

## **Antibacterial activity of water, ethanol and methanol extracts of *Ocimum gratissimum*, *Vernonia amygdalina* and *Aframomum melegueta***

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### **ABSTRACT**

The antibacterial efficiency of cold water and hot water, ethanol and methanol extracts of *Ocimum gratissimum*, *Vernonia amygdalina* and *Aframomum melegueta* on *Escherichia coli*, *Salmonella typhi* and *Klebsiella pneumoniae* were evaluated by disc diffusion technique. The cold water extract of *V. amygdalina* and *A. melegueta* slightly inhibited the growth of *S. typhi* and *E. coli* with inhibition zone diameter of 15 mm and 12 mm respectively. The hot water extract of *O. gratissimum*, *V. amygdalina* and *A. melegueta* moderately inhibited the growth of *S. typhi* and *E. coli* with inhibition zone diameter of 15 mm to 20 mm. While the ethanolic and methanolic extract of *O. gratissimum*, *V. amygdalina* and *A. melegueta* profoundly inhibited the growth of *S. typhi* and *E. coli* with inhibition zone diameter between 12 mm to 23 mm. The growths of *K. pneumoniae* were not inhibited by all the extracts of *O. gratissimum*, *V. amygdalina* and *A. melegueta*. This revealed that *O. gratissimum*, *V. amygdalina* and *A. melegueta* have antimicrobial activity on *E. coli* and *S. typhi* and probably could be used to control infections associated with these organisms.

**Keywords:** Antibacterial activities, medicinal plants, *O. gratissimum*, *V. amygdalina*, *A. melegueta*, *E. coli*, *S. typhi* and *K. pneumoniae*.

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### **INTRODUCTION**

According to World Health Organization medicinal plants would be the best source to obtain a variety of drugs. In developed countries about 80% of plants are used in traditional medicine. Therefore, such plants have been investigated for better understanding their medicinal properties. The antimicrobial properties of many plants have been investigated by a number of researcher's in worldwide (Adamu et al., 2005). Plants have been a source of medicine in pharmacopoeia (). Herbal medicine can be used as an alternative to some commercial drugs (Anyamene and Ezeadila, 2010). Medicinal plants provide inestimable projections for new drug discoveries because of the matchless availability of chemical range. The practice of herbal medicines in Asia signifies a long antiquity of human interactions with the environment (Sasidharan et al., 2011). Medical uses of plants range from the administration of the roots, barks, stems, leaves and seeds to the use of extracts and decoction from the plants (Ogbulie et al., 2007). Medicinal plants were used as excellent antimicrobial agents because it posses a variety of chemical constituent is nature recently much attention has directed towards extracts and biologically active compounds isolated from popular plant species (Prince and Prabakaran, 2011). Plants have ability to synthesize aromatic substances such as phenolic, (e.g. phenolic acids, flavonoids, quinones, coumarins, lignans, stilbenes,

tannins), nitrogen compounds (alkaloids, amines), vitamins, terpenoids (including carotenoids) and some other endogenous metabolites. These substances serve as plant defense mechanisms against predation by microbes, insects, herbivores (Bharathi et al., 2011)

There is a need to focus on the alternative sources of the antibiotics as the pathogenic microbes are gaining resistance against standard antibiotics (Tarun et al., 2012). Medicinal plants such as *Ocimum gratissimum*, *Vernonia amygdalina* and *Aframomum melegueta* have been ascertained to provide various culinary and medicinal properties. These medicinal properties exert bacteriostatic and bacteriocidal effects on some bacteria (Effraim et al., 2000; Okigbo and Mneka, 2006; Okeke et al., 2008; Funmilayo et al., 2010).

This study was undertaken to evaluate the antibacterial activity of *Ocimum gratissimum*, *Vernonia amygdalina* and *Aframomum melegueta* on *Escherichia coli*, *Salmonella typhi* and *Klebsiella pneumoniae*.

## MATERIALS AND METHODS

### Collection of plant material

Fresh samples of *Ocimum gratissimum* and *Vernonia amygdalina* leaves were collected from a gardens at Umuigboke, Oriuzor community in Ezza North local government area of Ebonyi state between the hours of 6-8 am at a prevailing temperature of about  $30\pm 2^{\circ}\text{C}$  while the dried seeds of *Aframomum melegueta* were bought from traditional healers at Abakpa Main market in Abakaliki, Ebonyi State. All the collections were done in the month of May. The plants were identified and authenticated by Prof. S.C.C. Onyekwelu (taxonomist) at the Department of Applied Biology, Ebonyi State University, Abakaliki, Ebonyi State.

### Preparation of extracts

**O. gratissimum:** Fresh leaves of *O. gratissimum* were thoroughly washed using tap water and rinsed with distilled water. The leaves were dried for 5 min in an oven at  $28^{\circ}\text{C}$  for one week and then pulverized to a fine powder with the aid of a Starlite blender (Model SL-999). Four solvents were used for the preparation of the extracts, namely cold distilled water, hot distilled water, ethanol 60% conc. and methanol 60% conc. The aqueous extract was prepared by weighing out (20 g) of the milled powdered leaves of *O. gratissimum* were soaked in 100 ml of cold water in a conical flask and stirring vigorously with a glass rod for proper extraction. The combination was allowed to settle for 24 hours at room temperature. For the hot water extract, 20 g of the pulverized powdered leaves of *O. gratissimum* and were soaked in 100 ml of hot water for 24 hours also. The extracts were then filtered using Whatman no.1 filter paper. The ethanol and methanol extracts were obtained by weighing out same fraction 20 g of the pulverized powdered leaves of the plant and soaking in 100 ml of the 60% ethanol and 60% ethanol. The extracts were then filtered using Whatman no.1 filter paper. All filtrates were air dried at  $28^{\circ}\text{C}$ . And the air dried filtrates were then reconstituted in 20 % Dimethylsulphoxide (DMSO) solution and then poured into amber bottle and kept in the refrigerator at  $4^{\circ}\text{C}$  prior to determination of the minimum inhibitory concentration (MIC).

**V. amygdalina:** The same procedure as in above was followed.

**A. melegueta:** The dried seeds were pulverized to a fine powder with the aid of a Starlite blender. The powder was extracted in 100 ml of hot distilled water, ethanol 60% conc. and methanol 60% conc. following the same procedure as for *O. gratissimum*.

### Preparation of test organisms

Clinical isolate of *E. coli* and *S. typhi* were obtained from stool samples of patients attending Ebonyi State University teaching Hospital (EBSUTH), Abakaliki, Nigeria while the strain of *K. pneumoniae* isolates was obtained from sputum of patients attending University of Nigeria Teaching Hospital (UNTH), Enugu, Nigeria. All isolates were identified using conventional technique (Cheesbrough, 2006). Pure cultures of this isolates were tested for antimicrobial activity of the three plants using Muller Hinton agar.

### Preparation of the disc

Each of the discs which are approximately 10 mm in diameter were cut from Whatman No.1 filter paper. The discs were put into a petri dish and then sterilized in the oven at  $160^{\circ}\text{C}$  for 2hr. The discs were then impregnated with the extract by soaking in the extract for 24 hours. Each of the disc contained approximately 200 mg/ml of the cold distilled water, hot distilled water, ethanolic and methanolic extract. With the help of sterilized forceps, each disc

was recovered from the extract, held for a few seconds for some of the ethanol to evaporate before being applied aseptically unto the agar surface in a plate, which had initially being inoculated with a pure culture of the test organism.

#### Determination of microbial activity of the extracts

Agar well diffusion technique as described by Cheesbrough (2006) was used to determine the antibacterial activity of the extracts. An 18 ml of Mueller Hinton agar plates that has been checked for sterility were seeded with 2 ml of an overnight broth culture of each bacterial isolate in sterile Petri-dish. The seeded plates were allowed to set after a uniform distribution of the bacterial isolate following slow rotation of the Petri dish. A standard sterile cork borer of 8 mm diameter was used to cut uniform wells on the surface of the agar. The wells filled with 2 ml of each extracts were with the aid of a sterile syringe. One of the well in each Muller Hinton agar plate is left unfilled as a control. The plates were then allowed to stand for 1 hour at room temperature to allow proper diffusion of the extract to occur. All the plates were incubated at 37 °C for 24 hours and observed for zones of inhibition. A zone of clearance round each well signifies inhibition and the diameter of such zones were measured in millimeter (mm). The minimum inhibitory concentration (MIC) in mg/ml was determined by comparing the different concentration of the extracts that have different zones of inhibition and then selecting the lowest concentration of each extract (Agaetmor, 2009).

#### Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of the plants that showed inhibition in the antimicrobial screening was used. The MIC was carried out by preparing the dried plants extract in different concentration 100 mg/ml, 200 mg/ml, 300 mg/ml, 400 mg/ml and 500 mg/ml by dissolving 0.1 g, 0.2 g, 0.3 g, 0.4 g and 0.5 g of the plant extract that showed inhibition in ml of distilled water. After each of the wells created on the inoculated Muller Hinton agar plates were filled with different concentration of the diluted extracts accordingly and then allowed to stand for 1 hour for proper diffusion of the extracts and then incubated after which the lowest concentration that showed inhibition was checked for.

## RESULTS

The various results for the tests done are shown below.

**Table I: Antibacterial activity of *O. gratissimum* (Nchuanwu), *V. amygdalina* (Bitter leaf) and *Aframomum melegueta* (Alligator pepper) on *Escherichia coli*, *Salmonella typhi* and *Klebsiella pneumoniae***

Plant Sp. Organism	Inhibition zone diameter (mm)											
	<i>O. gratissimum</i>				<i>V. amygdalina</i>				<i>A. melegueta</i>			
	CWE	HWE	EE	ME	CWE	HWE	EE	ME	CWE	HWE	EE	ME
<i>E. coli</i>	NI	NI	20	23	15	15	23	23	NI	NI	14	NI
<i>S. typhi</i>	NI	20	21	NI	NI	20	13	20	12	19	NI	12
<i>K. pneumoniae</i>	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI

Key: CWE = Cold water extract, HWE = Hot water extract, EE = Ethanol extract, ME = Methanol extract, NI = No inhibition.

**Table II: Minimum inhibitory concentration of *V. amygdalina* (Bitter leaf), *O. gratissimum* (Nchuanwu), and *A. melegueta* (Alligator pepper) on *E. coli***

Extracts	Concentrations (mg/ml)					
	500	400	300	200	100	MIC (mg/ml)
BLEE	11					500
BLHWE	18	12	16			400
APCWE						NI
APHWE						NI
APME						NI
NCHHWE	20	14	12			300
NCHEE	18	14	13	11		200

Key: BLEE = Bitter leaf ethanol, BLHWE = Bitter leaf hot water extract, APCWE = Alligator pepper cold water extract, APHWE = Alligator pepper hot water extract, APME = Alligator methanol extract, NCHHWE = Nchuanwu hot water extract, NCHEE = Nchuanwu ethanol extract, NI = No inhibition

**Table III: Minimum inhibitory concentration of *V. amygdalina* (Bitter leaf), *O. gratissimum* (Nchuanwu), and *A. melegueta* (Alligator pepper) on *S. Typhi***

Extracts	Concentrations (mg/ml)					MIC (mg/ml)
	500	400	300	200	100	
BLCWE						NI
BLHWE	14					500
BLEE	22	20	16	14		200
BLME	20	14	18			400
NCHME	16	18	20			500
APEE						NI

Key: BLCWE = Bitter leaf cold water extract, BLHWE = Bitter leaf hot water extract, BLEE = Bitter leaf ethanol extract, BLME = Bitter leaf methanol extract, NCHME = Nchuanwu methanol extract, APEE = Alligator pepper ethanol extract, NI = No inhibition.

### DISCUSSION

The result obtained from this work indicated that cold water, hot water, methanolic and ethanolic extract of *V. amygdalina*, *O. gratissimum* and *A. melegueta* inhibited the growth of *S. typhi* and *E. coli*. The results obtained also showed that cold water extract of *V. amygdalina* showed inhibition zone diameter of 15 mm only against *S. typhi* as shown in table I, which is similar to the findings of Ogbulie et al. (2007). The cold water extract of *V. amygdalina* could not inhibit the growth of *E. coli* which is contrary to the findings of Ogbulie et al. (2007). The hot water extract of *V. amygdalina* inhibited the growth of *S. typhi* and *E. coli* with the inhibition zone diameter of 15 mm and 20 mm respectively, which is in accordance with the findings of Ogbulie et al. (2007). While hot water extract did not show any inhibition against *S. typhi* and *E. coli*. The ethanolic extract showed inhibition against *S. typhi* and *E. coli* with inhibition zone diameter of 23 mm and 13 mm respectively, which is similar to the findings of Ogbulie et al. (2007). Okigbo and Mmeka (2008) also documented that *V. amygdalina* ethanol and aqueous extracts had inhibition on *E. coli*. This is also similar to Al-Magboul et al. (1997) research but contrary to Ashebir and Ashenafi (2007) observation of the plants' ability to inhibit *E. coli*. The methanolic extract of *V. amygdalina* also inhibited the growth of *S. typhi* and *E. coli* with the inhibition zone diameter 23 mm and 20 mm respectively. This is in line with what was reported by Akinpelu (1999) who said that *Vernonia amygdalina* leaf 60% methanol extract was found to be active at 25 mg/ml against *S. typhi* and *E. coli* isolates. But none of the *V. amygdalina* extract showed inhibition against the growth of *K. pneumoniae*.

The cold water extract of *O. gratissimum* did not inhibit the growth of all the test organisms, while the hot water only inhibited the growth of *E. coli* with inhibition zone diameter of 20 mm as shown in table I, which is contrary to the findings of Adebolu and Oladimeji (2005). But the ethanolic extract of *O. gratissimum* inhibited the growth of *S. typhi* and *E. coli* with inhibition zone diameter of 20 mm and 21 mm respectively. This is similar to the findings of Nwiyi et al. (2009) who reported that ethanol extracts of *O. gratissimum* showed more antibacterial activity against *E. coli*. And the methanolic extract of *O. gratissimum* only inhibited the growth of *Salmonella typhi* with an inhibition zone diameter of 23 mm. All the extracts of *O. gratissimum* did not inhibit the growth of *Klebsiella pneumoniae*.

The cold and hot water extract of *A. melegueta* only inhibited the growth of *E. coli* as 21 mm and 19 mm respectively, which is not in agreement with the findings of Ogbulie et al. (2007). The ethanolic extract of *A. melegueta* only inhibited the growth of *S. typhi* with inhibition zone diameter of 14 mm which is also contrary to the findings of Ogbulie et al. (2007). The methanolic extract inhibited the growth of *E. coli* with an inhibition zone diameter of 12 mm. While all the plant extracts in this work did not inhibit the growth of *K. pneumoniae*. Hence, the difference in antimicrobial properties of a plant extract might be attributable to the age of the plant used, freshness of plant materials, physical factors (temperature, light water), contamination by field microbes, adulteration and substitution of plants, incorrect preparation and dosage ( Calixto, 2000; Okigbo and Omodamiro, 2006; Okigbo and Igwe, 2007). In the same vein, Amadioha and Obi (1999), Okigbo and Ajale (2005a), Okigbo et al. (2005) reported that inactivity of plant extracts may be due to age of plant, extracting solvent, method of extraction and time of harvesting of plant materials. Subsequently, Chukwuka et al. 2011 also noted that medicinal plants with no antibacterial activity may be due to astringent properties possessed by extract of the plants.

Finally, very wide zone of inhibition of *O. gratissimum*, *V. amygdalina* and *A. melegueta* showed that it had great potential as a remedy for infections/diseases caused by *E. coli* and *S. typhi*.

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**CONCLUSION**

The result of this work indicated that ethanol and methanol are better solvent than water for the extraction of the active ingredients of these plants. The result also indicated that all the plant extract have no antibacterial effect on *Klebsiella pneumoniae*, showing that they do not contain active ingredients against the organism. The observed inhibition of *E. coli* by hot water extract of *V. amygdalina* and not the cold water extract of the same plant, showed that the active ingredients responsible for the antibacterial effect is best extracted with hot water, ethanol and methanol.

Finally, very wide zone of inhibition of *O. gratissimum*, *V. amygdalina* and *A. melegueta* on *E. coli* and *S. typhi* showed that it had great potential as a remedy for infections/diseases caused by *E. coli* and *S. typhi*.

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