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Therapeutic Effect of Ethanolic and Aqueous Extracts of Ocimum Gratissimum Leaves Against Gram-positive and Gram-negative Bacteria

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Abstract

Aim: This study is aimed at evaluating the therapeutic effect ethanolic and aqueous extracts of Ocimum gratissimum Leaves against gram-positive and gram-negative bacteria.

Materials and Methods: Fresh and healthy leaves of O. gratissimum were harvested, air dried and milled into powder and extracted using ethanol and water as solvents. The antibacterial activities of both extracts were determined by diffusion method. Nutrient agar medium was prepared using standard method. Pure cultures of Coliform bacillus, Staphylococcus epidermidis, Streptococcus viridans, Salmonella typhi and Escherichia coli were obtained from the Department of Veterinary Microbiology and Parasitology, Federal University of Agriculture, Abeokuta, Nigeria. The extracts were serially diluted to obtain 1.0%, 0.5%, 0.25%, and 0.125% solutions in sterile test tubes. Sterilized 9 mm filter paper disc soaked in the diluted extracts were placed on the plate and incubated for 24 hours at room temperature. The plates were examined for clear zones of inhibition. Presence of zones of inhibition indicated activity.

Results: The results showed that both ethanolic and aqueous extracts of O. gratissimum leaves has antibacterial potential against all the test organisms used in this study and also exhibited inhibitory activity against them. However, the inhibitory activity of the ethanolic extract was greater than that of the aqueous extract.

Conclusion: The result of this present study showed that O. gratissimum extracts possess antibacterial potential against both gram positive (S. epidemidis and S. viridans) and gram negative bacteria (C. bacillus, E. coli and S. typhi). However, both extracts have a greater inhibitory activity against gram positive bacteria than gram negative bacteria. In the same vein, ethanolic extract of O. gratissimum leaves possess higher inhibitory activity than its corresponding aqueous extracts.

Keywords: Ocimum gratissimum, C. bacillus, E. coli, S. epidemidis, S. typhi, S. viridians

1. INTRODUCTION

The search for newer sources of antibiotics is a global challenge pre-occupying research institutions, pharmaceutical companies, and academia, since many infectious agents are

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becoming resistant to synthetic drugs. In recent years, the growing demand for herbal products has led to a quantum increase in volume of plant materials traded across the countries [1]. However, the use and history of herbs dates back to the time of early man, who had the crudest tools as his implements and use stones to start his fire [1]. They used herbs in their raw and cooked forms to keep fit. Since that time, the use of herbs has been known and accepted by all nations and has been known also as the first line of treatment available to man [2]. The importance of herbs in the management of human ailments cannot be overemphasized. It is clear that the plant kingdom harbours an inexhaustible source of active ingredients invaluable in the management of many intractable diseases [3]. Furthermore, the active components of herbal remedies have the advantage of being combined with other substances that appear to be inactive. However, these complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components [1].

Ocimum gratissimum is an herbaceous plant which belongs to the Labiatae family. It is commonly referred to as scent leave. The plant is indigenous to tropical areas especially India and it is also in West Africa. In Nigeria, it is found in the Savannah and coastal areas. It is cultivated in Ceylon, South Sea Islands, and also within Nepal, Bengal, Chittagong and Deccan [4]. It is known by various names in different parts of the world. In India it is known by its several vernacular names, the most commonly used ones being Vriddhutulsi (Sanskrit), Ram tulsi (Hindi), Nimma tulasi (Kannada). In Nigeria, the plant is called "effinrin-nla" by the Yorubas, "Ahuji" or "Nchuanwu" by the Igbos, "Daidoya" by the Hausas [5]. O. gratissimum has been reported to have immense health benefit such as treatment of diarrhea, chronic dysentery and vomiting. It is used in treatment of oral infection, fungal infections, fever, cold and catarrh [5]. It is also used for the prevention and treatment of malaria, earache and colon pains [6]. It can be used for the treatment of urinary infections, gonorrhea infection, vaginal douches for vaginitis [7]. Airaodion et al. [8] reported that the plant is rich in phytochemical constituents and possesses antioxidant potential. This present study therefore sought to evaluate the therapeutic effect of ethanolic and aqueous extracts of O. gratissimum leaves against gram-positive and gram-negative bacteria.

2. MATERIALS AND METHODS

2.1 Collection and Extraction of Plant Materials

Fresh and health leaves of O. gratissimum free from disease were harvested from the Institute of Agricultural Research and Training, Moor Plantation, Ibadan and were identified by a botanist. They were washed in running water to remove contaminants. They were air dried at room temperature in an open laboratory space for 14 days and milled into powder using an electric blender (Moulinex). The extraction was done using soxhlet apparatus and ethanol as the solvent according to the method described by Airaodion et al. [9,10]. About 25g of the powder was packed into the thimble of the soxhlet extractor. 250mL of ethanol was added to a round bottom flask, which was attached to the soxhlet extractor and condenser on a heating mantle solvent was heated using the heating mantle and began to evaporate moving through the apparatus to the

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condenser. The condensate dripped into the reservoir housing the thimble containing the sample. Once the level of the solvent reached the siphon, it poured back into the round bottom flask and the cycle began again. The process was allowed to run for a total of 18 hours. Once the process was completed, the ethanol was evaporated in a rotary evaporator at 35 oC. The aqueous extract was obtained by the method described by Taiwo [11]. About 25 g of the powder O. gratissimum leaves was soaked in 250 mL of water in a conical flask. The mixture was stirred, covered, and allowed to stand for 24 hours, and filtered using sterile Whatman No.1 filter paper. The filtrate was concentrated to 20 ml on a water bath and evaporated to dryness at room temperature. The various extracts were used for the analysis of antibacterial activities and bacterial inhibition assay.

2.2 Determination of Antibacterial activity

The antibacterial activity of extracts of O. gratissimum leaves was determined by the diffusion method of Kirby Bauer described by Duguid et al. [12].

2.3 Preparation of the Nutrient Medium

Nutrient agar medium was prepared according to the method described by Taiwo [11]. 2.8 g of nutrient agar was dissolved in 100 mL distilled water. The solution was sterilized in an autoclave at 121 oC at 1.1N pressure for 15 minutes. The suspension was cooled and poured into sterile Petri-dishes to solidify. The agar depth of the medium was 4.0 mm.

2.4 Preparation Cultures and Inoculation

Pure cultures of Coliform bacillus, Staphylococcus epidermidis, Streptococcus viridans, Salmonella typhi and Escherichia coli obtained from the Department of Veterinary Microbiology and Parasitology, Federal University of Agriculture, Abeokuta, Nigeria were separately used to inoculate the Petri-dishes. This was done by streaking the surface of the plates in a zigzag manner until the entire surface was then covered. The inoculated plates were then incubated at room temperature for 24 hours [11].

2.5 Assay of Bacterial Inhibition Activity

Both ethanolic and aqueous extracts of O. gratissimum leave were serially diluted to obtain 1.0%, 0.5%, 0.25%, and 0.125% solutions in sterile test tubes according to Taiwo [11]. Sterilized 9 mm filter paper disc soaked in the diluted extracts was placed on the plate and incubated for 24 hours at room temperature. The plates were examined for clear zones of inhibition. Presence of zones of inhibition indicated activity. The zones were measured.

3. RESULTS

The results of antibacterial activity and inhibition of bacterial growth by both ethanolic and aqueous extracts of O. gratissimum leaves are presented in tables 1 and 2 respectively.

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Table 1: The Antibacterial Activity of O. gratissimum Leaf Extracts

Test Organism	Ethanolic Extract	Aqueous Extract
Coliform bacillus	+	+
Staphylococcus epidemidis	+	+
Streptococcus viridans	+	+
Salmonella typhi	+	+
Escherichia coli	+	+

Table 2: Inhibition of Bacterial Growth by O. gratissimum Leaf Extracts

Test Organism	Dilution (%)	Zone of Inhibition (mm)	
		Ethanolic Extract	Aqueous Extract
Coliform bacillus	1.00	4.00	3.50
	0.50	3.50	2.00
	0.25	3.00	1.50
	0.125	2.00	1.00
Straphylococcus epidermidis	1.00	7.50	7.00
	0.50	5.50	5.50
	0.25	4.00	3.50
	0.125	2.50	2.00
Streptococcus viridans	1.00	6.50	5.00
	0.50	5.00	4.00
	0.25	3.50	2.50
	0.125	2.50	2.00
Salmonella typhi	1.00	5.50	3.00
	0.50	3.50	2.50
	0.25	2.00	1.00
	0.125	1.00	0.00
Escherichia coli	1.00	5.00	4.50
	0.50	4.00	3.00
	0.25	2.50	2.50
	0.125	1.50	1.00

4. DISCUSSION

Emergence of resistant strains of pathogenic microorganism has continued to pose a major health concern about the potency and efficacy of several drugs, most importantly antibiotics currently in use [13]. Thus, attention has been shifted to medicinal plants. Sofowora [14] and Balandrin et al. [15] defined medicinal plants as plants in which one or more organs contain substances that can be used for therapeutic purposes or which it precursors for the manufacturing of drugs are useful for disease therapy. Since medicinal plants do not nearly save people from feeling pain but permit them to emerge unscathed, they deserve investigation. The local use of natural plants as primary health remedies, due to their pharmacological properties, is quite common in Asia, Latin

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America, and Africa [16]. This present study is aimed at evaluating the therapeutic effect of ethanolic and aqueous extracts of O. gratissimum leaves against gram-positive and gram-negative bacteria

In this present study, both ethanolic and aqueous extracts of O. gratissimum leaves were observed to have high range of antibacterial potential against both gram positive (S. epidemidis and S. viridans) and gram negative bacteria (C. bacillus, E. coli and S. typhi) (table 1). Both extracts were also effective against antibiotic resistant bacteria and their toxic products. This effect might be due to the constituents of O. gratissimum leaves [8]. This is in agreement with the findings of Ogunjobi and Elizabeth [17] but contradicts that of Airaodion et al. [18] who reported that both ethanolic and aqueous extracts of Carica papaya leaves showed no antibacterial activity against S. typhi. S. typhi is a gram-negative bacterium that is responsible for typhoid fever and has been a burden on developing nations for generations [19]. Therefore, O. gratissimum leaves might be useful in the management of typhoid fever.

The results of the inhibition of bacterial growth by the extracts of O. gratissimum leaves showed that both extracts exhibited highest antibacterial activity against gram positive organisms. This showed that the gram-negative bacteria were relatively more resistant to the extracts than the gram-positive bacteria. The susceptibility of bacteria to antibiotic chemical is expressed in minimum inhibitory concentration (MIC) or high zone of inhibition [20]. MIC is the lowest concentration of a chemical, usually a drug, which prevents visible growth of a bacterium or bacteria. MIC depends on the microorganism, the affected organism (in vivo only), and the antibiotic itself [21]. The susceptibility of bacterial strains also depend on their structural composition, particularly gram-positive bacteria contain lower percentage of lipid than the gram-negative bacteria [1]. Thus, the lipid content of the membranes will have an effect on the permeability of hydrophobic and volatile bioactive substances in O. gratissimum leaves. Hence this phenomenon may favor the destruction of the cell wall and genetic material of gram-positive bacteria than that of gram-negative bacteria [22]. Therefore, the lower inhibitory action of O. gratissimum leaves on gram-negative bacteria in this study might mean that the outer membrane of gram-negative bacteria make it less susceptible to antimicrobials than gram positive bacteria [23]. This is agreement in with the findings of Wolde et al. [22]. This result justified the folkloric use of O. gratissimum in the treatment of dysentery and diarrhea.

It was also observed in this study that ethanolic extract of O. gratissimum leaves possessed greater inhibitory activity than its corresponding aqueous extracts in all the test organisms. This might be due to its low viscosity which has reciprocal relationship with the rates of diffusion. Thus, the molecule of ethanol extracts of O. gratissimum leaves inhibits the bacterial growth. It has also been reported that active pharmacological agents of plant extracts are more soluble in ethanol than water [22].

5. CONCLUSION

The result of this present study showed that O. gratissimum extracts possesses antibacterial potential against both gram positive (S. epidemidis and S. viridans) and gram negative bacteria

(C. bacillus, E. coli and S. typhi). However, both extracts have a greater inhibitory activity against gram positive bacteria than gram negative bacteria. In the same vein, ethanolic extract of O. gratissimum leaves possess higher inhibitory activity than its corresponding aqueous extracts

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