

Full Length Research Paper

Antibacterial effects of extracts of *Ocimum gratissimum* and *piper guineense* on *Escherichia coli* and *Staphylococcus aureus*

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The upsurge in the prevalence of side effects of many synthetic antimicrobial agents and incidence of multidrug resistant bacteria has spurred scientists on the research for plant based antimicrobial of therapeutic potentials. *Ocimum gratissimum* and *Piper guineense* present such potential of high medicinal value. These plants are used in Nigeria traditionally as condiments and for treatment of various ailments such as pyorrhea, dysentery and bronchitis. Aqueous and ethanol extracts of *O. gratissimum* and *P. guineense* leaves were screened for antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. Both extracts were found to exhibit selective inhibition against the isolates. The diameter zones of inhibition exhibited by the extracts were between $2 \pm 0.01 - 10 \pm 0.10$ mm. The minimum inhibitory concentration (MIC) determined by the agar diffusion method was between 10.00 and 2.50 mg/ml⁻¹. Ethanol extracts showed more inhibitory effect compared to the aqueous extracts. Results obtained show that the extracts of *O. gratissimum* and *P. guineense* possess some level of antibacterial activities against *E. coli* and *S. aureus*.

Key words: *Ocimum gratissimum*, *Piper guineense*, antibacterial activity, minimum inhibitory concentration (MIC), *Escherichia coli*, *Staphylococcus aureus*.

INTRODUCTION

There is growing interest in exploiting plants for medicinal purposes especially in Africa. This stems from the fact that microorganisms are developing resistance to many drugs and as such created situation where some of the common and less expensive antimicrobial agents are losing effectiveness (Montefore et al., 1989). Herbal medicine which uses medicinal plants primarily presents as an alternative to such situation (Sofowora, 1993). These medicinal plants have immensely contributed to the development of human health and welfare. Concomitantly, there is an increase in data and huge

patronage to herbal products round the world (Eisenberg et al., 1990; Omoseyindemi, 2003). Medicinal plants such as *Ocimum gratissimum* and *Piper guineense* have been asserted to provide various culinary and medicinal properties. These medicinal properties exert bacteriostatic and bacteriocidal effects on some bacteria. These effects have been attributed to the peptides, alkalonoids, essential oils, phenols and flavonols which are major components in these plants (Okigbo and Igwe, 2007). *O. gratissimum* belongs to the family *leguminocaeae*, commonly known as "alfavaca". It is naturally used in the treatment of different diseases which include: upper respiratory tract infections, diarrhea, headache, conjunctivitis, skin disease, pneumonia tooth

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and gum disorder, fever and as mosquito repellants (Onajobi, 1986; Ilori et al., 1996; Okigbo and Mneka, 2006). *O. gratissimum* is found in the tropical and warm temperature regions such as India and Nigeria (Okigbo and Ogbonnaya, 2006). Some of the vernacular names in Nigeria include: (Ncho-anwu, Ahuji) Igbo, (Efinrin,) Yoruba, (Aramogbo) Edo and (Daidoya) Hausa (Effraim et al., 2000). *O. gratissimum* has been described to have other species in the flora of tropical West Africa. These include: *Ocimum viride* Linn, *Ocimum suave* Linn, *Ocimum basilicum* Linn and *Ocimum canum* Sims. Mshana et al. (2000) reported their numerous medical uses.

The *Ocimum* oil has been described to be active against several species of bacteria and fungi. These include *Listeria monocytogenes*, *Shigella*, *Salmonella* and *Proteus*, for fungi *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Cryptococcus neoformans*, *Penicillium islandicum*, and *Candida albicans* (Begum et al., 1993; Nwosu and Okafor, 1995; Akinyemi et al., 2004; Janine de Aquino Lemos et al., 2005; Lopez et al., 2005). From recent findings, *O. gratissimum* has proved to be useful in the medication for people living with Human Immuno deficiency Virus (HIV), and Acquired Immuno Deficiency Syndrome virus AIDs (Elujoba, 2000).

In Congo, *O. gratissimum* decoction is used for gonorrhoeal infection, vaginal douches for metritis and vaginitis and used in treatment of mental illness (Abdulrahma, 1992).

P. guineense commonly referred to as African black pepper or Ashanti pepper is very similar to *Piper nigrum* which is the true pepper of commerce from which black and white peppers are processed (Isawumi, 1984). *P. guineense* belongs to the family *piperaceae*. It has more than 700 species throughout the tropical and subtropical regions of the world. It is known with different vernacular names in Nigeria: Igbo (Uziza), and Yoruba (iyere). *P. guineense* has culinary, medicinal, cosmetic and insecticidal uses (Dalziel, 1955; Okwute, 1992). *P. guineense* insecticidal activity against *Zonocerus variegatus* is attributable to the piperine-amide composed by the plant. The leaves are considered aperitive, carminative and eupeptic. They are also used for the treatment of cough, bronchitis, intestinal diseases and rheumatism (Sumathykutty et al., 1999).

Escherichia coli and *Staphylococcus aureus* are organisms associated with the gastrointestinal tract of man and animals. They occur in other environment. *S. aureus* is among the invasive gram positive known as pyogenic cocci implicated in several diseases of human. It has been found to be normal flora of upper respiratory tract and vagina. *S. aureus* has been known to produce heat stable toxins which are implicated in illnesses and stomach upset (CDC, 1989; Mosset et al., 1990). From literatures, *S. aureus* had shown to be very resistant to a wide variety of antibiotics (Nwinyi et al., 2008). *E. coli*, a

facultative anaerobe of wide distribution in the environment, has been implicated in the cause of urinary tract infections, meningitis, sepsis wound infections, noscomial pneumonia and arthritis. A subgroup enterohemorrhagic *E. coli* (EHEC) can cause severe potentially fatal illness known as hemorrhagic colitis with symptoms of blood diarrhea and severe abdominal pain (Dolore et al., 2001). Thus, this study seeks to investigate the scientific basis for the traditional use, of these medicinal plants among the local people in Nigeria, for treating ailments associated with *E. coli* and *S. aureus* infections.

MATERIALS AND METHODS

Collection of plant material

Fresh samples of *O. gratissimum* leaves were collected from a forest at Alor town in Anambra State between the hours of 6-8 am at a prevailing temperature of about $28 \pm 2^\circ\text{C}$ while the fresh leaves of *P. guineense* were bought from traditional healers at a local market in Awka, Anambra State. All the collections were done in the month of May. The plants were identified and authenticated at the Herbarium of the Department of Biological Sciences, Nnamdi Azikiwe University, Awka, Anambra State. A voucher specimen was deposited at the Herbarium for reference purposes.

Preparation of extracts

Fresh leaves of *O. gratissimum* and *P. guineense* were thoroughly washed using tap water and rinsed with distilled water. The leaves were dried for 5 min in an oven at 60°C to stop enzyme activity (Effraim et al., 2000). They were then air dried to a constant weight and milled to a fine powder with the aid of a Binatone blender (Model BLG-401). Two solvent were used for the preparation of the extracts, namely distilled deionized water and ethanol 60% conc. The aqueous extract was prepared by weighing out (250 g) of the milled powdered leaves of *O. gratissimum* and *P. guineense* and adding in 200 ml of distilled deionized water respectively in a 500 mL beaker and stirring vigorously with a glass rod. The combination was allowed to settle for 3 h using the infusion method. The extracts were then filtered using Whatman no.1 filter paper. The ethanol extracts were obtained by weighing out same fraction 250 g of the different plants and wrapping it in Whatman no.1 filter paper and placed in the holding chamber of the soxhlet extractor. About 500 mL of the 60% ethanol was used as solvent for the extraction of the leaves using the reflux method for a period of 48 h. This was carried out exhaustively. From literature, Soxhlet extraction has been found to give a higher yield of the extracts. The extracts were then concentrated by evaporating to dryness using rotary evaporator at a temperature 40°C . A dark- green colored mass for *P. guineense* and *O. gratissimum* were obtained and stored in airtight bottles at 4°C in a refrigerator until ready for use. The stored extract was reconstituted using the corresponding solvent to obtain extracts of several concentrations 10.0, 5.0, 2.5, 1.25 mg/ml^{-1} and stored at 4°C prior to determination of the minimum inhibitory concentration.

Preparation of test organisms

Clinical isolate of *S. aureus* was obtained from Glanson labora-

Table 1. Antibacterial activity of the aqueous extracts of *Ocimum gratissimum* and *Piper guineense* against *Escherichia coli* and *Staphylococcus aureus*.

Plant	Test organism	Mean zones of Inhibition (mm)				
		Control (water)	10.0	5.0,	2.5,	1.25
<i>Ocimum gratissimum</i>	<i>Escherichia coli</i>	-	3± 0.09	-	-	-
	<i>Staphylococcus aureus</i>	-	7.5± 0.10	-	-	-
<i>Piper guineense</i>	<i>Escherichia coli</i>	-	2 ± 0.07	-	-	-
	<i>Staphylococcus aureus</i>	-	4± 0.10	-	-	-

(-), No inhibition of growth; (10.0, 5.0, 2.5, 1.25) mg/ml⁻¹, different concentrations.

Table 2. Antibacterial activity of the ethanol extracts of *Ocimum gratissimum* and *Piper guineense* against *Escherichia coli* and *Staphylococcus aureus*.

Plant	Test organism	Mean zones of Inhibition (mm)				
		Control (ethanol)	10.0,	5.0,	2.5,	1.25
<i>Ocimum gratissimum</i>	<i>Escherichia coli</i>	-	5± 0.09	-	-	-
	<i>Staphylococcus aureus</i>	-	8 ± 0.11	7.5± 0.11	6 ± 0.06	-
<i>Piper guineense</i>	<i>Escherichia coli</i>	-	10 ± 0.10	8± 0.10	7± 0.11	-
	<i>Staphylococcus aureus</i>	-	2± 0.01	-	-	-

(-), No inhibition of growth; (10.0, 5.0, 2.5, 1.25 mg/ml⁻¹), different concentration.

tories, Awka, Anambra State, Nigeria while the strain of *E. coli* was obtained from a culture collection centre at Sammlung von Mikroorganismen (DSM) Germany. The isolates were tested for viability by resuscitating the organisms in buffered peptone broth, after which it was sub-cultured into nutrient agar medium and incubated at 37°C for 24 h. The isolates were sub-cultured in a nutrient broth at 37°C for 8 h prior to antibacterial testing.

Determination of antibacterial activity of the aqueous and ethanolic extracts

Agar well diffusion technique as described by Adeniyi et al. (1996) was used to determine the antibacterial activity of the extracts. An 18 mL of Mueller Hinton agar plates (MHA Difco, France) were seeded with 2 mL of an overnight broth culture of each bacterial isolate (equivalent to 10⁷ – 10⁸ CFU mL⁻¹) 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 Pasteur pipette. The dishes were allowed to stand for 45 min at room temperature to allow proper diffusion of the extract to occur. The control experiments were setup with 2 mL of ethanol (60%) and distilled deionized water which served as controls were also put in separate wells.

All the plates were incubated at 37°C for 24 h. The assay was conducted at regular intervals of 24 h until marked decline in the potency of the extracts to inhibit the growth of the test organisms was noticed. Zones of clearance round each well means inhibition and the diameter of such zones was 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).

RESULTS

Antibacterial activity of the extracts (aqueous and ethanol) extracts.

The results of the antibacterial activity of the extracts against the test organisms namely *E. coli*, *S. aureus* are shown in Tables 1 and 2. The mean zones of inhibition of growth of the isolates are a function of relative antibacterial activity of the extracts. The extracts showed selective levels of activities against the isolates. The highest growth inhibitory activity was obtained from the ethanol extracts. Ethanol and distilled deionized water served as control and were inactive against both test organisms.

The minimum inhibitory concentration of the aqueous and ethanol extracts

Results of the minimum inhibitory concentration (MIC) are summarized in Table 3. Generally, less values were obtained for the aqueous extracts. Higher concentrations of the *O. gratissimum* extract were needed to inhibit *E. coli* when compared to *S. aureus*.

The MIC of the aqueous extract of *O. gratissimum* was 10.00 and 10.00 mg ml⁻¹ for *E. coli* and *S. aureus*, respectively whereas that of the ethanol extract ranged between 10.00 and 2.50 mg ml⁻¹ for *E. coli* and *S. aureus*, respectively.

Table 3. Minimum inhibitory concentration (MIC) of the aqueous and ethanol extracts of *Ocimum gratissimum* and *Piper guineense*.

Test organism	<i>Ocimum gratissimum</i>		<i>Piper guineense</i>	
	Aqueous extract (mg ml ⁻¹)	Ethanol extract (mg ml ⁻¹)	Aqueous extract (mg ml ⁻¹)	Ethanol extract (mg ml ⁻¹)
<i>Escherichia coli</i>	10.00	10.00	10.00	2.50
<i>Staphylococcus aureus</i>	10.00	2.50	10.00	10.00

DISCUSSION

From this investigation, the aqueous extracts of *P. guineense* and *O. gratissimum* showed minimal antibacterial activity against the isolates at 10 mg/ml⁻¹ concentrations. This observed difference between these plants extracts may be due to insolubility of active compounds in water or the presence of inhibitors to the antimicrobial components (Okigbo and Ogbonnanya, 2006). Amadioha and Obi (1999), Okigbo and Ajale.(2005a), Okigbo et al. (2005b) 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. Conversely, the ethanol extracts of *P. guineense* and *O. gratissimum* showed a concentration dependent gradient decrease in the level of inhibition against isolates. From the results there is variation in the degrees of antibacterial activities of the extracts on the isolates. The variation is presumed to be due to different active compounds present in these plants. Ethanol extracts of *O. gratissimum* showed more antibacterial activity against *S. aureus* than *E. coli*. This result is in agreement to Agatemor (2009) where it was reported that gram negative bacteria are more resistant than gram positive bacteria to the essential oil which are antimicrobial agents. Nweze et al. (2004) reported phytochemical screening of *O. gratissimum* in the presence of alkaloids, tannins, glycoside, saponin, resins, cardiac glycoside, steroidal terpenes and flavonoids. Flavonoids are reported to exhibit antioxidant activity (Ramanathan, et al., 1989) and are effective scavengers of superoxide anions (Robak and Gryglewski, 1988). Thus this can significantly affect the cell wall of *S. aureus* which invariably may lead to the collapse of the cell wall and overall, affect the entire mechanism of the organism.

E. coli a gram negative organism contains a high level of lipid material. These materials are thought to make a substantial contribution to the mechanism whereby injurious chemicals are prevented from reaching their sites of action within the cell. *P. guineense* ethanol extract showed significant inhibition to *E. coli* due to high content of essential oils such as monoterpenes and sesquiterpene and minimal effect was observed on *S. aureus*. This result agrees to Obafemi et al. (2006) where sesquiterpene was tested against *S. aureus*.

From this study, it was observed that ethanol extracts exhibited high inhibitory activity on the test organisms.

This can be deduced to the ability of ethanol to extract more of the essential oils and secondary plant metabolites which are believed to exert antibacterial activity on the test organisms. This study however can justify the use of the leaves in traditional medicine practice as a therapeutic agent and can explain the long history use of these plants.

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