial activity of *Ocimum gratissimum* and *Vernonia amygdalina* on selected bacterial species, as well as comparing the antibacterial activities of their fresh and dried leaves. Fresh leave samples of the plants

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were collected from Okwu, Uratta in Owerri North, Imo State, Nigeria. The plants were identified and ethanolic and aqueous extractions of the dried and fresh leave samples were done using standard procedures. Antibacterial activity of the extracts was tested invitro against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Streptococcus pyogenes* using agar diffusion, punch method. The pattern of inhibition varied with plant extract, solvent used for extraction and test organisms. For the dried leave samples, all the extracts showed zones of inhibition above 5mm diameter against all the isolates, except hot water extract which had no activity for all. Ethanol extracts showed the highest zone of 20mm of *O. gratissimum* against *Staphylococcus aureus* and highest zone of 40mm by *V. amygdalina* against *Pseudomonas aeruginosa*. However, for the fresh leave samples, only Ethanolic extract of *O. gratissimum* was active against all the isolates. None of the fresh leave sample extracts of *V. amygdalina* was active against all the isolates, except the cold water extract which showed zones of inhibition of 10mm against *E. coli* and *Streptococcus pyogenes* each. It was also observed that *Staphylococcus aureus* had the lowest Minimum Inhibitory Concentration (MIC) of 0.48µg/ml and 0.82µg/ml against *O. gratissimum* and *V. amygdalina* respectively, and Minimum Bactericidal Concentration (MBC) of 0.28µg/ml and 0.61µg/ml respectively. From this work, it has been noted that the leaves of *Ocimum gratissimum* and *Vernonia amygdalina* have antibacterial properties. Government is therefore advised to invest money into more pharmacological researches on these extracts in order to make them better alternative to modern medicine for the treatment of infection caused by these pathogens.

Keywords: Herbal; Ocimum gratissimum; Vernonia amygdalina; antibacterial; extracts.

1. INTRODUCTION

Plants are sources of medicine in pharmacopoeia. Herbal medicine can be used as an alternative to some commercial drugs. As a result, some people resort to natural leaves for the treatment of infection. The antimicrobial properties of many plants have been investigated by a number of researchers word wide [1]. Medicinal use of plants ranges from the administration of the roots, barks, stems, leaves and seeds to the use of extracts and decoctions from the plants [2].

Medicinal plants are used as excellent antimicrobial agent because they posses variety of chemical constituents in nature. Plants have the ability to synthesize aromatic substances such as phenolic, (e.g), phenolic acid, flavonoids, quinines, coumarins, lignans, stibenes, tannins), Nitrogen compound (alkaloids, amines) vitamins, terpenoids (including carotenoids) and some other endogenous metabolites. These substances serve as plants defense mechanism against predation by microbes, insects, and herbivores [3].

Medicinal plants such as *Ocimum gratissimum* and *Vernonia amygdalina* have been ascertained to provide various culinary and medicinal properties. These medicinal properties exert bacteristatic and bactericidal effects on some bacteria [4].

Ocimum gratissimum belongs to the plants family “Labiatae” which is one of the most employed medicinal plants as a worldwide source of spices and also as a consolidated source of extracts with strong antibacterial and antioxidant properties. It is a perennial plant that is woody at the base. It has an average height of 1-3m high. The leaves are broad and mainly ovate, usually 5-13cm long and3-9cm wide. It is known locally as “Nchu anwu”.

Vernonia amygdalina on the other hand, belongs to the plant family "compositeae". It is a small tree of about 2-5m with petiolate leaf of about 6mm diameter and elliptic shape. The leaves are green with a characteristic odour and a bitter taste.

In developing countries which Nigeria is part of, it could be seen that herbal medicines play a vital role in the health care for large sections of the populations, in many cases, would bridge the gap between the availability of and the demand for modern medicine [5]. Nearly one quarter of drugs prescribed in the United States of America are of plant origin. He estimated the cost for pharmaceuticals derived from plants at US$9millions per year in the US alone [6].

This work therefore, is aimed at evaluating the antibacterial effect of *Ocimum gratissimum* and *Vernonia amygdalina* on selected bacterial species, as well as to compare the antibacterial activities of fresh and dried leaves of the two plants on the selected bacterial pathogens of common infections.

2. MATERIALS AND METHODS

2.1 Sample Collection

Fresh samples of both *Ocimum gratissimum* and *Vernonia amygdalina* leaves were harvested from matured plants from a farm land at Okwu-Uratta Owerri, Imo State, Nigeria. The leaves were authentically identified in the department of plant science Imo State University, Owerri.

After identification, the samples were transported to the microbiology laboratory of the department of medical laboratory science, Imo State University, Owerri for analysis. The leave samples were thoroughly rinsed with sterile distilled water and sodium hypochloride for surface sterilization, part of the samples was dried in hot air oven at 28ºc for one week.

2.2 Extraction

The extracts (from both dry and fresh leaves) were prepared as described by Madunagu et al. [7] 20g of ground pulp of each plant leaves were added to 100ml of cold sterile distilled water, Hot sterile distilled water and ethanol (95%) respectively and kept for 72hrs. The extracts were filtered using what man No 1 filter paper and membrane filter for sterilization. The extracts were concentrated with rotary evaporator before being stored in the refrigerator at 4ºC prior to use.

2.3 Test Bacteria

The organisms used were *Escherichia coli, Pseudomonas aerugenosa, Staphylococus aureus* and *Streptococcus pyogenes* obtained from the Microbiology Laboratory of the Department of medical laboratory science, Imo State University, Owerri. The isolates were reconfirmed prior to use for this work, in the same laboratory, following the conventional method of identification, such as morphological features, Grams staining reaction, motility and Biochemical characteristics.

Their susceptibility tests were done using standard discs of gentamicin, penicillin and ampicillin respectively, with the zones of inhibition of more than 15mm diameter.

2.4 Susceptibility Testing

Plate diffusion technique using the punch-hole method was adopted, on Nutrient agar medium (oxoid) which was prepared and sterilized according to the manufactures instruction.

The plates were inoculated with the organism after dilution to give about 10^6cfu/ml in triplets. Punch holes were made with the help of sterile cock borer of 10mm diameter. Each hole was labeled with the leaf extract concentration and filled, allowed to stay for about 30 minutes before incubation at 37ºC for 18 hours. Zones of inhibition were measured using mm rule. Any leaf extract concentration giving an inhibition zone of more than 2mm on either side of the hole was regarded as having antibacterial activity.

2.5 Minimum Inhibitory Concentration (MIC)

To confirm the resistivity of the organisms to *Ocimum gratissimum* and *Vernonia amygdalina*, minimum inhibitory concentration (MIC) of *Ocimum gratissimum* and *Vernonia amygdalina* for the isolates by agar dilution method was determined. A two-fold serial dilution for series of the *Ocimum gratissimum* and *Vernonia amygdalina* (Ethanol extract) were prepared in 30ml containers including extract free controls. 19ml of molten Mueller–Hinton agar at temperature of 50℃ was added to each of the containers, mixed thoroughly and poured into pre-labeled sterile petridishes on a level surface. It was allowed to solidify at room temperature and was dried in the incubator to remove moisture. Then, from a 10^4cfu density (McFerland Standard) of the organisms broth already prepared, the plates, including the control were inoculated using a standard wire loop. The inoculums spots were allowed to dry at room temperature before inverting the plates for incubation at 37℃ for 18hrs under aerobic condition.

2.6 Minimum Bactericidal Concentration (MBC)

The MBCs were determined by first selecting the plates that showed no growth during MIC determination, a loopful from each tube was sub-cultured onto extract free agar plates, incubated for further 24hours at 37℃. The least concentration at which no growth was observed was noted as the MBC.

3. RESULTS

The result of Antibacterial activity of *Ocimum gratissimum* (Nchu-Anwu) and *Vernonia amygdalina* (Bitter leaf) as shown in Table 1 indicated that isolates showed variable response to various extraction methods to the dried leave samples of *Ocimum gratissimum* and *Vernonia amygdalina.* All the extracts showed zones of inhibition above 5mm diameter against all the isolates, except hot water extraction which had 00mm for all the isolates. Ethanol extraction showed the highest zone of 20mm of *O. gratissimum* against *Staph. aureus* and the highest zone of 40mm of *V. amygdalina* against *Pseudo aeruginosa.*

Table 2, shows the antibacterial activity of fresh leaves of *O. gratissimum* and *V. amygdalina* on the selected bacterial isolates. Only Ethanol extractions of *O. gratissimum* was active against all the isolates. None of the extractions of *V. amygdalina* was active against all the isolates, except the cold water extracts which showed zones of inhibition of 10mm against *E. coli* and *Strep. Pyogenes* each.

Table 3 Shows minimum inhibitory concentrate (MIC) of the dry (Ethanolic extract) of *O. gratissimum* and *V. amygdalina* on selected Bacterial isolates. *E coli* has MIC of (17.2mm and 36.00mm) for *O. gratissimum* and *V. amygdalina* respectively, others are *P. aeruginosa* (18.8mm and 16.2mm), *Staphylococcus aureus* (0.48mm and 0.82mm) and *Streptococcus pyogenes* (7.0mm and 16.0mm) respectively.

Table 4 Shows Minimum Bactericidal Concentration (MBC) of the dry (Ethanol extract) of *O. gratissimum and V. amygdalina* on selected Bacterial isolates. *E. coli* has MBC of (9.40 and 18.00)µg/ml, for *O. gratissimum and V. amygdalina* respectively, *P. aeruginosa* (7.10 and 10.30)µg/ml, *Staphylococcus aureus* (0.28 and 0.61)µg/ml and *Streptococcus pyogenes* (4.20 and 9.10)µg/ml respectively.

Table 1. Antibacterial activity of dry *O. gratissmum* and *V. amygdalina* leaves on selected bacterial isolates

Organism	Zones of inhibition (mm)					
	***O. gratissimum* (200mg/ml)**			***V. amygdalina* (200mg/ml)**		
	CDWE	**HWE**	**EE**	**CDWE**	**HWE**	**EE**
E. coli	10	00	10	10	00	00
P. aeruginosa	10	00	06	00	00	40
Staphylococcus aureus	06	00	20	00	00	06
Streptococcus pyognese	10	00	06	00	00	00

KEY: CDWE = Cold Distilled Water Extracts, HWE: = Hot Water Extract, EE = Ethanol Extract

Table 2. Antibacterial activity of Fresh *O. gratissimum* and *V. amygdalina* leaves on selected bacteria isolates

Organism	Zones of inhibition (mm)					
	O. gratissimum (200mg/ml)			**V. amygdalina (200mg/ml)**		
	CDWE	**HWE**	**EE**	**CDWE**	**HWE**	**EE**
E. coli	00	00	20	10	00	00
P. aeruginosa	00	00	40	00	00	00
Staph. aureus	00	20	10	00	00	00
Strep. pyognes	00	00	10	10	00	00

Table 3. Minimum Inhibition Concentration (MIC) of Dry (ethanol extract) of *O. gratissimum* and *V. amygdalina* on selected bacterial Isolates (µg/ml)

Organism	O. gratissimum	V. amygdalina
E. coli	17.20	36.00
P. aerogenosa	18.40	16.20
Staph. aureus	0.48	0.82
Strep. pyogenes	7.00	16.00

Table 4. Minimum Bactericidal Concentration (MBC) of Dry (ethanol extract) of *O. gratissimum* and *V. amygdalina* on selected bacterial Isolates (µg/ml)

Organism	O. gratissimum	V. amygdalina
E. coli	9.40	18.00
P. aeruginosa	7.10	10.30
Staph. aureus	0.28	0.61
Strep. pyogenes	4.20	9.10

4. DISCUSSION

In the 20th century, one of the goals of research work is to discover chemotherapeutic agents that will be effective in every infectious disease [8]. In the developing countries like Nigeria, it could be seen that herbal medicines play a vital role in the health care delivery for large sections of the population. In many cases would bridge the gap between the availability of and the demand for modern medicine [5].

From this study, it is observed that *Ocimum gratissimum* was highly active against the four organism with diameter of zones of inhibition as *E. coli, P. aeruginosa, Staph aureus* and *strep pyogenes*. This peculiar high levels of zones of inhibition reveals the antibacterial efficacies of these plants. This is in agreement with Adebolu and Salu [9] who demonstrated the antimicrobial activity of some medicinal plants against bacteria using the extracts of *O. gratissimum* which proved active.

Again this work reveals that ethanol could probably be among the best solvent for the extraction of both dry and fresh leaves of *Ocimum gratissimum* and *Vernonia amygdalina*. This could be because ethanol diffuses easier into the medium than cold or hot water. This is in agreement with Okigbo and Mmeka, [10] who attributed the good antibacterial properties of ethanol extraction to isolates to low concentration of plant extract used. Again it is in agreement with the findings of Obi and Onuoha [11], who reported alcohol to be the best solvent for the extraction of most plant active principles of medical importance.

Leaf extract of *Ocimum gratissismum* (both dry and fresh) has more antibacterial activity than *Vernonia amygdalina* even though both of them provided various culinary and medicinal properties. This is in agreement with Elujoba [12] who linked the antibacterial activity of *Ocimum gratissimum* to its numerous properties, such as the tanins and sweet smelling volatile oil known as essential oil.

It is also observed that dry leaf extracts were more active against the isolates than the fresh leaf extracts, this could be attributed to the fact that vaporizers tend to work best when the material is very dry and finely ground up. This is in agreement with a scholarly research in which it was stated that moisture left in herbs or material will lessen the amount of vapour produced and will cause the vapour to be harsher than it should be [13]. They also stated that when ground up super fine, there will be increase in the area that the heat from the vape can reach.

The low MICs and MBCs observed for ethanolic extracts of the dry leaves of both *O. gratissimum and V. amygdalina* on the selected isolates is an indicator that they may be used as an alternative treatment to orthodox antibiotics in the treatment of disease due to the isolates. This is in agreement Mbata and Saikia [14] who suggested the same especially

he said “as they frequently develop resistance to known antibiotic and will reduce the cost of obtaining health care.

5. CONCLUSION AND RECOMMENDATION

In conclusion, this research work has confirmed that ethanol is the best solvent in the extraction of the active ingredients of plats and that dry leaf extracts are more active against bacterial than fresh leaf extracts.

The work also reveals that *Ocimum gratissimum* and *Vernonia amygdalina* plants possess antibacterial properties however, that *O. gratissimum* is more active than *V. amygdalina.*

We therefore recommend the use of *O. gratissimum and V. amygdalina* inthe treatment of infections/diseases caused by; *E. coli, Staph aureus, P. aeruginosa* and *Strep. Pyogenese.*

Also recommend that Government should sponsor more researches on these plants so as to make them better alternatives to modern medicine.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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