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Phytochemical Analysis and Antibacterial Activities of Ocimum Gratissimumleaves

[1,]OLANREWAJU , C. A. [2,]UJU, B. C.
Department of Biological Sciences, University Of Abuja,
PMB 117, Abuja, Nigeria.

ABSTRACT
The phytochemical analysis and antibacterial activities of Ocimumgratissimum leaf extracts were carried out in the month of July, 2012.The phytochemical screening of Ocimumgratissimumleaves revealed the presence of steroids, saponins, alkaloids in the aqueous extract while flavonoids, alkaloids, cardiac glycosides and tannins were found in the ethanolic extract. The bacterial strains used in this study were pure clinical isolates of Staphylococcus aureusand Escherichia coli obtained from the Microbiology Laboratory, University of Abuja Teaching Hospital, Abuja. The isolates were tested for viability by sub-culture into nutrient broth at 37^{0}C and kept in the incubator for 24 hours prior to antibacterial testing.The antibacterial activities of the plant extracts were tested on the test isolates using Agar-well diffusion techniques. The MIC (Minimum Inhibitory Concentration) of the potent extracts was determined according to the macro broth dilution technique. The Minimum BactericidalConcentration (MBC) was also carried out. Escherichia coli and Staphylococcus aureus showed inhibition zones ranging from 11.0 to 20.0mm. From this study, it was observed that ethanol extracts exhibited high inhibitory activity on Escherichia coli; a representative of enteric coliforms and Gram negative bacteria and Staphylococcus aureus; a representative of Gram positive bacteria than the aqueous extract. 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 for the maintenance of health and can explain the long use of this plant. Further experimental research efforts on the plant and its extracts are needed to be able to ascertain the safety of the plant.

***KEY WORDS**: nutrient broth, agar well diffusion, MIC, MBC, E.coli, S. areus*

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I. INTRODUCTION

Progress over the centuries towards a better understanding of a plant derived medicine has depended on two factors that have gone hand in hand. One has been the development of increasingly strict criteria for proof that a medicine really does what it is claimed to do and the other has been the identification and chemical analysis of the active compound in the plant (Holiman, 1989). Medicinal plants are of great importance to the health of individual and the communities. The medicinal value of some plants lies in some chemical substances that produce definite physiological actions in the human body. The most important of these bioactive constituents are alkaloids, tannins, flavonoids and phenolic compounds. According to Obute (2007), many traditional herbal practitioners tend to hide the identity of plants used for different ailments largely for fear of lack of patronage should the patients learn to cure themselves. Thus to mystify their trade, cultivation of the plant is not encouraged, consequently collection is virtually from the wild. The discovery of medicinal plants has usually depended on the experience of the populace based on long and dangerous self-experiment. Progress over the centuries towards a better understanding of a plant derived medicine has depended on two factors that have gone hand in hand. One has been the development of increasingly strict criteria for proof that a medicine really does what it is claimed to do and the other has been the identification and chemical analysis of the active compound in the plant (Holiman, 1989). Many of these indigenous medicinal ethno botanical and ubiquitous plants serve as rich natural resources of natural drugs for research and development (Okwu, 1999, 2001;Kong *et al*., 2008). Medicinal plants based drugs owe the advantage of being simple, effective and exhibit broad spectrum activity. Many published reports have shown the effectiveness of traditional herb against microorganisms, as a result plants are one of the bedrocks for modern medicine to attain new principles (Evans *et al*., 2002).The role of plants in the development of new drugs are; they may become the base for the development of a medicine, a natural blueprint for the development of new drugs, and a phyto-medicine to be used for the treatment of disease. Medicinal uses of plant ranges from the administration of the roots, stem,

leaves and seeds to the use of extract and decoction from the plant (Ogbulie*et al*., 2004). As a result of factors like high cost of synthetic drugs, their side effects, development of antibiotic resistant strains of microorganism, poverty etc. majority of the population in some developing countries including Nigeria depends largely on medicinal plants for their health needs (Jawetz*et al*., 1991).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.*Ocimumgratissimum*is also known as African Basil. It belongs to the Kingdom: plantae, order: lamiales, family: lamiacea, genus: *1cimum* and species:*Ocimumgratissimum*. Its vernacular names include "Nchonwu" in Igbo,"Effinrin" in Yoruba, "Aramogbo" in Edo and "Daidoya" in Hausa.It is naturally used in the treatment of different diseases which includes: upper respiratory tract infections, diarrhoea, headache, conjunctivitis, skin diseases, pneumonia, tooth and gum disorder, fever, and as mosquito repellants (Ilori*et al.,* 1996).

II. MATERIALS AND METHODS

Collection and preparation of plant materials

Fresh leaves of *Ocimumgratissimum* (scent leaf) was collected from Gwagwalada market in the month of July2012 and was identified and authenticated in the botany section of Departmentof Biological Sciences, University of Abuja by a taxonomist.The fresh plants were properly washed in running tap water and then rinsed in sterile distilled water. The fresh plant was air dried for two weeks at room temperature. The plant was pulverized using mortar and pestle to obtain the powder form.

Aqueous Extraction

Fifty grams (50g) of the powdered leaves was suspended in 500ml of distilled water in one litre conical flask. It was shaken vigorously for 30minutes and allowed to stand for 48hours at room temperature. Muslin cloth was used to filter the plant and the filtrate was further purified by filtration through Whatman No1 filter paper under aseptic conditions. The filtrate collected was evaporated to dryness using a water bath. The extract was collected in fresh sterile universal bottles and stored in the refrigerator at 4^0C until when required for use (Atata*et al*., 2003).

Ethanol Extraction

Fifty grams (50g) of the powdered leaves was suspended in 500ml of 95% ethanol in one litre conical flask. It was shaken vigorously for 30minutes and allowed to stand for 48hours at room temperature. Muslin cloth was used to filter the plant and the filtrate was further purified by filtration through Whattman No1 filter paper under aseptic conditions. The filtrate collected was evaporated to dryness using a water bath. The extract was collected in fresh sterile universal bottles and stored in the refrigerator at 4^0C until when required for use (Atata*et al*., 2003).

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

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

Test for phlobatannins

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

Test for saponins

0.1g of each extract was dissolved in 5ml of distilled water and shaken vigorously.The formation of fronthing bubbles which lasted for 10minutes indicate the presence of saponin.

Test for alkaloids

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

Test for flavonoids

0.2g of each extract was dissolved in 2ml 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

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

Test for steroids

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

Test for reducing sugar

0.1g of each extract was dissolved in 2ml 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 121^0C at 15 psi for 15 minutes 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 15minutes, 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, University of Abuja Teaching Hospital, Abuja. The isolates were strains of *Staphylococcus aureus*and *Escherichia coli*. The isolates were tested for viability by sub-culture into nutrient broth at 37^0C in an incubator for 24 hour prior to antibacterial testing.

Biochemical Identification of the Test Organism

Escherichia coli

The E. coli was placed on Eosine Methylene Blue agar for 18 hours. 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 Manitol Salt Agar (MSA) for 18 hours. Smooth circular colonies with yellow colour indicate a positive result for S. aureus (Oyeleke and Manga, 2008).

Extract Dilution

The method of Akujobi*et al.*(2004) was adopted. The crude extract was diluted with the $DMSO_4$ to obtain concentration of 200, 150, 100, and 50mg/ml respectively.

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. Four wells were aseptically bored on each agar plate using a sterile cork borer (6mm) 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. A control well was in the centre with 0.01 ml of the extracting solvent. The plates were allowed on the bench for 40 minutes for pre-diffusion of the extract to occur and then incubated at 37^0C for 24 hours. 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 37^0C for 24 hours. 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 hours at 37^0C. The least concentration, at which no growth was observed, was noted as the MBC (Koche*et al*., 2012).

III. RESULT AND DISCUSSION

The result of the phytochemical screening of *Ocimumgratissimum*leaves revealed the presence of steroids, saponins, alkaloids in the aqueous extract while flavonoids, alkaloids, cardiac glycosides and tannins were found in theethanolic extract (Table 1).

Table 1: Phytochemical analysis of dried leaf extract of *Ocimumgratissimum*

Phytochemicals	Ethanol leaf extract	Aqueous leaf extract
Tannins	+	-
Saponins	-	+
Flavonoids	+	-
Steroids	-	+
Cardiac glycosides	+	-
Alkaloids	+	+
Reducing sugar	-	-
phlobatannin	-	-

Key: + = present
- = Absent

The phytochemical analysis result of the leaf of this plant is similar to that of Nweze *et al*.(2004). The 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 leaves of *Ocimumgratissimum*justifies the local use of this plant for the treatment of various ailments. Flavonoids are compounds that are biologically active against liver toxins, microorganisms, inflammation, tumor and free radicals (Okwu, 2004). Saponins are natural glycosides that act as hypo-glycemic, antifungal and serum cholesterol lowering agents in animals (Sapna*et al*., 2009). Saponins are essential elements in ensuring hormonal balance and synthesis of sex hormones (Okwu, 2003). Tannins are bitter polyphenolic compounds that hasten the healing of wounds. They also possess anti-diuretic and anti-diarrhea properties (Okwu, 2004). Conversely, condensed tannins can inhibit herbivore digestion by binding to consumed proteins, thereby making it indigestible for animals. Its concentration in the leaves might be the reason why animals do not graze on this plant (Edeoga*et al*., 2006). The result of this work also showed that the ethanolic extract revealed high inhibitory zones than aqueous extract (Tables 2, 3&4).

Table 2 Determination of antimicrobial activity (inhibitory zones) of ethanol and aqueous extracts of *Ocimumgratissimum* leaf on *S.aureus* and *E.coli.*

Micro organism	**Zone of inhibition (mm)**								
	Concentration of ETE (mg/ml)				Concentration of AQE (mg/ml)				Control (mg/ml
	200	100	50	25	200	100	50	25	20
Staphylococcus aureus	20	17	13	13	17	15	13	11	22
Escherichia coli	19	18	11.6	11	15	14	12	11	24

Key: ETE = Ethanol extract

AQE = Aqueous extract

Table 3: Minimum inhibitory concentration (MIC) of ethanol and aqueous extracts of *Ocimumgratissimum* on *S. aureus* and *E. coli.*

Extract	Test organism	Concentration in (mg/ml)					
		200	100	50	25	12.5	6.25
Ethanol	*S. aureus*						
Aqueous		-	-	-	+	+	+
		-	+	+	+	+	+
	E. coli						
Ethanol		-	-	-	+	+	+
Aqueous		-	-	+	+	+	+

Key: - = No growth (clear)
+ = Bacteria growth (Very turbid)

Table 4: Minimum bactericidal concentration (MBC) of ethanol and aqueous extract of *Ocimumgratissimum* against *S. aureus*and E.*coli*.

Extract	Test organism	Concentration in (mg/ml)					
		200	100	50	25	12.5	6.25
	S. aureus						
Ethanol		-	+	+	+	+	+
Aqueous		-	+	+	+	+	+
	E. coli						
Ethanol		-	-	+	+	+	+
Aqueous		-	+	+	+	+	+

- Key: + = Bacteria growth of colonies
- - = No growth of bacteria colonies

This observed difference between these plants extracts may be due to insolubility of active compounds in water, the presence of inhibitors to the antimicrobial components in the ethanolic extract or the antibacterial activity of the solvent, ethanol. Okigbo and Ogbonnanya(2006) attributed this observation to the high volatility of ethanol which tends to extract more active compound from the sample than water. The ethanol and aqueous extracts of *O. gratissimum* showed a concentration gradient decrease in the level of inhibition against isolates. *Escherichia coli* and *Staphylococcus aureus* showed inhibition zones ranging from 11 to 20mm and 11-17mm respectively. Other reports such as Nwinyi*et al*. (2009) and Agatemor (2009) have shown results similar to this work. From this study, it was observed that ethanol and aqueous extracts exhibited high inhibitory activities on *Escherichia coli*; a representative of enteric coliforms and Gram negative bacteria and*Staphylococcus aureus* ; a representative of Gram positive bacteria. Ethanolic extract had higher inhibition compared to the aqueous extract. 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 for the maintenance of health and can explain the long use of this plant.

IV. RECOMMENDATION

This study draws attention to the need for further studies on this plant for the treatment of diseases. Experimental research efforts on the plant and its extracts are needed to be able to ascertain the safety of the plant.

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