PHYTOCHEMICAL AND ANTIBACTERIAL ACTIVITIES OF *Ocimum gratissimum* ON SOME SELECTED DRUG RESISTANT BACTERIA

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Abstract: The use of *Ocimum gratissimum* (scent leaf) as food and medicine could be attributed to its phytochemical and antimicrobial properties. This work is aimed at evaluating the phytochemicals and antibacterial activities of *Ocimum gratissimum* on some selected drug resistant bacteria. The antibacterial activities of the plant potent extracts were tested on the test isolates using Agar-well diffusion techniques. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extracts were determined according to the macro broth dilution technique. Phytochemical analysis of fresh and dried leaf extracts revealed the presence of tannins, alkaloids, flavonoids saponin, and terpeniods. Results obtained revealed that the cold water extracts of the fresh leaves was most potent, inhibiting all isolates with diameter zones of inhibition ranging from 16 to 20 mm, followed by ethanol extract of the dry leaves with zone range of 15 to 18 mm and hot water fresh leaf 11 to 18 mm, but *E. coli* showed total resistance to the cold water dry leaf extract. The extracts inhibited the growth of the bacterial isolates in a concentration dependent manner with MICs ranging between 50.5–150 mg/ml, while MBC ranged from 12.5–100 mg/ml. The findings from this study seem to provide the *in vitro* evidence that might justify *O. gratissimum* as a good candidate medicinal plant for further investigations, and that the active principles of the plant may be more polar in nature.

Keywords: Antibacterial, alkaloids, flavonoids, *Ocimum gratissimum*, phytochemicals

Introduction

The use of plant whether herbs, shrubs or tree, in parts or whole in the treatment and management of diseases dated back to pre-historic times. Plants extracts have been used in folk medicinal practices for the treatment of different types of ailments since antiquity (Kong *et al.*, 2008). During the last century, the practice of herbalism became mainstream throughout the world. In spite of the great advances achieved in modern medicine, plants still make an important contribution to health care. This is due to the recognition of the value of traditional medicinal systems.

World Health Organization (WHO, 1993) describes a medicinal plant as any plant in which oneor more of its organs contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs.

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plant are alkaloids, tannins, flavonoids, saponins and phenolic compounds (Mojab *et al.*, 2003).

The local use of natural plants as natural care remedies due to their pharmacological properties is quite common in Asia, Latin America and Africa (Bibitha *et al.*, 2002). In Nigeria, there are over five thousand local medicinal plants available as gifts from nature and used in the treatment of various diseases (Awosika, 1991).

Ocimum gratissimum popularly called scent leafis indigenous to India, but grown widely in West Africa including Nigeria. It belongs to the Kingdom: plantae, order: lamiales, family: lamiacea, genus: *Ocimum* and species: *Ocimum gratissimum*. It is often used in traditional medicine specifically grown in most compounds and sold in the market places. Spice basil, scientifically called Ocimumis commonly called sweet basil, tea bush. To the Igbos, it is called Nchuanwu, Effirin in Yoruba, Daidoga in Hausa, Ufuo-yibo in Urhobo, Ebaromwokhor in Bini, Bassilic in French, Tulsi in India and Basilica in Italian, (Elujoba, 2000). It is commonly used in folk medicine to treat different diseases of upper respiratory tract, diarrhea, headache, skin disease, pneumonia, fever and conjunctivitis (Oboh and Masodje, 2009). Recent studies on *Ocimum gratissium* proved it to be a useful medication for people living with Human Immune Deficiency Virus (HIV), and acquired Immune Deficiency Syndrome AIDS (Elujoba, 2000).

The leaves of the plant contain volatile oil which has been shown to contain some antibacterial properties and the vapour of the oil was reported to kill protozoa (El Said *et al.*, 1970). *Ocimum gratissimum* has also been reported to be active against several species of bacteria and fungi (Nwosu and Okafor, 1995; Nakaruma *et al.*, 1999)

The knowledge of the chemical constituents of plants could further be valuable in discovering the actual value of folkloric remedies (Mojab *et al*., 2003). Chemical constituent may be therapeutically active or inactive, the ones which are active are called active constituents and the inactive ones are called inert chemical constituents.

The aim of this present work therefore is to study the phytochemical constituents and the antibacterial activities of *Ocimum gratissium* (scent leaf) on some selected drug resistant bacteria.

Materials and Methods

Collection and preparation of plant materials

Fresh leaves of *Ocimum gratissimum* (scent leaf) was collected from Ariaramain market Aba, Abia State and was identified and authenticated at the Nnamdi Azikiwe University Herbarium Awka, by a taxonomist Egboka Tochukwu with Voucher number of NAUH182A.

Processing of plant samples

The fresh plants were properly washed in running tap water and then rinsed in sterile distilled water. The leaves were divided into two equal parts; one part was dried in the hot air oven at 40ºC for 3 days, while the second portion was blended fresh using electric blender. The dry leaves were pulverized using sterile laboratory mortar and pestle. It was stored in airtight glass containers protected from sunlight until required for extraction.

Extraction of plant material

Cold and hot extraction with water and soxhlet extraction with ethanol (99%)as described in AOAC (1980) were adopted for

the study. 20 g of each sample was weighed into 100 ml of the solvent (water and ethanol). For cold extraction, the samples and the solvent were stirred every 30 min for 3 h and allowed to stand for 24 h, while for hot extraction, the samples and solvent were heated for 30 min at 100ºC and stirred every 30 min for 3 h and allowed to stand for 24 h.

Extract dilution

After preparation of the crude extract as described, the aqueous and the ethanolic extracts were reconstituted using sterile distilled H_2O to obtain concentrations of 200, 150, 100 and 50 mg/ml.

Phytochemical screening

Phytochemical screening were carried out to test for the presence of secondary metabolites which include tannins, phlobatannins, alkaloids, flavonoids, saponins, glycoside, reducing sugar and steroids on both extracts (aqueous and ethanol) using standard procedures (Trease and Evans, 1983; 1978; Brain and Turner, 1975; Oyeleke and Manga, 2008).

Test for tannins

Two gram (2 g) of each extract was dissolved in 10 ml of distilled water in separate test tubes and 3 drops of 10% ferric chloride ($FeCl_3$) was added to 2 ml of the solution. The occurrence of blackish-blue, green or blackish green coloration indicates the presence of tannins.

Test for phlobatannins

Each extract of 0.2 g was boiled with an equal volume of 1% HCl, the deposition of a red precipitate indicate the presence of phlobatannins.

Test for saponins

Each extract of 0.1 g was dissolved in 5ml of distilled water and shaken vigorously. The formation of frothing bubbles which lasted for 10 min indicates the presence of saponin.

Test for alkaloids

Each extract of 0.5 g was dissolved in 3 drops of Dragendoffs reagent. An orange precipitate indicates the presence of alkaloid.

Test for flavonoids

Each extract of 0.2 g was dissolved in 2 ml of sodium hydroxide solution. The occurrence of a yellow solution which disappears on addition of Hcl acid indicates the presence of flavonoids.

Test for cardiac glycoside

Each extract of 0.5 g was dissolved in 3 ml of Fehling solution. A brick red precipitate indicates the presence of glycosides.

Test for steroids

Five (5) drops of concentrated H_2S0_4 was added to 0.1 g of each extract in test tube, a reddish brown coloration indicates the presence of steroids.

Test for reducing sugar

Each extract of 0.1 g was dissolved in 2 ml of distilled water in separate test tubes. This was followed by addition of Fehling solution (A + B), and then the mixture was warmed. A brick red precipitate at the bottom of the test tube indicates the presence of reducing sugar.

Sterilization of materials

Glass wares used were properly washed and sterilized in an autoclave at 121ºC at 15 psi for 15 min before use. The work was carried out under aseptic condition. The work bench was disinfected with 70% ethanol.

Media preparation

Nutrient agar medium and nutrient broth was used during the course of this work; it was prepared according to manufacturer's instructions.It was sterilized by autoclaving at 121^0C at 15psi for 15 min, after which it was allowed to cool and then poured into sterile plate and allowed to solidify.

Test microorganism

The bacterial strains used in this study were pure clinical isolates obtained from the Microbiology Laboratory, Braithwhyte Memorial Hospital Port-Harcourt, Rivers State. The isolates were strains of *Staphylococcus aureus* and *Escherichia coli*. The isolates were tested for viability by sub-culture into nutrient broth at 37ºC in an incubator for 24 h prior to antibacterial testing.

Biochemical identification of the test organism

Escherichia coli

The *E. coli* was placed on Eosine Methylene Blue agar for 18 h. Colonies with green metallic sheen were observed which indicate a positive result for *E. coli* (Oyeleke and Manga, 2008).

Staphylococcus aureus

The *S. aureus* was placed on Mannitol Salt Agar (MSA) for 18 h. Smooth circular colonies with yellow colour indicate a positive result for *S. aureus* (Oyeleke and Manga, 2008).

Antibacterial assay

The antibacterial assay of the plant extracts were carried out on the test isolates using Agar-well diffusion Technique. The isolates were inoculated on the surface of freshly gelled sterile nutrient agar plates by streaking using sterilized swab stick. Six wells were aseptically bored on each agar plate using a sterile cork borer (6 mm) and wells were properly labelled. Fixed volumes (0.1 ml) of different concentrations of the extracts (aqueous and ethanol) were then introduced into the wells in the plates respectively. The 5^{th} and 6^{th} well were used as positive control well (filed with Gentamicin) and a negative control well (filled with sterile water) respectively. The plates were allowed on the bench for 40 min for pre-diffusion of the extract to occur and then incubated at 37ºC for 24 h. The resulting zone diameter of inhibition was measured using a transparent ruler calibrated in millimetres. The readings were taken to be the zone diameter of inhibition of the bacterial isolate in question at that particular concentration (Koche *et al.*, 2012).

Minimum inhibitory concentration (MIC)

The MIC of the potent extracts was determined according to the macro broth dilution technique. Standardized suspensions of the test organism was inoculated into a series of sterile tubes of nutrient broth containing two-fold dilutions of leaf extracts and incubated at 37ºC for 24 h. The MICs were read as the least concentration that inhibited the growth of the test organisms (Koche *et al.*, 2012). The lowest or least concentration of the extract that shows no growth in the test tubes is the MIC of the extract tested.

Minimum bactericidal concentration (MBC)

The MBCs were determined by first selecting tubes that showed no growth during MIC determination; a loopful from each tube was sub-cultured onto already gelled nutrient agar plates using spread plate technique and incubated for 24 h at 37ºC. The least concentration, at which no growth was observed, was noted as the MBC (Koche *et al*., 2012).

Mode of action of the extracts

All plates showing no visible growth on the nutrient agar (NA) indicated bactericidal effect of the concentration of the extract used. Plates showing light growth indicated the bacteriostatic effects of the extract concentration. Concentrations of the extracts showing moderate and heavy growth were considered to have no inhibitory effect on the organism (Puyveld, 1986).

Results and Discussion

Table 1 shows the Phytochemical analysis of leaf extracts of *Ocimum gratissimum*. From the table, alkaloid, was found to be present in CWFL, HWFL, CWDL, HWDL, EXFL and EXDL. Cardiac glycosides was only found in CWDL and absent in HWFL, CWDL, HWDL, EXFL and EXDL.

Table 1: Phytochemical analysis of Leave extracts of *Ocimium gratissimum*

Extract compounds	CWFL	HWFL	CWDL	HWDL	EXFL	EXDL
Alkaloid	++	+	+	-	+	+
Tanins	++	+	+	-	+	-
Saponins	++	+	+	-	+	+
Flavonoids	++	+	+	+	+	+
Steroids	+	-	+	-	+	-
Anthraquinone	+	-	-	-	-	-
Terpenoids	+	+	+	+	+	+
Cardiac glycosides	+	-	-	-	-	-

– = Absent; + = Present in trace quantity; ++ = Present in appreciable quantity; CWFL = Cold water fresh leaf; HWFL = Hot water fresh leaf; CWDL = Cold water dry leaf; HWDL = Hot water dry leaf; EXFL = Ethanol extract fresh leaf; EXDL = Ethanol extract dry leaf

Table 2: Antibacterial activities of the fresh and dry leaf extract of *Ocimium gratissimum* on *S. aureus* and *E. coli*

Isolates	Mean zone diameter of inhibition (mm)					Extracts
S. aureus 19	18	17	16	20	0	CWFL
S. aureus 10	9	6	1	20	0	HWFL
S. aureus 8	5	2	0	16	0	CWDL
S. aureus 0	0	0	0	17	0	HWDL
S. aureus 4	2	0	0	18	0	EXFL
S. aureus 7	6	1	0	19	0	EXDL
E. coli 18	17	17	16	19	0	CWFL
E. coli 18	16	15	11	19	0	HWFL
E. coli 0	0	0	0	18	0	CWDL
E. coli 11	8	5	0	17	0	HWDL
E. coli 17	16	14	13	17	0	EXFL
E. coli 18	18	17	15	17	0	EXDL
	200	150	100	50	+C	-C

CWFL = Cold water fresh leaf; HWFL = Hot water fresh leaf; CWDL = Cold water dry leaf; HWDL = Hot water dry leaf; EXFL = Ethanol extract fresh leaf; EXDL = Ethanol extract dry leaf; +C = Positive control; –C = Negative control

The antibacterial activities of the fresh and dry leaf extract of *O. gratissimum* on *S. aureus* and *E. coli* is found on Table 2. The mean zone diameter of inhibition for *S. aureus* on the different extracts was between the ranges of 0 to 19 mm while that of *E. coli* was between 0 to 18 mm.

The MIC of leaf extracts of *Ocimum gratissimum* on *S. aureus* and *E. coli* is found on Table 3. The MIC of *S. aureus* was between 50 mg/ml while that of *E. coli* was between 50 to 150 mg/ml.

Table 3: Minimum inhibitory concentration (MIC) of leaf extracts of *O. gratissimum* on *S. aureus* and *E. coli*

Isolates	Concentration of Extracts(mg/ml)								Extracts
MIC	200	150	100	50	25	12.5	6.25	3.13	
S. aureus	-	-	-	-	+	+	+	+	CWFL 50
S. aureus	-	-	-	+	+	+	+	+	HWFL 100
S. aureus	-	-	-	-	+	+	+	+	EXFL 50
E. coli	-	-	-	-	+	+	+	+	CWFL 50
E. coli	-	-	+	+	+	+	+	+	HWFL 150
E. coli	-	-	-	-	+	+	+	+	EXFL 50

CWFL = Cold water fresh leaf; HWFL = Hot water fresh leaf; EXFL = Ethanol extract fresh leaf

Table 4: Minimum bactericidal concentration (MBC) of leaf extracts of *O. gratissimum on S. aureus* and *E. coli*

Isolates	Concentration of extracts (mg/ml)								Extracts
MBC	200	150	100	50	25	12.5	6.25	3.13	
S. aureus	-	-	-	-	-	+	++	++	CWFL 25
S. aureus	-	-	-	+	+	+	++	++	HWFL 100
S. aureus	-	-	-	-	-	-	++	++	EXFL 12.5
E. coli	-	-	-	-	-	++	++	++	CWFL 25
E. coli	-	-	-	-	-	++	++	++	HWFL 25
E. coli	-	-	-	+	+	+	++	++	EXFL 100

CWFL = Cold water fresh leaf; HWFL = Hot water fresh leaf; EXFL = Ethanol extract fresh leaf

The MBC of leaf extracts of *O. gratissimum* on *S. aureus* and *E. coli*is found on Table 4. The MBC for *S. aureus* isolates was between the range of 12.5 to 100 mg/ml while that of *E. coli* was between the range of 25 to 100 mg/ml.

The positive results for alkaloids, anthraquinnes, cardiac glycosides, saponins, tannins, flavonoids, steroids and terpenoids confirms the presence of these secondary metabolites in the cold water fresh leaf extract, hot water fresh leaf and cold water dry leaves except anthraquinones and cardiac glycosides of *O. gratissimum*. Antimicrobial effects of plant extracts have been attributed to the presence of these secondary metabolites (Oboh and Masodje, 2009). The presence of these metabolites in the investigated plant part account for its usefulness as a medicinal plant.

*Ocimumgratissimum*is known for its various uses in traditional medicine. Indeed, the drug is reported for its action against gastrointestinal infections (diarrhoea, dysentery), infections of the skin (dermatitis, eczema, scabies), infections of the upper respiratory tract, associated with cough, asthma and bronchitis, wounds and sores, insect bites, nosebleeds, stroke, anaemia (Elujoba, 2000). This could be attributed to the presence of essential oil.

Ocimum gratissimum contains eugenol and shows some evidence of antibacterial activity (Nweze and Eze, 2009). Spices are aromatic flavourings made from parts of plants. The "Spice" is a culinary term not a botanical category, it does not refer to a specific kind of plant or plant part (Farrell, 1990). Each spice has a unique aroma and flavour which derive from compounds known as phytochemicals or secondary compounds. These chemicals evolved in plants to protect them against herbivorous insects, vertebrates, fungi, pathogens, and parasites (Walker, 1994). Spices are used as substances that increase the taste and variation of food (Bulduk, 2000). It frequently also includes herbs, which are the fragrant leaves of herbaceous plants, many of which are native to temperate regions.

The results obtained in this study revealed the antimicrobial efficacy of cold water extract of the fresh leaf *Ocimum gratissimum* on all the test isolates. Its highest inhibitory activity, suggests that the active component of this plant may be a highly polar compound. This is similar to the findings of Ije h*et al.* (2005), but in contrast to the report of Obi and Onuoha (2000), who reported alcohol to be the best solvent for the extraction of most plant active principles of medical importance. From this study alcohol could not have been the best plant solvent, since the entire test isolates were completely resistant to ethanol fresh extracts, except for *E. coli* that was particularly susceptible to ethanol dried extracts with zones of inhibition ranged from 16-20 mm. The susceptibility of *E. coli* to extracts of fresh and dried leaf, confirms the antimicrobial activity reported by (Sofowora, 1982) using both fresh and dried leaf of the plant, but the none inhibitory activity of the dried leaf extract, suggest that active principle of the plant may be heat labile, and likely lost during drying. It is not unusual to observe antimicrobial activity of

plant extract to have been contributed by solvents of extraction, but in this study solvents used toreconstitute extracts were observed not to possess any antibacterial effect.
The minimum inhibitory concentrations observed for cold water, hot water and ethanol extracts of the fresh leaf ranged from 50.5–150 mg/ml while minimum bactericidal concentration (MBC) gave a range of 12.5–100 mg/ml. The variation in results imply that the MBC results obtained after plating on various dilutions of extracts is more reliable compared to MIC results obtained usually using turbidity as an index.

Conclusion

Phytochemical screening revealed that alkaloid was found to be present in CWFL, HWFL, CWDL, HWDL, EXFL and EXDL. Cardiac glycosides were only found in CWDL and absent in HWFL, CWDL, HWDL, EXFL and EXDL.
The antibacterial results revealed the highest inhibitory efficacy of cold water extract of the fresh leaf of *Ocimum gratissimum* on all the test isolates. Its highest inhibitory activity, suggests that the active component of this plant may be a highly polar compound.
The variation in results obtained for the minimum inhibitory concentrations of cold water, hot water and ethanol extracts of the fresh leaf compared to the minimum bactericidal concentration (MBC), imply that the MBC results obtained after plating on various dilutions of extracts is more reliable compared to MIC results obtained using turbidity as an index.
The demonstration of antimicrobial activity is an indication that the plant is a potential source for the production of drugs with a broad spectrum of activity (Oboh and Masodje, 2009). The result of this study also supports the traditional application of the leaves of the plant in treating health related issues and suggests that the plant extract possesses compounds with antibacterial properties that can be used as antibacterial agents in novel drugs for the treatment of health issues associated with drug resistant bacteria (Oboh and Masodje, 2009). Further pharmacological evaluations, toxicological studies and possible mechanism of action processes are the future challenges that require further research.

Conflicts of Interest

Authors declare that there is no conflict of interest.

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