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ANTIBACTERIAL ACTIVITY OF EXTRACTS OF *OCIMUM GRATISSIMUM* ON BACTERIA ASSOCIATED WITH DIARRHOEA

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ABSTRACT
The antibacterial potential of cold water, hot water (100°C), ethanol and chloroform extracts of Ocimum gratisssimum leaves were tested against some pathogenic bacteria known to cause diaorrhoea; Staphylococcus aureus, Escherichia coli, Shigella sp. and Salmonella sp. Cold water and chloroform extracts did not show any effect on the test organisms. Only ethanol extract and hot water (100°C) extracts had inhibitory effects on the test organisms. All the test organisms were susceptible to ethanol extract with zones of inhibition which ranged from 6mm - 11mm and minimum inhibitory concentration ranging from 30 - 60mg/ml. The test organisms were also susceptible to hot water extract with zones of inhibition ranging from 5mm - 10mm and minimum inhibitory concentration of range 25 - 70mg/ml. Due to the antimicrobial activity of Ocimum gratisssimum, it is therefore conceivable that it should be used to treat cases of diarrhea caused by the test organisms.
Keywords: Staphylococcus aureus, Antimicrobial activity, extracts, Diarrhoea, Bacteria

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 becoming resistant to synthetic drugs (Latha amd Kannabiram, 2006). The situation has further been complicated with the rapid development of multi-drug resistance by the microorganism to the antimicrobial agents available. Over the past 20 years, there has been an increased interest in the development of resistance of pathogens against antibiotics caused by the indiscriminate use of modern antibiotics (Dash *et al.,* 2011)

The use of medicinal plants in the treatment of diseases has been in practice since ancient times in different parts of the world. Plants have the major advantage of still being the most effective and cheaper alternative sources of drugs (Pretorious and Watt, 2001). In effect, different extracts from traditional medicinal plants have been tested to identify the sources of the therapeutics effect (Parekh and Chanda, 2007). Historically, plants have provided a source of inspiration for novel drug compounds as plant derived medicines have contributed largely to human health and well being. Their importance is still growing, although it varies depending on the ethnological, medical and historical background of each country (Lin, 2005).

The local use of natural plants as natural care remedies due to their pharmacological properties is quite common in Asia, Latin America and Africa (Bibitha *et al*., 2002). In Nigeria, there are over five thousand local medicinal plants available as gifts from nature and used in the treatment of various diseases (Awosika, 1991). The list is by no means complete as there are many plants that are yet to be discovered. *Ocimum gratissimum* L which is of the family *Labiatae* is widely distributed in tropical and warm temperate regions. According to Gills (1992), it has an English name of "Tea Bush". It is commonly called "scent leaf" because it usually gives out sweet scent and pleasant aroma. One can make use of the whole plant but the leaves are mostly used. The leaves of the plant was thought to contain both thymol and eugenol (El said *et al*., 1970).

The leaves of the plant contain volatile oil which has been shown to contain some antibacterial properties and the vapour of the oil was reported to kill protozoa (El said *et al*., 1970). *Ocimum gratissimum* has also been reported to be active against several species of bacteria and fungi (Nwosu and Okafor,, 1995; Nakaruma *et al.,* 1999). This study was therefore designed with the objectives of confirming the antibacterial activity of different extracts of the leaves of *Ocimum gratissimum* against some bacterial pathogens, which are known to be among the etiologic agents of diarrhea.

MATERIALS AND METHODS

Sample Collection and Processing

Fresh leaves of *Ocimum gratissimum* Linn were collected from a garden in Ekpoma and were identified and authenticated at the Botany Department of Ambrose Alli University, Ekpoma, Edo State. The leaves were rinsed with distilled water and macerated using sterile laboratory mortar and pestle for bioactive compound extraction.

Test Microorganisms

Isolates of *Staphylococcus aureus, Escherichia coli, Shigella* sp. *and Salmonella* sp. were all clinical isolates obtained from Somos Medical Laboratory, Ekpoma. Purity plates of each of the bacterial isolates were obtained by sub-culturing on their respective selective media. Biochemical tests were performed to re-identify and confirm the identity of the isolates. Fresh plates of the test bacteria were made from the isolate cultures obtained on agar slants.

Discrete colonies of fresh cultures of the different bacterial isolates were then picked and suspended in 5ml Nutrient broth in well-labelled sterile Bijou bottles, and incubated for 24 hrs at 37°C prior to antimicrobial susceptibility testing.

Preparation of Extracts

Cold water, hot water, chloroform and ethanol solvent extraction of the macerated leaves of *Ocimum gratissimum* using the method as described by Morris *et al*., (1988) and suitably modified by Junaid *et al*., (2006) was carried out. Five grams (5g) of the macerated leaves was weighed into 10ml each of the solvent (cold water, hot water, chloroform and ethanol). For cold water extraction at room temperature, the samples and solvent were stirred every 30mins for 3hours using a sterile tiny metal spatula and allowed to stand for 24hours. While for hot water extraction, the sample and solvent were heated for 30mins at 100^0C and stirred every 30mins for 3hours and then allowed to stand for 24hours. For chloroform and ethanol extraction, the sample and solvents were also stirred every 30mins for 3hours and then allowed to stand for 24hours. They were each filtered and solvent concentrated.

Determination of Antimicrobial Activity

The different extracts (aqueous and organic) were tested for antimicrobial activity against the test organisms using the agar diffusion method of Ver-poorte *et al*. (1988). Wells were made on the nutrient agar plates with a sterile cork borer of 6mm diameter. The punched plates were then seeded with 10^5 - 10^6 of the test organisms and the wells were subsequently filled with the various extracts. The plates were covered and incubated at 37°C for 24 hours. The antimicrobial activity was determined by measuring the zone of inhibition around each well at the end of the incubation period.

Determination of Minimum Inhibitory Concentration (MIC)

The broth dilution method of Bailey and Elvyn (1970) was employed in the determination of the minimum inhibitory concentration (MIC) of the leaf extracts of *Ocimum gratissimum* against the test organisms. To each 5ml of the various extracts in different test tubes was added 5ml of nutrient broth each and serially diluted out to various concentrations ranging from 70 – 25mg/ml. A loop full of each test organisms was inoculated into each of the test tubes and incubated at 37°C for 24 hours. The MIC was the lowest concentration of the leaf extracts that inhibited growth.

RESULTS

Table 1 shows the results of the antibacterial susceptibility test of the extracts against the test organisms. From the results, the diameter of zone of inhibition among the test organisms by various extracts ranges from 2.0 – 11.0mm.

Table 2 shows the minimum inhibitory concentration of the leaf extracts against the test organisms. The lowest MIC of 25mg/ml was demonstrated by the hot water extract against *S. aureus* while the highest MIC of 70mg/ml was demonstrated by the hot water extract against *Salmonella* sp.

Table 1: Antibacterial activity of leaf extracts of *Ocimum gratissimum* on the test organisms

Test Organisms	Diameter of zone of inhibition (mm)			
	Cold water	Hot water	Ethanol	Chloroform
Staphylococcus aureus	-	10.0	11.0	-
Escherichia coli	-	6.0	9.0	2.0
Shigalla sp.	-	7.0	8.0	3.0
Samonella sp.	-	5.0	6.0	-

Key: - : negative, 1.0 - 3.0: resistant, 4.0 - 11.0: susceptible

Table 2: Minimum inhibitory concentration (MIC) of *Ocimum gratissimum* leaf extracts on the test organisms

Test Organisms	Concentration (mg/ml)			
	Cold water	Hot water	Ethanol	Chloroform
Staphylococcus aureus	++	25	30	++
Escherichia coli	++	60	30	++
Shigalla sp.	++	60	40	++
Samonella sp.	++	70	60	++

Key: ++: high growth, 20-50: highly susceptible, 60-70: moderately susceptible.

DISCUSSION

Today, there is a renewed interest in traditional medicine and an increasing demand for more drugs from plant sources. This revival of interest in plant-derived drugs is mainly due to the current widespread belief that green medicine is safe and more dependable than the costly synthetic drugs, many of which have adverse side effects (Agbafor *et al.,* 2011). The results of this investigation showed that the leaves of *Ocimum gratissimum* exhibited antibacterial activity. All the test organisms were susceptible to hot water and ethanol extracts while they were highly resistant to cold water and chloroform extracts. *Staphylococcus aureus* was most susceptible of all test organisms with the highest zone of inhibition ranging from 10mm - 11mm. *Salmonella* sp. was the least susceptible of all test organisms to hot water and ethanol extracts with zone of inhibition ranging from 5mm - 6mm. This research has shown that the hot water and ethanol extracts of the leaves of *O. gratissimum* possesses potential factor which confers measurable in-vitro antibacterial activity on the test organisms. This is because hot water and ethanol are better extractants of the volatile oil of the leaves of *O. gratissimum.*

This work agrees with the findings of Orafidiya *et al*., (2000) who showed that the volatile oil of the leaves of *O. gratissimum* was active against enteroaggregative *E. coli*. Sofowora (1982) found that one of the active component which impacts antibacterial activity on *O. gratissimum* is thymol and its content is sufficient explanation for the antidiarrhoea activity of *O. gratissimum.* Eugenol could also have impacted the antimicrobial property on *O. gratissimum* as it has been shown to have both antibacterial (Nakaruma *et al*., 1999) and antihelminthic activities (Pessoa *et al*., 2002).

The result further shows that the organic extracts (ethanol) would be slightly more than the aqueous extracts (hot water) in terms of activity. This may be due to the better solubility of the active components in organic solvents (de Boer *et al*., 2005). It may also be that ethanol is a better extractant of the bioactive components of the leaves of *O. gratissimum* which confers upon it its antimicrobial activity.

The fact that the extracts were active against both gram negative and gram positive bacteria tested may indicate a broad spectrum of activity. This observation is very significant because of the possibility of developing therapeutic substances that will be active against multi-drug resistant organisms. The resistance of the test organism to the cold water and chloroform extracts may be due to insufficient release of the volatile oil of *O. gratissimum* during extraction. The generally low MIC value is an indication of their antibacterial potential. *Staphylococcus aureus* had the lowest MIC of 25mg/ml while *Salmonella* sp. had the highest MIC of 70mg/ml.

The demonstration of antimicrobial activity is an indication that the plant is a potential source for the production of drugs with a broad spectrum of activity. The result of this study also supports the traditional application of the leaves of the plant in treating diarrhea and suggests that the plant extract possesses compounds with antibacterial properties that can be used as antibacterial agents in novel drugs for the treatment of diarrhea cases. Further pharmacological evaluations, toxicological studies and possible mechanism of action processes are the future challenges that require further research.

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