

SENSITIVITY OF THREE GASTRO-INTESTINAL ORGANISMS TO AQUEOUS EXTRACT OF LEAF OF *Ocimum gratissimum*

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ABSTRACT

The aqueous extract of the leaf of *Ocimum gratissimum* (scent leaf) has been claimed according to indigenous pharmacopoea to be useful in the treatment of infections such as those of the upper respiratory tract, diarrhoea, skin, cough, fever and conjunctivitis. On an account to validate some of such claims, an investigation was carried out to determine the effect of the aqueous extract of the plant on micro-organisms implicated in gastroenteritis (*Salmonella typhimurium*, *Escherichia coli* and *Shigella dysenteriae*). The antibacterial susceptibility assay was carried out using the Agar well diffusion technique. The susceptibility of the tested bacterial isolates was determined by subjecting them to extract concentrations of 250mg/ml, 200mg/ml, 150mg/ml, 100mg/ml, 50mg/ml, 25mg/ml and 10mg/ml. The activity of the extract against the isolates was compared with that of standard antibiotics. Minimum inhibitory concentration was determined by measuring the turbidity of the macro broth dilution assay tubes relative to its absorbance using a spectrophotometer. Minimum bactericidal concentration was observed by selecting tubes that showed no growth during MIC and plating them on fresh agar plates. Phytochemical screening was done to ascertain the presence of bioactive components present. The antibacterial investigation revealed that *shigella dysenteriae* was the most susceptible of the tested isolates at 10mg/ml while *salmonella typhimurium* was the most resistant and had minimum inhibitory concentration at 125mg/ml. The minimum inhibitory and bactericidal concentration assays revealed that extract inhibited the growth of isolates in a concentration dependent manner with MICs ranging from (7.81 - 125) mg/ml. Phytochemical analysis revealed the presence of active principles such as flavonoids, terpenoids, tannins e.t.c. The ability of the aqueous extract of the leaf of *Ocimum gratissimum* to inhibit the bacterial isolates was well demonstrated and hence supports the use of infusions from the plant as useful in indigenous pharmacopoeia.

Keywords : *Ocimum gratissimum*, phytochemicals, susceptibility, bactericidal, Agar well assay

INTRODUCTION

The search for agents to cure infectious diseases began long before people were aware of the existence of microbes (Sofowora, 1982). These early attempts used natural substances, usually native plants or their extracts and many of such herbal remedies proved successful (Sofowora, 1993). The use of plants for medicinal purposes dates back to several thousand years ago in which various medicinal plants were known to cure various ailments. In recent years, extracts from medicinal plants have been purified and used for the production of purely synthetic drugs based on natural plant products and western medicine also is moving in this direction. Today globally the dilemma of emerging resistant diseases is starring the face of the human race and we are in a battle with microbes, which calls for urgent alternative approaches especially from nature's own pharmacy. Also,

in the present scenario where a larger percentage of the world population have no access to orthodox medication due to financial constraints, it is quite necessary to emphasize the relevance of traditional remedies which constitute a major part of the health care system in the developing countries and are also entering the therapeutics in the developed countries (Borris, 1996).

New efforts in the area of health care have been initiated globally and the newly emerging scientific discipline of ethnopharmacology forms part of it. The plant used in this study is *Ocimum gratissimum*. It is also known as African basil and belongs to the genus *Ocimum* of the family Lamiaceae, sub-family Napetoideae of the tribe Ocimeae.

Ocimum consists of between 50-150 species of aromatic annual and perennial herbs and shrubs from the tropical region of Africa, Asia, Central and South America. (Darrah, 1980). Morphologically the stem is erect, round and quadrangular, much branched, glabrous or pubescent, woody at the base often with epidermis peeling at the base. *Ocimum gratissimum* is widely planted by most people on nearby farmlands, towns and villages in Nigeria as vegetables mostly for soups. They are heat and drought tolerant (i.e. they do not require high humidity). It is a source of essential oils and aromatic compounds, culinary herb, and an attractive fragrant ornamental. The seeds also contain edible oils (Morales and Simon, 1996). It is widely grown in Nigeria and commonly known as scent leaf or "Nchu anwu" by the Igbos, "effirin-nla" by the Yorubas and "doya tagida" by the Hausas (Orwa *et al.*, 2009; Okoli *et al.*, 2010). The English people call it wild basil or tree basil, in French it is called 'menthe gabonaise' (Orwa *et al.*, 2009). Ethnopharmacologically, *O. gratissimum* has been used extensively in the traditional system of medicine in many countries. In the North east of Brazil, it is used for medicinal, condiment and culinary purposes.

Ocimum gratissimum is used by the Ibos of Southeastern Nigeria in the management of the baby's cord, to keep the wound surfaces sterile. It is also used in the treatment of fungal infections, fever, cold and catarrh (Ijeh *et al.*, 2004). The Yoruba tribe of Nigeria use decoctions of this plant in the treatment of abdominal disorders.

This work aims at examining the in vitro- sensitivity of gastrointestinal organisms to the aqueous extract of the leaf of *Ocimum gratissimum* with the view of validating claims of its efficacy in traditional medicinal practice.

MATERIALS AND METHODS

Source of plant material

Fresh leaves of the plant were obtained in the early hours of the morning at Asa-dam area within Ilorin metropolis of Kwara State, Nigeria. Confirmation and botanical naming, was done at the Herbarium of the Department of Plant Biology of the University of Ilorin, Nigeria. Sample was deposited and Voucher Specimen No. UIH 984 obtained at the Herbarium.

Sample preparation and extraction procedure

Aqueous extraction was adopted for the study. One hundred and twenty gramms (120g) of the fresh leaf was weighed and properly rinsed in sterile distilled water, pulverized by hand and soaked into 600ml of distilled water. This was left on an orbital shaker and allowed to stand for 4 hours. The filtrate was decanted and concentrated using a rotary evaporator. Afterwards, the concentrate was weighed to obtain a mass of 4.06g. Reconstitution was done using distilled water to obtain concentrations of 250, 200, 150, 100, 50, 25 and 10mg/ml (Akujobi *et al.*, 2004).

Test Isolates

The microorganisms used in this assay were *Escherichia coli*, *Salmonella typhimurium* and *Shigella dysenteriae*. They were obtained from the culture collection unit of the Department of Microbiology, University of Ilorin, Nigeria.

Antimicrobial sensitivity assay (agar diffusion technique)

The antimicrobial screening of the aqueous extract was carried out using the agar diffusion technique. Nutrient agar was poured in sterile Petri dishes and allowed to solidify. One millilitre of the test culture was streaked on the solidified agar using swab sticks. Wells of approximately 5mm in diameter were bored on the surface of the agar medium with the aid of a sterile cork borer. Each well was filled with 0.5ml of the aqueous extract. The plates were incubated at 37°C for 24 hours. Sensitivity of the organisms to the extract was recorded by reading zones of inhibition around the wells (Bauer *et al.*, 1966; Barry and Thornsberry, 1985).

Comparison of extract with standard antibiotics

The method of Bauer *et al.*, 1966 was adopted. The plates were prepared as done for the sensitivity assay except that wells were not bored at the centre of the plates rather, antibiotic discs were placed on the inoculated agar plates using sterile forceps. The antibiotic discs were gently pressed to ensure even contact with the medium. The plates were incubated at 37°C for 24 hours. Sensitivity of the organisms to antibiotics was recorded as above.

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration was determined by measuring the turbidity of the macro broth dilution assay tubes relative to its absorbance using a spectrophotometer (model type: Genesis.20, model no.: 4001/4 thermo fisher scientific, France). Ten test tubes were filled with 9ml of nutrient broth. 250mg/ml of the extract was added and serially diluted into tubes 2 – 9 to obtain concentrations of 125, 62.50, 31.28, 15.63, 7.81, 3.90, 1.95 and 0.95mg/ml. Tube 10 was used as a positive control in which no extract was added, while tube

11 served as the negative control containing broth only. Standardized suspensions of the test organisms were inoculated into the test tubes containing the various concentrations of extract, and incubated at 37°C for 24 h. The MICs were read as the least concentration of the extract that showed no turbidity translating to inhibition of growth of the test organisms (Black,1996).

Minimum bactericidal concentration (MBC)

MBCs were determined by first selecting tubes that showed no growth during MIC determination, a loopful from such tube was subcultured onto extract free agar plates, incubated for further 24 hr. at 37°C. The absence of growth on the agar plates was noted as the MBC (Black,1996).

Phytochemical screening

Phytochemical analysis was done on the extract to ascertain the presence of bioactive components present as described by Trease and Evans (1983) and Sofowora (1982).

RESULTS

Influence of different concentrations of aqueous extract on test isolates

The aqueous extract of the leaf of *Ocimum gratissimum* showed activity against all the test isolates with maximum inhibition rate of the plant extract observed with *Shigella dysenteriae* at a concentration of 250mg/ml. Also with lower concentrations of the extract *S. dysenteriae* exhibited the highest zones of inhibition compared to the other tested isolates. (Table 1). *Escherichia coli* and *Salmonella typhimurium* demonstrated higher resistance to the extract especially at lower concentrations such as from 10 – 50 mg/ml.

Table 1: Sensitivity profile of test isolates against aqueous extract of leaf of *Ocimum gratissimum*

Extract concentration (mg/ml)	Mean zone of inhibition (mm)		
	<i>Escherichia coli</i>	<i>Salmonella typhimurium</i>	<i>Shigella dysenteriae</i>
10	1.45±0.91 ^a	2.41±0.27 ^a	3.01±0.01 ^a
25	1.85±0.87 ^{a,b}	2.70±0.27 ^a	3.45±0.26 ^a
50	2.48±0.81 ^{a,b}	3.16±0.30 ^{a,b}	3.99±1.15 ^a
100	3.75±0.68 ^{b,c}	4.10±0.33 ^{b,c}	5.48±0.30 ^b
150	5.02±0.57 ^{c,d}	5.02±0.57 ^{c,d}	8.99±0.01 ^c
200	6.26±0.43 ^{d,e}	5.98±0.01 ^{d,e}	11.05±0.55 ^d
250	7.55±0.32 ^e	6.89±0.64 ^e	15.00±0.00 ^e

KEY: Each value is expressed as means of 3 replicates $n = 3$, $\bar{x} \pm SD$. Test values carrying superscripts different from each other across each parameter are significantly different at ($p < 0.05$).

Influence of standard antibiotics on test isolates.

The standard antibiotics used showed significant activity against the test isolates (Table 2) than did the plant extract. However, *Shigella dysenteriae* was again the most susceptible to relatively all the antibiotics used as observed for the extract assay. Although some of the antibiotics used (such as ampiclox) were not effective against the test isolates hence resulting in assay without sensitivity.

Table 2: Sensitivity profile of test isolates against standard antibiotics

Antibiotic	Mean zone of inhibition (mm)		
	<i>E coli</i>	<i>S. typhimurium</i>	<i>Shigella dysenteriae</i>
Gentamycin	8.74±0.73 ^d	0.00 ^a	5.20±0.12 ^b
Ciproflavin	15.00±0.00 ^f	0.00 ^a	19.70 ±2.14 ^e
Ciprofloxacin	10.00±0.00 ^e	12.65±1.36 ^d	7.32±0.39 ^{b,c}
Ampiclox	0.00 ^a	0.00 ^a	6.02±0.01 ^{b,c}
Augumentine	0.00 ^a	0.00 ^a	0.00 ^a
Chloramphenicol	6.73±0.01 ^c	6.57±0.34 ^c	14.20±1.04 ^d
Pefloxin	5.24±0.44 ^b	4.53±0.02 ^b	8.05±0.61 ^c
Ofloxacin	5.79±0.24 ^b	5.14±0.08 ^{b,c}	6.26±0.15 ^{b,c}

KEY: Each value is expressed as means of 3 replicates $n = 3$, $\bar{x} \pm SD$. Test values carrying superscripts different from each other across each parameter are significantly different at ($p < 0.05$).

Analysis of the minimum inhibitory and bactericidal concentrations of the aqueous leaf extract of *Ocimum gratissimum*

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) results are shown in Tables 3 and 4. Table 3 shows that the minimum inhibitory concentration of the aqueous extract against *Escherichia coli* and *Salmonella typhimurium* was obtained at a value of 125mg/ml while *Shigella dysenteriae* demonstrated highest susceptibility at a concentration of 7.81mg/ml.

Table 3: Minimum inhibitory concentrations (MIC) of aqueous leaf extract of *Ocimum gratissimum*

Organisms	Extract concentration (mg/ml)									MIC (mg/ml)
	250	125	62.50	31.28	15.63	7.81	3.90	1.95	0.95	
<i>E. coli</i>	-	-	+	+	+	+	++	++	++	125
<i>S. typhi</i>	-	-	+	+	+	++	++	++	++	125
<i>S. dysenteriae</i>	-	-	-	-	-	-	+	++	++	7.81

KEY: - = No growth, + = Growth ++ = Heavy growth

Table 4: Minimum bactericidal concentrations (MBC) of aqueous leaf extract of *Ocimum gratissimum*

Organisms	Extract concentration (mg/ml)									MIC (mg/ml)
	250	125	62.50	31.28	15.63	7.81	3.90	1.95	0.95	
<i>E. coli</i>	-	-	+	+	+	+	++	++	++	125
<i>S. typhi</i>	-	-	+	+	+	++	++	++	++	125
<i>S. dysenteriae</i>	-	-	-	-	-	-	+	++	++	7.81

KEY: - = No growth, + = Growth, ++ = Heavy growth

Phytochemical analysis

Phytochemical investigation of the leaf extract is presented in Table 5. This shows the presence of flavonoids, Tannins, saponins, terpenoids, and phlebotannins.

TABLE 5: Phytochemical analysis of the plant leaf extract.

Constituents	Result
Alkaloids	-
Flavonoids	+
Terpenoids	+
Tannins	+
Saponins	+
Phlobatannins	+

Key: - = Absent, + = Present

DISCUSSION

The result of this work shows that the aqueous extract of *Ocimum gratissimum* exhibits considerable antibacterial activity against all the test isolates. It was observed that generally *Shigella dysenteriae* was the most susceptible of the three isolates to all the concentrations of the plant extract, while lesser antibacterial activities were observed for *Escherichia coli* and *Salmonella typhimurium*. According to the assay higher activity was demonstrated with increased concentration of the extract. This could be attributed to the presence of more of the crude plant material in lesser volume of the diluent (water) which may have allowed for the concentration of the active ingredients in a kind of matrix in the environment medium. The reduced susceptibility of *E. coli* and *Salmonella typhimurium* to the extract (Table 1) could be due to the fact that the capsular polysaccharide of their outer cell membranes prevented the invasion of *Ocimum gratissimum* extract into the cells. This is in accordance with the work of Tegos *et al.* (2002) that *Salmonella typhimurium* has a high level of intrinsic resistance to several antimicrobials, due to the combination of a very restrictive outer membrane barrier. Another reason may be their possession of multi-drug resistance efflux pumps, which extrude amphipathic toxins and foreign molecules across the outer membrane (Tegos *et al.*, 2002). In the comparison of the standard antibiotic with the crude extract, conventional antibiotics produced larger zones of inhibition (Table 2) than the plant extract when assayed against the test isolates. This could be attributed to purity of conventional antibiotics which was not carried out on the crude extract thereby permitting certain inactive substances to disrupt the activity of the extract.

The analysis of the minimum inhibitory concentrations and Bactericidal concentration of the test isolates (Tables 3 and 4) showed that the highest MIC was produced by *Shigella dysenteriae*. This could be that the level of intrinsic resistance of this organism to antimicrobials is not high enough to prevent the invasion of the extract. Also, MICs and MBCs were got at higher concentrations of the extract. This could be attributed to more of the crude extract at such higher concentrations resulting in faster mortality of the isolates.

The inhibitory properties of the aqueous leaf extract suggests that it contains some active principles of medicinal values which are actually capable of exterminating the three isolates. As observed in this study from the phytochemical screening of the aqueous extract as shown in Table 5, the extract revealed the presence of Flavonoids, Terpenoids, Tannins, Saponins and Phlobatannins. These phytochemicals which are secondary metabolic products of plants, confer innate immunity to the plants as they inhibit the development of invading pathogens. More so, they have been shown to be present in reasonable amounts which have also been the reason for their peculiar scent. This is similar to the investigation made by Okwu, (2003) and Edeoga *et al.* (2005). Flavonoids have been shown to have a broad spectrum of activity against pathogenic micro organisms. The presence of terpenoids could also have added to the inhibitory effect of the aqueous extract, as studies have shown that terpenoids inhibit the growth of some enteric pathogenic micro organisms and as such are classified under the beta-lactam group of antibiotics as they diffuse through the medium and destroy the integrity of the cell membrane of microorganisms. Tannins which are frequently encountered in food product of plant-vegetable origin such as tea and many fruits, could have also contributed to the inhibitory effect of the aqueous plant extract, as it has the ability to shrink proteins, and thus capable of destroying the cells of microorganisms (Barnabas *et al.*, 1998). They are also known to be bitter and astringent to taste (Barnabas *et al.*, 1998). Tannins have also been shown to have oxidative ability due to the presence of gallic and diagallic acids (Roy *et al.*, 2006). Saponins which has found wide applications in both pharmaceutical and medical areas, could also influence the activity or inhibitory effect of the aqueous plant extract against the test organisms. Its foaming ability and frothing effect makes it useful in the manufacture of soaps and shampoos (Okwu, 2003).

Phlobatannins and cardiac glycosides also have their own contributing effect to the inhibitory properties of the plant leaf extract (Okwu, 2003).

CONCLUSION

Based on the results of this study, the aqueous extract of *Ocimum gratissimum* is indeed capable of inhibiting the growth of some enteric microorganisms and water is inferably a good solvent capable of extracting the active phytochemicals in the leaf of the plant. This study however justifies the claims of efficacy of infusions of the leaf and its use in traditional medical practice as a therapeutic agent.

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