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ANTIBACTERIAL PROPERTIES AND PRELIMINARY PHYTOCHEMICAL ANALYSIS OF METHANOLIC EXTRACT OF *OCIMUM GRATISSIUM* (SCENT LEAVES)

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ABSTRACT
Methanolic extract of the leaves of Ocimum gratissium (scent leaves) was screened for its phytochemical and antibacterial properties on some clinical bacterial isolates, visa vie as Staphylococcus aureus, Escherichia coli, Proteus mirabilis and Pseudomonas aeruginosa. The agar diffusion technique was used to assay the growth inhibition against the four bacterial isolates. The antibacterial effect of the methanolic extract of Ocimum gratissium was compared to that of some selected commercially available antibiotics. The inhibitory effect of the methanolic extract was comparable at all concentrations tested to the commercially available antibiotics indicating the high potency of the methanolic extract O. gratissium. Results obtained showed, the extract inhibits the growth of the test isolates with diameter of zones of inhibition of 5mm to 10mm for P. aeruginosa, P. mirabilis, E. coli and S. aureus respectively. The Broth microdilution assay gave minimal inhibitory concentration values ranging from 13.5μg/ml to 15.0μg/ml. The result of the phytochemical analysis of the dried leaves extracts revealed the presence of alkaloids, carbohydrate and saponins.

Keywords: Herbalism, Phytochemicals, Scent, Benin

INTRODUCTION

Rich storehouses of medicinal plants exist everywhere especially in Africa which offers a vast reservoir of plants that has been categorized (Aluyi *et al.,* 2003). The medicinal properties of various plant materials and extracts have been recognized since the beginning of the 5th century (Kay, 1986).

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 (Okanla *et al*., 1990). 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) (1976) describes a medicinal plant as any plant in which one or 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 (Hill, 1952). *Ocimum gratissium* is indigenous to India, but grown widely in West Africa including Nigeria. It is often used in traditional medicine specifically grown in most compounds and sold in the market places. Spice basil, scientifically called *Ocimum* is commonly called sweet basil, tea bush. In the Igbos, it is called Nchuanwu, Effirin in Yoruba, Bassilic in French, Tulsi in India, Basilica in Italian, Ufuo-yibo in Urhobo and Ebaromwokhor in Bini (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 (Correa, 1932). 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).

This study was aimed at investigating the antibacterial properties and preliminary photochemical analysis of methanolic extract of *Ocimum gratissium*.

MATERIALS AND METHODS

Sources of *Ocimum gratissium*

The leaves of the plant used for the research were collected from farmland at Agho Street, Off Ekenwan Road, Benin City, Edo State, Nigeria.

Sources of test bacterial isolate

The clinical isolates of *Staphylococcus aureus*, *Proteus mirabilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*, were obtained from the Diagnostic and Bacteriology Laboratory (Medical Microbiology Laboratory), University of Benin Teaching Hospital (UBTH), Benin City, Edo State, Nigeria.

The four bacterial isolates of common nosocomial infections obtained from University of Benin Teaching Hospital (UBTH), Benin City, were maintained and identified according to The Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974). Pure cultures of each of the bacterial isolates was obtained by culturing the isolates on their selective media. The biochemical and physiological tests were performed to re-identify and confirm the purity of the isolates.

Preparation of Extract

The fresh leaves of *Ocimum gratissium* were harvested and carefully washed in running tap water and then rinsed in sterile distilled water. The leaves were oven-dried at 37^0C before grinding into fine powder using sterile mortar and pistle. The provided powdered obtained was stored in airtight glass containers protected from sunlight until required for analysis.

Partial purification of the crude extracts

Partial purification of the crude extract was done by one-dimensional thin layer chromatography (TLC). Silical gel was used as the coating material (0.2mm thick) while the developing solvents were chloroform and methanol (4:1). The chromatography was viewed under the UV light. The separated components were eluted with 90% alcohol and subjected to infrared spectrophotometer (Pye-Unicam SP3-200) and ultraviolet – visible spectrophotometer (120 – UV/VIS spectrophotometer) analyses.

Photochemical Screening

Chemical tests were carried out on the extract to ascertain the presence of the bioactive components present in *Ocimum gratissium*. The presence of alkaloids, resins, saponins, tannis, flavonoids, carbohydrates and phenolic group were determined as described by Sofowora (1993); Edeoja, *et al.,* (2005).

Test for antibacterial property of *Ocimum gratissium*:

Susceptibility tests were carried out using the modified agar diffusion method of Garrod, *et al.,* (1981) and Irobi (1992). Commercial antibiotics were used as positive reference standard to determine the sensitivity of the isolates.

Determination of minimum inhibition concentration (MIC)

The minimum inhibitory concentration was determined using the method of Rusell and Fur (1977). The plant extract (methanolic extract) at concentrations of 35mg/ml, 75mg/ml, 135mg/ml and 150mg/ml were added to molten sterile nutrient agar (Oxoid) aseptically, and thoroughly mixed together in a sterile Petri dish. This was then allowed to set, the surface of the agar was allowed to dry properly before streaking with the appropriate bacterial isolate. The plates were then incubated at 37^0C for 72hrs. The lowest concentration preventing all visible growth was taken as the minimal inhibitory concentration.

RESULTS

The methanolic extract of *Ocimum gratissium* showed antibacterial activity against all the test organisms with the highest activity on *Staphylococcus aureus* and the least with *Pseudomonas aeruginosa* (Table 1). Partial purification of the crude extract by TLC showed two components with R_f values: 0.74cm and 0.79cm (Table 2) respectively.

The phytochemical screening of the crude extract revealed the presence of carbohydrate, saponins and alkaloids (Table 3). The presence of these bioactive components is a confirmation of the importance of *O. gratissium*, serving as a good purpose on the treatment and management of common nosocomial infections.

Table 1: Susceptibility of test organism to the crude extract and the standard reference antibiotics

Test organisms/ crude extract and antibiotics	Diameter zone of inhibitions (mm)			
	Staphylococcus aureus	***Escherichia coli***	***Pseudomonas aeruginosa***	***Proteus mirabilis***
Methanolic crude Extract	10.0	9.50	5.0	6.0
Ampicillin	10.0	17.0	-	17.0
Oxacillin	20.0	-	-	-
Nitrofurentin	-	17.0	-	15.0
Tetracycline	17.0	-	-	-
Vancomycin	17.0	-	-	-
Piperacillin	-	-	18.0	-

Key: - = no zone of inhibition

Table 2: Phytochemical Composition of *O. gratissium* extract

Phytochemical Compounds	Status
Alkaloid	+
Carbohydrate	+
Reducing group	-
Saponin	+
Tannin	-
Phenolic compound	-

Key: + = Positive, - = negative

Table 3: Purified spots of the two components the R_f factor

Spot	$R_{f\text{ - factor}}$
C_1	0.74
C_2	0.79

DISCUSSION

Naturally occurring substance 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 (Ekhaise and Okoruwa, 2001). The speed of resistance depends on the respective class of antibiotics and their product use.

For many years, medicine depended exclusively on leaves, flowers and barks of plants, only recently have synthetic drugs come into use and in many instance, these are carbon copies of chemical identified in plants (Conway, 1973). In orthodox medicine, a plant may be subjected to several chemical processes before its active ingredients are extracted, while in traditional medicine, a plant is simply eaten raw, cooked or infused in water or native wine or even prepared as food (Conway, 1973).

The results presented in this paper shows that the crude extracts of *Ocimum gratissium* possesses antibacterial activity against the common gram-positive and gram-negative organisms, thus confirming the use of the plant in the treatment of common nosocomial infections. Several researches have found that extract from a number of plants possess antimicrobial activity against bacteria causing common human infections (Okanla *et al.*, 1990; Irobi, 1992). The observed antibacterial effects of the methanolic extracts of *O. gratissium* 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 nosocomial infections, supporting its ethnomedical importance. This findings is in agreement with Ekhaise and Okoruwa (2001), Aluyi *et al.* (2003) and Nwze *et al.* (2004), who found that various extracts of plants inhibited the growth of some hospital isolates.

The phytochemical screening and quantitative estimation of the crude extract of the chemical constituents of the plant studied showed that the extract was rich in alkaloids, saponins and carbohydrates. These components have been known to show medicinal activity as well as physiological activities (Sofowora, 1992; Nwze *et al.* 2004). The plant studies here can be seen as a potential source of useful drugs. Further studies are on going to isolate, identity, characterize and elucidate the structure of the bioactive components.

CONCLUSION

African medicinal plants have been screened for their *invitro* antibacterial activities. It could be inferred that the extract tested had pronounced inhibitory effect against all test organisms. The test gave validity to the traditional use as a natural antibacterial.

It is therefore, recommended that further studies should be carried out on the efficacy of the plant extract, to enhance the primary health care delivery systems in the developing countries. The importance of ethnomedical system in health care delivery system cannot be over emphasizes, hence intense research should be encourage to enhance the health system.

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