



Original Research Article

Antimicrobial activity and quantitative analysis of *Ocimum Gratissimum* on some pathogenic bacteria

Received 4 June, 2018

Revised 19 July, 2018

Accepted 23 July, 2018

Published 21 September, 2018

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Ocimum gratissimum commonly called scent leaf is a herbaceous plant belong to the family of lamiacea . It is a well-known medicinal plant amongst farmer and rural dwellers in Nigeria. An investigation of the antimicrobial activity and phyto constituent of *Ocimum gratissimum* root and leaf was conducted against some clinical isolates of *Pseudomonas auroginosa*, *Klebsiella sp* , *Bacillus subtilis* and type culture of *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC20923). The water extract of both root and leaf were effective against the test organisms. They had no effect on *Bacillus subtilis* and *Pseudomonas auroginosa*. The ethanol extract of the root showed no action against the test organisms because no zone of inhibition was observed. The effect of aqueous (water) and ethanol extracts of the leaves and roots of *Ocimum gratissimum* were compared with a standard antibiotic (ciprofloxacin). The result showed that the extracts had a strong antimicrobial activity. The minimum inhibitory concentrations (MIC) ranges from 5mg/ml to 20mg/ml. *Ocimum gratissimum*, possesses some important phytochemicals which includes alkaloids, tannins, saponins, flavonoids, and quercetin.

Key words: *Ocimum gratissimum*, *Pseudomonas auroginosa*, *Klebsiella sp*, *Bacillus subtilis*, *Escherichia coli*, ciprofloxacin, phytochemicals.

INTRODUCTION

Ocimum gratissimum L. commonly called scent leaf originated from Africa and southern Asia. It is a perennial scrub belonging to the family of Lamiaceae. It grows up to 1-3m high, the stem which bears the leaves are dark brown. *Ocimum. gratissimum* leaves are opposite to each other, narrow and oval in shape and they (leaves) grow to about 5-13cm in length and 3-9cm in width.

Ocimum gratissimum has been used extensively in the traditional system of medicine in many countries. In the Northeast of Brazil, it is used for medicinal, condiment and culinary purpose. The flowers and the leaves of this plant are rich in essential oils which makes it an excellent recipe in the preparation of teas and infusion (Rabelo et al., 2003). Brazilian tropical forest inhabitants use a decoction of *Ocimum gratissimum* roots as a sedative for children (Freire et al., 2006). People of Kenyan and sub Saharan African communities' use *Ocimum gratissimum* for various purposes like, the leaves are rubbed between the palms and

sniffed as a treatment for blocked nostrils, they are also used for abdominal pains, sore eyes, ear infections, coughs, fever, convulsions, tooth ache and regulation of menstruation (Matasyoh et al., 2007). In India, the whole plant has been used for the treatment of sunstroke, headache, influenza, as a diaphoretic, antipyretic and for its anti-inflammatory activity (Prajapati et al., 2003). In the coastal areas of Nigeria, the plant is used in the treatment of epilepsy, high fever and diarrhea (Effraim et al., 2003). In the Savannah areas, decoctions of the leaves are used to treat mental illness (Akinmoladun et al., 2007). *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., 2005).

There are many tribes in Nigeria that use the leaves extract in the treatment of diarrhea, while the cold leaves infusion is used for relief of stomach upset and hemorrhoids (Kabir et al., 2005). The plant is commonly used in folk medicine to treat different diseases such as

upper respiratory tract infections, diarrhea, headache, diseases of the eye, skin diseases, pneumonia, cough, fever and conjunctivitis (Adebolu et al., 2005). *Ocimum gratissimum* in the coastal areas of Nigeria is used in the treatment of epilepsy (Osifor, 1992), high fever (Oliver, 1980) and diarrhea (Oliver, 1980; Sofowora, 1999). The whole plant is used as an antibacterial agent throughout West Africa (Iwu et al., 1999). The study was aimed at investigating the antimicrobial activities and quantitative analysis of aqueous and ethanol extracts of *Ocimum gratissimum*.

MATERIALS AND METHODS

Plant material

The leaves and roots of *Ocimum gratissimum* were obtained from a farm land in Olomoro community of Delta State.

The glass wares used include; beakers, conical flasks, bottles (Mar cartney and universal), test tubes, thermometer and pipettes. The reagents used include; water, ethanol and dimethyl Sulfoxide (DMSO). Other materials include; autoclave, disposable Petri dishes, disposable syringes, disposable hand gloves, core borer, sterile swab sticks, Mueller Hinton agar, spirit lamp, inoculating needle, aluminum foil paper and cotton wool.

Preparation of water leaf/root extracts

Dried leaves and roots of *Ocimum gratissimum* were blended with a warring blender and thirty grams of the powder was soaked in 100ml of distilled water. 2ml of chloroform were added to prevent microbial contamination and left to stand for 24 hours on a laboratory work bench. The mixture was filtered using whatman No.1 filter paper.

Preparation of ethanol leaf/ root extracts

The leaves and roots of *Ocimum gratissimum* were pounded in a mortar. Fifty (50) grams of each was weighed and placed in a soxhlet extractor. A 100ml of ethanol was added to each and left on the laboratory bench for 8 hours. The mixture (the mashed leaves and roots in ethanol) were evaporated to dryness using a rotary evaporator. This was repeated several times to get desirable quantities for the experiment.

Preparation of agar medium

Powdered Mueller Hinton agar weighing 38grams was put into a conical flask containing 1 liter of distilled water. This mixture was swirled vigorously for 5 minutes left for 10 minutes on the laboratory bench. The mixture was then sterilized by autoclaving at 121°C for 15 minutes. The solution was allowed to cool down to ambient temperature of 32.6°C before pouring into the Petri dishes.

Purification of isolates

Pseudomonas aeruginosa, *Bacillus subtilis* and *Klebsiella sp* were clinical isolates obtained from the microbiology laboratory of University of Benin Teaching Hospital (UBTH) while *Escherichia coli* (ATC25922) and *Staphylococcus aureus* (ATCC20923) were America typed culture obtained from pharmaceutical microbiology department of University of Benin (UNIBEN). These isolates were further purified to ascertain their authenticity by culturing them on different media and observing their colony characteristics such as colour, elevation edge etc (Figure 1-5).

Determination of antimicrobial activities of *occimum gratissimum* at 400 mg/ml concentration.

Aqueous and ethanol extracts of the leaves of *Ocimum gratissimum* of 4gram each was dissolved in 10ml of water. The mixture was swirled vigorously until the substance dissolved. The ethanol and water extracts of the roots were insoluble in sterile water. Therefore, 4grams of both extracts was dissolved in 6mls of DMSO (Dimethyl sulfoxide). Then, 4ml of sterile water was later added to make it up to 10ml. This was again swirled vigorously until the extracts dissolved. The stock was made up of 4mg of the extracts in 10ml of sterile distilled water. The stock was used to test the clinical isolates of the bacteria.

Preparation of 0.5 mcfarland turbidity of colony suspension

An inoculum of each isolate was transferred with the aid of a flamed wire loop into 1ml of normal saline to give a suspension of 10^8 cfu which is the equivalent to 0.5 mcfarland turbidity.

Antimicrobial susceptibility assay

The agar well diffusion method described by (Orafidiya et al., 2002) was employed for the antimicrobial screening of the aqueous and ethanol extracts of the roots and leaves of *Ocimum gratissimum*. The prepared Mueller Hinton agar medium was poured into sterile Petri dishes and allowed to solidify. A sterile swab stick was dipped into the colony suspension of the isolates and used to streak the entire surface of the solidified Mueller Hinton agar medium in the Petri dishes. Wells of approximately 10mm in diameter were made on the surfaces of the already streaked Petri dishes using sterile core borer. The Petri dishes were inverted and labeled with a marker. The base of the wells were sealed with the molten Mueller Hinton agar using pipette. Each well was filled with 0.2 ml of the extracts using micro pipette. A sensitivity ciprofloxacin disc was picked using sterile forceps and placed on the surface of the agar medium (this served as standard) while water was used as control. The plates were incubated at 37°C for 24hours. Sensitivity of the organisms to the extracts, the standard and the control were recorded.



Figure 1: *Bacillus subtilis*



Figure 4: *Klebsiella* sp.



Figure 2: *Escherichia coli*



Figure 5: *Pseudomonas aeruginosa*



Figure 3: *Staphylococcus aureus*

Determination of minimum inhibitory concentration (MIC) of the extracts

The agar dilution method was used for the determination of the minimum inhibitory concentration (MIC) of the extracts. Mueller Hinton agar medium was prepared following the manufacturer's instructions. From the stock (4mg of the extracts of the roots/leaves of *Occimum gratissimum* each in 10ml of sterile distilled water to give 400 mg/ml conc.), different concentrations were prepared as follows: 5 ml of extract in 15 ml of Mueller Hinton agar to give 100 mg/ml conc., 2.5ml of extract in 17.5ml of Mueller Hinton agar to give 50mg/ml conc., 1ml of extract in 19ml of Mueller Hinton agar to give 20mg/ml conc., 0.25 ml of extract in 19.5ml of Mueller Hinton agar to give 10mg/ml conc., 2.5ml of extract in 19.75ml of Mueller

Table 1. Inhibition Zone diameter of *Ocimum gratissimum* Extracts against test micro organism

Microbes	Wt extract (L)	Et extract (R)	Wt extract (L)	Et extract (R)	Cipro
<i>E. coli</i>	15.33±0.33	20.33±0.33	16.67±0.33	-	25.00±0.00
<i>S.aureus</i>	19.67±0.88	17.67±0.33	16.00±0.00	-	30.00±0.00
<i>Klebsiella</i>	15.00±0.57	20.67±0.33	16.00±1.00	-	25.00±0.00
<i>B. subtilis</i>	-	-	12.00±0.00	-	25.00±0.00
<i>P.aurogenosa</i>	-	-	-	-	-

Key: - means no zone of inhibition, L means leaf, R means root, Wt means water, Et means ethanol

Table 2. Minimum inhibitory concentration of active extract against test organisms in mg/ml

Microbes	Wt extract (L)	Et extract(L)	Wt extracts (R)	Et extract (R)
<i>E. coli</i>	10	10	20	NA
<i>S. aureus</i>	10	20	10	NA
<i>Klebsiella</i>	5	10	20	NA
<i>B. subtilis</i>	10	20	20	NA

Key: NA means not applicable

Hinton agar to give 5mg/ml conc. and 0.13ml of extract in 19.86ml of Mueller Hinton agar to give 2.5mg/ml conc. The solutions of the extracts were filtered, and at different volumes were incorporated into molten agar [(melted agar before solidifying). The mixtures (agar and extracts) were then poured into separate sterile Petri dishes and allowed to solidify. The organisms were streaked onto the solidified agar extract medium. All plates were incubated for 24 hours at 37°C.

Determination of phytochemicals constituents

Quantitative analysis was carried out on the extracts of roots and leaves of *Ocimum gratissimum* to ascertain the present of active compound. The presence of alkaloid, flavonoid, tannin, terpenoid and oxalate were determined using the method described by Sofowora (1999), Edeoga et al., (2005).

RESULTS

All values are expressed in mean ± S.E.M. (standard error of mean) for three replicate. One-way ANOVA with Turkey Multiple Comparison Test was performed. A statistical significance of $p > 0.05$ was used in all cases.

DISCUSSION

Plants extracts have been used in folk and even modern medical practices for the treatment of different ailments, most of which are due to microbial activities (Irobi, 1992). Bacterial infection seems controllable especially when good hygienic practice and effective antibacterial drugs are used. The development of resistance to antibiotics is an almost

inevitable consequence of their application (Ekhaie and Okoruwa, 2001).

Extracts of *Ocimum gratissimum* have been reported to show more antibacterial activity against *S. aureus* than *E. coli* (Agatemor 2009). Other studies also showed the effect of the extract of *O. gratissimum* against *S. aureus*, *Listeria monocytogenes*, *Escherichia coli*, *Shigella*, *Salmonella* and *Proteus* (Nwosu and Okafor, 1995; Akinyemi et al., 2005; Lopez et al., 2005).

The result from this study shows that crude extracts of *O. gratissimum* possess antimicrobial activities against both gram negative and gram-positive organism. Hence, confirming its uses in the treatment of common nosocomial infection. Several researches have also found that extracts from several plants possess antimicrobial activities against bacteria causing human infections (Irobi, 1992).

The ethanol leaves extract of *Ocimum gratissimum* had higher inhibitory zone of 21mm against *E. coli*, and *klebsiella* sp than the water extract of the leaves and root while the water extract of leaves had a zone of inhibition of 20mm against *S. aureus*. The ethanol extract of roots was inactive against the test organisms because no zone of inhibition was recorded. This could probably be that ethanol is not the right solvent for root extraction (Table 1). This is in agreement with (Oladimeji, 2005) who demonstrated the antimicrobial activity of *Ocimum gratissimum* against the same bacteria isolates. However, these authors did not determine the antibacterial effect of the root extract of *Ocimum gratissimum*.

The potency of the ethanol extract over the water extract may be due to ability of ethanol to extract bioactive compound and because ethanol diffuses easier into the medium than water (Okigbo and Mmeka, 2008; Obi and Onuoha. 2000).

The minimum inhibitory concentration (MIC) values obtained varies from 5mg/ml to 20mg/ml (Table 2). These

Table 3. Phytochemical Analysis of the Leaves Extract of *Ocimum Gratissimum*

Phytochemical constituent	Ethanol extract (%)	Water extract (%)
Tannins	1.72	0.19
Saponin	1.21	0.11
Flavonnioid	0.48	-
Steroids	0.71	0.1
Terpenoids	0.11	-
Alkaloids	1.97	1.67
Oxalate	0.13	-
Phylate	-	-
Antharquinone	0.19	-

Key: - means absent

Table 4. Phytochemical Analysis of Roots Extract of *Ocimum Gratissimum*

Phytochemical constituent	Ethanol extract (%)	Water extract (%)
Tannins	1.12	0.11
Saponin	0.21	-
Steroids	-	-
Terpenoids	0.15	-
Alkaloids	1.67	0.71
Oxalate	-	-
Phvlate	-	-
Antharquinone	-	-

Key: - means absent

variations may be due to genetic differences between strains.

The result from this study also revealed the presence of the phytochemical agents in the leaves and roots of the plant (Table 3 and 4) . Phytochemicals in medicinal plants have been reported to be the active principles responsible for the pharmacological potentials of medicinal plants (Edeoga et al., 2005). The presence of these chemicals in the leaf of *Ocimum gratissimum* justifies the local use of this plant for the treatment of various diseases. The leaves are rich in flavonoids, saponins and tannins, with considerable amount of phenolics and alkaloids. The high level of flavonoids in the leaves might be responsible for the use of the plant by traditional healers to treat diabetes.

Saponins are natural glycosides that act as hypoglycemic, antifungal and serum cholesterol lowering agents in animals. This substantiates its use as a local condiment for the nursing mothers in some tribes in Nigeria. Tannins are bitter polyphenolic compounds that hasten the healing of wounds. They also possess anti-diuretic and anti-diarrhea properties (Okwu, 2004). The significant amount of tannins in the leaves of *O. gratissimum* might be responsible for its use by the local herbalists to treat gastrointestinal disorders (Khoobchandani et al., 2010). The concentration of phenolics in the leaves of *O. gratissimu* shown in this study indicates that it can be a source of antioxidants. Alkaloids are chemicals which help plants to repel some predators. The concentration of alkaloid in the

leaves as shown in this study could contribute to the use of the leaves as an insect repellent.

CONCLUSION

The role of medicinal plants in the health care delivery system in Nigeria cannot be over emphasized. The antimicrobial actions of the aqueous (water) and ethanol extract of *Occimum gratissimum* revealed their inhibitory effects against the test organisms. The antimicrobial action justifies the traditional use of *Occimum gratissimum* (*scent leave*) in the treatment of various bacterial infections. The present result also revealed the possible active compounds to which its medicinal value may be attributed. Thus, there is a crave need for further research to be conducted on the applications of *Occimum gratissimum* in medicine folk. This will further determine its efficacy in the enhancement of primary health care delivery system, in developing countries and Africa in particular.

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