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ANTIMICROBIAL ACTIVITIES OF SCENT LEAVES (OCIMUM GRATISSIMUM) ON *ESCHERICHIACOLI* AND *SALMONELLATYPHI*

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ABSTRACT
Essential oils or volatile oils from medicinal plants have potentially very strong anti microbial activities for the control of pathogenic micro organisms. Ocimum gratissimum is one of the medicinal plants which are widely used as sources of extracts with strong antibacterial and anti oxidant properties. In this study the leaf extract of Ocimum gratissimum was tested for its antibacterial activity against some human pathogenic bacteria (salmonella typhi and Escherichia coli). Antibacterial activity test was carried out by using the agar plate diffusion method. The plants materials were dried and extracted with 90% ethanol and water. The tested extract exhibited a significant antimicrobial activity against both strains. The antimicrobial activity was determined by measuring the zones of inhibition. The most significant inhibitory activity was observed against E. coli (22mm zone and 18mm zone) at 100mg/ml for both ethanolic and aqueous extracts. The physical properties of the extracts of Ocimum gratissimum were evaluated. The colours of the extracts were greenish and brownish for ethanol and aqueous extracts respectively. Phytochemical properties of the plants were analysed and the plant was confirmed to contain alkaloids, cyanide, flavonoids, saponis, carbohydrates, tannins, oxalates and phytates.

Keywords: Ocimum gratissimum, Antibacterial, Aqueous, Ethanolic, Extract.

INTRODUCTION
A vast number of infections are of microorganism origin. Diseases have diverse causes, which can he classified into two broad groups: infectious and non-infectious. Infectious diseases can spread from one person to another and arc caused by microscopic organisms that invade the body. Non-infectious diseases

are not communicated from person to person and do not have, or are not known to involve infectious agents (Microsoft Encarta, 2010). Infections are injurious contamination of the body or parts of the body by pathogenic agents, such as fungi, bacteria, protozoa, rickettsiae or viruses, or by the toxins that these agents may produce. These are mostly microorganisms therefore, making the studies of methods of preventing their survival and proliferation very important. The prevention of the growth and development of these organisms are achieved mostly through the use of' antimicrobial agent formulations either in the form of synthetic drugs or plant extracts.

Over 50% of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programmes. The World Health Organization (WHO) estimates that approximately 80% of the world's inhabitants rely on traditional or herbal medicines for their primary health care and plants have long formed the basis of sophisticated traditional medicine systems and purportedly provide excellent leads for new drug developments (WHO, 2008). Herbal medicine is the oldest form of healthcare known to mankind and over 50% of all modern clinical drugs are of natural products origin and natural products play important roles in drug development in the pharmaceutical industry (preethi et al., 2010). The use of herbal products for medicinal benefits has an important role in nearly every culture on earth. Herbal medicine was practiced by the ancient people of Asia, Europe and the Americas (Wargovish et al., 2001).

Ocirnum gratissirnum (Scent leaf) is widely distributed in tropical and warm temperate regions. The plant is commonly used in folk medicine to treat different diseases such as upper respiratory tract infections, diarrhea, headache, skin diseases, pneumonia, cough, fever and conjunctivitis

(Onajobi, 1986). Moreover, a lot of work has been done to show the antimicrobial properties of this plant to some selected pathogens. For example, *O. gratissimum* has been reported to be active against several species of bacteria and fungi (Nwosu and Okafor, 1995).

Over the years, plant materials and their extracts have been used in the treatment and prevention of diseases and infections of microorganism origins but some of these extracts produce adverse allergies in their consumers. This study therefore seeks to examine comparatively, the antimicrobial activities of both aqueous and ethanolic extracts of the scent leaf plant.

MATERIALS AND METHOD

COLLECTION AND PROCESSING OF PLANT MATERIAL

Ocimum gratissimum was collected from the off-campus area of The Federal Polytechnic Bida, Niger state. The fresh plants were thoroughly washed with tap water and spread on a clean sack and was allowed to dry completely for about four weeks at room temperature before using them for this study. The plant (*Ocimum gratissimum*) was identified at the department of Science Laboratory Technology, The Federal Polytechnic, Bida, Niger state. After drying, it was grounded to powder using a sterile bLender. This was done to enhance the penetration of the extracting solvent into the plant cells thus facilitating extraction of the active ingredient from the plant.

SOURCE OF TEST ORGANISM

The Test organisms for this study were members of the family Enteriobacteriaceae namely *Escherichia coil* and *Salmonella spp*. The pure clinical isolates were obtained from the laboratory of the Federal University of Technology Minna, Niger state. All the clinical isolates were checked for purity and were maintained in nutrient broth until it was required for use.

SAMPLE PREPARATION

The extraction of the active substances of the plant material was carried out as described by Atata et al., (2013).

About 50g of the powdered plant material were macerated in 500m1 of 90% ethanol and also in 500ml distilled water for about 48hours with agitation at regular intervals. After 48hours, the macerated materials were filtered using Muslin cloth and then Whatmann No. I filter paper. The filtrates were then concentrated to dryness at 40°C using water bath (Gellenkamp 4B 6233 D). The following concentrations of the extracts were prepared using distilled water: 0.2g/l0ml, 0.4g/l0ml, 0.6g/10rnl, 0.8g/10rnI and 1g/l0rnl for 20mg/ml, 40 mg/ml, 60 mg/ml, 80 mg/ml and 100 mg/ml.

The various concentrations were then collected in fresh sterile universal bottles and sterilized in hot air oven after which they were tested for sterility as described by Ronald (1995) by introducing 2m1 of the supposed sterile extract into I Omi nutrient broth, Incubation was done at 37°C for 24hours. A sterile extract was indicated by absence of turbidity after the incubation.

STANDARDIZATION OF BACTERIAL CELL SUSPENSION

The nutrient broth cultures of the organisms for this study were taken and inoculated on a fresh agar plate for 24hours. Sterile distilled water was poured on it and then mixed with the inoculums, 1ml each was taken and were transferred into 9m1 of sterile distilled water and dilute to 10^4 fold. One hundred microlitre of this were taken and poured on the surface of the n spread evenly with the use of a spreader on the plate to be used for this study. Well were bored on the agar triplicates using sterile cork borer (No. 4) of about 6mm in diameter and a drop of sterile nutrient agar was used to seal up the base of the bored holes.

AINTIMICROBIAL TESTING OF PLANT EXTRACT

About 0.1 ml of the various concentration of the plant extracts were introduced into the bored wells and were allowed to stand on the bench for about 10 - I5minutes to allow for diffusion of the extract then the plates were incubated at 37°C for 24hours after which they were examined for presence or absence of zones of inhibition.

The Minimum Inhibitory Concentration (MIC) was determined as described by Ayogu and

Amadi (2008). l'he plates with the 100mg/mi of plant extracts were considered the MICs. Fresh

Nutrient agar medium plates were prepared and the inoculums were made from the MIC plates.

RESULT AND DISCUSSION

RESULT

The result of the antimicrobial activities of ethanol and aqueous extracts of *Ocimum gratisslinum* on isolates of *Escherichja coil* and *Salmonella typhi* revealed that the extract had significant antimicrobial activity on *E. coli* which had a mean zone of inhibition of 14.0 and *Salmonella ryplii* had a mean zone of inhibition of 6mm and 10mm for both ethanol and aqueous extracts at a concentration of 100mg/mi.

The lowest concentration preventing all visible growth was taken as the Minimum Inhibitory concentration (MICs), this were I00mg/mI for both *S. typhi* and *E. coil* in both the ethanol and aqueous extracts.

Table 4a: Phytochemical of *O. gratissimum*

PHYTOCHEMICAL COMPOUNDS	STAUS
Alkaloids	+
Carbohydrates	+
Cyanide	+
Flavonoids	+
Oxalates	+
Tanins	+
Saponins	+
Reducing groups	-

Table 4b: Inhibitory Activities of Different Concentrations of Ethanolic Extracts of *Ocimum gratissimum* on the Test Organisms

ORGANISM	EXTRAT CONCENTRATION (mg/ml)	Zone of inhibition (mm)
Salmonella typhi	20	6.0
	40	7.2
	60	8.0
	80	8.6
	100	10.0
E. coli	20	12.0
	40	15.0
	60	17.6
	80	19.8
	100	22.0

Table 4b: Inhibitory Activities of Different Concentrations of Ethanolic Extracts of *Ocimum gratissimum* on the Test Organisms

ORGANISM	EXTRAT CONCENTRATION (mg/ml)	Zone of inhibition (mm)
Salmonella typhi	20	7.0
	40	9.4
	60	10.0
	80	7.0
	100	14.0
E. coli	20	8.3
	40	9.0
	60	12.7
	80	14.0
	100	18.0

Discussion

Naturally occurring substances of plant origin have been reported to inhibit the growth of microorganisms. 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 especially controllable due to good hygiene and the availability of effective antibacterial drugs. The development of resistance to antibiotics is an almost inevitable consequence of their application. The speed of resistance depends on the respective class of antibiotics and their product LISC (Ekhaise and Okoruwa. 2011).

The results presented in this research work shows that crude extracts of *Ocimiim gratissimum* possesses antibacterial activities against common gram-positive and gram-negative organisms thus confirming the use of the plant in the treatment of common infections. The observed antibacterial effects of both ethanolic and aqueous extracts of *Ocimum gralissimum* on the bacterial isolates used, though *invitro* appear interesting and promising. This is an indication that the plant extract may indeed be effective in the management of common infections, supporting its ethno-medical importance. This finding is in agreement with Aluyi *el al.* (2003) and Nwze *el al.* (2004), who found that various extracts of plants inhibited the growth of some isolates. The phytochemical screening of the crude extract of the plant studied showed that the extract was rich in

alkaloids, flavonoids, saponins, tannins, phytates, carbohydrates and oxalates have been known to show medicinal activities as well as physiological activities (Sofowora, 1993; Nwze *el al*. 2004). The plant studied here can be seen as a potential source of useful drugs.

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