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ANTIMICROBIAL EFFICACY OF *OCIMUM GRATISSIMUM* AND*VERNONIA AMYGDALINA* ON GASTROINTESTINAL BACTERIA

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ABSTRACT

This study was carried out to determine the antibacterial potency of *Ocimum gratissimum* and *Vernonia amygdalina* against isolates of *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis* and *Streptococcus faecalis* using sensitivity disc diffusion assay. Extraction was achieved by drying and blending into powdery form, which was separately mixed with distilled water and 70% ethanol in respective flasks. This was properly sieved and stored. The antibacterial properties of extracts of both samples were separately determined against the test isolates. The phytochemical test conducted revealed that the extracts of both samples possessed different biologically active constituents, namely: tannins, flavonoids, saponins, anthocyanins and phlobatannins, while betacyanins were found absent in both extracts. Physicochemical analyses of both extracts showed that a pH range of between 6.0 and 7.5 provided a broad spectrum of activities against a wide range of bacterial infections. Results of antibacterial screenings revealed that the water extracts of both samples were more efficacious than the ethanol extracts. However, both were found to be very active against the test organisms. It was further observed that water extracts were more active against *Bacillus subtilis, Staphylococcus aureus* and least against *Pseudomonas aeruginosa.* On the other hand, ethanol extracts were more efficacious against *Escherichia coli* and *Streptococcus faecalis.* It has been concluded in this study that *O. gratissimum* and *V. amygdalina* can be extensively used tradomedically in the Nigerian folk medicine to treat several bacterial infections, and are thus, recommended as a source of natural product for future use in the management and cure of multi-drug resistant bacterial infections in African continent and the world at large.

Keywords: Gastrointestinal, antimicrobials, bacteria, plants, medicinal.

INTRODUCTION

Microorganisms have been found to be the causal agents of many diseases, a fact which has incited the interest of many scientists to detect substances which can be used to control the pathogens. These substances are known as antimicrobial agents. The term 'anti-microbial agent' is a general term for all substances that can be used systematically (introduced into the body) to inhibit or kill microbial pathogens regardless of their origin (Norton, 1991). Nature has endowed mankind with a rich store-house of natural antimicrobial agents – the plants. Plants are vital parts of man's existence, the most essential to his well being. Plants are able to synthesize a wide range of chemical substances which had been of tremendous value in the treatment and prevention of diseases. Studies carried out on them have revealed that the active ingredients in many of such are known to contain alkaloids, phenolics, saponins and tannins (Odebiyi and Sofowora, 1998). Medicinal plants are used in the treatments of diseases either alone or in combination with other plants parts. They are used as anti-infective agents, laxatives, cardiovascular and nervous remedies, proteolytic ferments, steroid sources, sweeteners, anti-tumour drugs, and a source of anti-malaria in dosage form (Sofowora, 1982; Gbile and Adesina, 1996; Owonubi, 1998). The research is therefore still on for a naturally occurring and readily available effective antibiotics of plant origin.

As a result of research into the active components contained in some plants extracts, considerable information has surfaced into the world of chemotherapy. For instance, Kusoje *et al.* (1968) obtained antifungal activity from "*rice bran ter*" highly potent in the treatment of eczema. Vegetable extracts of garlic, onion, turnip, green pepper and radishes were found to inhibit the growth of *Escherichia coli, Salmonella typhi, Shigella dysenteriae* and *Staphylococcus aureus* (Aldemy and Alli, 1970). A preparation from avocado pear has been shown to inhibit thirteen different species of bacteria (Neemen and Kashman, 1990).

Ocimum gratissimum; family Labiaceae is an herbaceous plant commonly found in tropical Asia, especially in India where it is used for aromatic baths of fumigations in the treatment of rheumatism and paralysis. It is widely distributed in tropical and temperate regions. The plant is also found in West Africa. In Nigeria, it is found in the savannah and coastal areas where it is used in the

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treatment of high fever (Oliver, 1980), epilepsy (Osifo, 1992) and diarrhoea (Oliver, 1980; Sofowora, 1993). Decoctions of the leaves have also been used in treatment of mental illness (Abdulrahman, 1992). The leaves of the plant are used as condiments in food. It is called "Efinrin" by Yoruba tribe of South-Western Nigeria , Ebavbokho in Benin (Delta State), Aai doya ta gida in Hausa (Northern Nigeria) and Nchonwu in Igbo (South Eastern Nigeria) (Owulade, 2004).

Lima *et al.* (1993) tested in vitro antifungal activity of thirteen essential oil obtained from some plants against dermatophytes in which that of *O. gratissimum* was found to be the most active, inhibiting 80% of the dermatophyte strains tested and producing zone greater than 10 mm in diameter. Nwosu and Okafor (1995) reported the antifungal activities of extracts of ten medicinal plants collected from southeastern Nigeria against seven pathogenic fungi. According to these authors, *O. gratissimum* inhibited the growth of *Trichophyton rubrum* and *Trichophyton mentagrophytes*. Ilori *et al.* (1996) reported the anti-diarrhoeal activities of leaf extracts of *O. gratissimum* that the extracts were active against *Aeromonas sobria, Escherichia coli, Salmonella typhi* and *Salmonella dysenteriae.* The authors have shown that the MIC for these organisms ranged from 8-50mg/ml while MBC were from 8-62mg/ml.

Vernonia amygdalina is a shrub or small tree of 2-5m with petiolate leaf of about 6 mm diameter and elliptic shape that grow predominantly in the tropical Africa. In Nigeria, the plant is locally called bitter leaf due to its very bitter taste. No seeds are produced and the three has to be distributed through cutting. They grow under a range of ecological zones in Africa, produce large mass of forage and are drought tolerant. There about 200 species of Vernonia (Bonsi *et al.*, 1995a).

These plants are qualified to be called medicinal plants by virtue of the fact that they have been in therapeutic use against various diseases like parasitic infection, URTI, Syphilis, other venereal diseases like gonorrhea, pneumonia and enteric fever respectively, for over one hundred years (Lewis and Elvin-Lewis, 1977). Just a few studies in Africa are available on the phytochemistry, dosage of administration and contraindication of medicinal plants compared to the array of available medicinal plants (Gundiza, 1985; Ebana *et al.*, 1991; Kola *et al.*, 2002). This explains why certain less potent toxic synthetic chemicals from the west are recognized and preferred to these more potent, less toxic medicinal plants. The potentials for multiple resistances by the isolates used in this research have been demonstrated by many researchers around the world (Diep *et al.*, 2008). Many isolates of *Escherichia coli* (and *Staphylococcus aureus*) for instance, are resistant to ampicillin, amoxicillin, tetracycline and trimethoprim-sulfamethoxazole (Aibinu *et al.*, 2004). In the year 2000, 7.1% cases of multiple drug resistant bacterial isolates to conventional antibiotics were reported (Sahm *et al.*, 2001). Umolu *et al.* (2006) reported that 67% of the resident isolates exhibited multiple drug resistance. The therapeutic failure of antibiotics in Nigeria, Africa and indeed all parts of the world buttresses the need to support the use of local medicinal plants.

In this study, five different genera of bacteria were employed against the antimicrobial efficacies of the plants' extracts. These are two gram-negative bacteria: *Escherichia coli* and *Pseudomonas aeruginosa*, and three gram-positive bacteria: *Staphylococcus aureus, Bacillus subtilis* and *Streptococcus faecalis*. These groups of bacteria are normal inhabitant of the gastrointestinal tract of man and animals. Therefore, since *O. gratissimum* and *V. amygdalina* are herbaceous plants and are known to have some medicinal efficacies, and results of studies on their medicinal potentials have not been extensively documented in the past. It is on this basis of the little available information on the antimicrobial significance of these plants that the present study was aimed at achieving the antibacterial efficacy of the water and ethanol extracts of *O. gratissimum* and *V. amygdalina* on selected bacteria species, phytochemical analysis on the plants extracts speculate on the significance of both extracts and hence, their chemotherapeutic functions in the treatment of diseases.

MATERIALS AND METHODS

Collection of plant samples

The medicinal plants used in this study were purchased on consultation of practitioners of ethno-medicine from Oshodi and Bariga Markets in Lagos State, and Oja-Oba and Bodija Markets in Oyo State, South- Western Nigeria. The samples were authenticated by experts in the School of Pharmacy/Botany, University of Lagos, Nigeria. They were kept in the refrigerator at 4^0C until used.

Test organisms

Two gram-negative bacteria: *Escherichia coli* and *Pseudomonas aeruginosa*, and three gram-positive bacteria: *Staphylococcus aureus, Bacillus subtilis* and *Streptococcus faecalis* were obtained as type strains from the culture collection unit of the Department of Biotechnology, Federal Institute of Industrial Research Oshodi (FIIRO), Lagos, Nigeria. The cultures were maintained on nutrient agar slants and sub-cultured fortnightly throughout the period of research.

Preparation of *Ocimum gratissimum* and *Vernonia amygdalina* leaves extracts

The leaves of the plants were dried in the oven at 50^0C for 48hrs. The dried samples were blended using milling

machine and later sieved to obtain the flour samples from both plants (Ugorji *et al.,* 2000). 200g of the flour samples of each plant was weighed and separately mixed with 1 litre (1000mls) of distilled water and 70% ethanol in respective flasks (Alade and Irobi, 1993; Ugorji *et al.*, 2000). Each flask was then allowed to stand for 72 hrs at room temperature with occasional agitation. The mixture was then filtered, and thereafter centrifuged at 5000 rpm for 20 minutes. The volume extracts obtained were then stored in screw cap bottles in the refrigerator at 4^oC (Ugorji *et al.,* 2000).

Phytochemical analysis of samples extracts

The preliminary phytochemical analysis was carried out employing the method of Culer (1982), Sofowora (1993), Odebiyi and Sofowora (1998) and Trease and Evans (2002). The extracts were screened for the presence of biological active constituents such as Tannins, Saponins, Flavonoids, Betacyanins, Anthocyanins and Phlobatannins.

Determination of the physicochemical parameters of samples' extracts

The pH of the extracts were determined using the pH meter (Unicam 9450) after its initial standard standardization using appropriate buffers. A graduated mercury bulb thermometer was employed to determine the temperatures of extracts. These were recorded accordingly.

Determination of antibacterial properties of extracts

Sensitivity disc method was employed as described by Kela and Kufeji (1995). The test isolates of bacteria were cultured in peptone water for 24hrs. 0.2ml of each suspension was taken and mixed with 10ml of nutrient agar in sterile petridishes. Discs of equal diameters were soaked in water-dissolved and ethanol-dissolved extracts' suspensions for 3hrs. The different discs from the different suspensions were removed and placed on the culture surface, and incubated at 37^0C for 24hrs. The culture media were examined for zones of inhibition.

RESULTS AND DISCUSSION

Table 1 showed the results of the phytochemical analyses of water extracts of *O. gratissimum* and *V. amygdalina*. These results revealed the presence of many biologically active constituents namely: tannins, flavonoids, saponnins, anthocyanins, phlobatanins, with the absence of betacyanins in the water extracts. These same active constituents were found present in the ethanol extracts of the samples but in different degrees (Table 2). The presence of these active constituents actually helped to confer the antibacterial properties on the extracts of both samples. The result is in line with the observation made by Kela and Kufeji (1995) while experimenting on the efficacy of *Moringa oleifera* and *Mitracarpus scaber*. They observed that the efficacy of these plant extracts was due to the presence of tannins, saponins and anthocyanins in the extracts. Alade and Irobi (1993) made similar observation when they carried out analyses on the antimicrobial activities of leaf extract of *Acalypha weikeisiana* against some selected organisms. They related the antimicrobial activities of the leaf extract to the presence of active constituents such as phenol and saponin. These bioactive compounds have been reported to possess antimicrobial potency (Sofowora, 1993).

The pH of water and ethanol extracts of *O. gratissimum* were 6.50 and 7.20 respectively with temperatures

Table 1. Phytochemical analyses of water extracts of *O. gratissimum* and *V. amygdalina.*

	Tannins	Flavonoids	Saponins	Anthocyanins	Phlobatannins	Betacyanins
O. gratissimum	+++	+++	+++	+++	++	-
V. amygdalina	++	++	+++	+++	+	-

Key: +++ = strongly positive; ++ = moderate positive + = trace positive; - = negative

Table 2. Phytochemical analyses of ethanol extracts of *O. gratissimum* and *V. amygdalina.*

	Tannins	Flavonoids	Saponins	Anthocyanins	Phlobatannins	Betacyanins
O. gratissimum	++	++	++	++	+	-
V. amygdalina	+	++	++	++	+	-

Key: ++ = moderate positive; + = trace positive; - = negative

Table 3. Physicochemical analyses of water and ethanol extracts of *O. gratissimum.*

Parameter	Extracts of *O. gratissimum*	
	Water Extract	Ethanol Extract
pH	6.50	7.20
Temperature	28 ± 2^0C	28 ± 2^oC

Table 4. Physicochemical analyses of water and ethanol extracts of *V. amygdalina*.

Parameter	Extracts of *V. amygdalina*	
	Water Extract	Ethanol Extract
pH	6.62	7.40
Temperature	28 ± 2^0C	28 ± 2^oC

Table 5. Antibacterial activities of water and ethanol extracts of *O. gratissimum* against some bacterial species.

Extract	Size of Zone of Inhibition (mm)				
	Escherichia coli	*Streptococcus faecalis*	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Staphylococcus aureus*
Water	8.0	9.0	6.0	10.0	10.0
Ethanol	6.0	6.0	8.0	9.0	7.0

ranging from 28 ± 2^0C (Table 3). Similarly, the pH of the water and ethanol extracts of *V. amygdalina* measured 6.62 and 7.40 respectively with the same temperature of 28 ± 2^oC (Table 4).

The antibacterial analyses of the extracts from both samples revealed that water extracts have higher efficacy in their inhibitory activities on the various test organisms than the ethanol extracts (Tables 5 and 6). The higher bactericidal effect of the water extract could be attributed to the concentration of the biologically active constituents following extraction. This confirmed water as a better solvent than ethanol thereby enhancing extraction, hence a higher concentration of the constituents. The passage of the active constituents across the bacterial membrane depends on the size of their particles, and it is probable that the active constituents extracted by water had finer particles which made them penetrate more easily through the membranes of the bacterial cells than the ethanol extract. It was, however, observed that the ethanol extract of *O. gratissimum* has greater efficacy on *Pseudomonas aeruginosa* than the water extract (Table 7). Also, ethanol extract of *O. gratissimum* was very efficacious against *Bacillus subtilis* but least effective against *Escherichia coli* and *Streptococcus faecalis* (Table 5). The results showed that the water extracts of *O. gratissimum* and *V. amygdalina* were more active against *Bacillus subtilis, Staphylococcus aureus* but least against P*seudomonas aeruginosa* (Tables 5 and 6). Furthermore, ethanol extract of *V. amygdalina* was also found to be highly active against *Staphylococcus aureus* and least in *Streptococcus faecalis* and *Pseudomonas aeruginosa* (Table 6). It was observed in this study that the antibacterial action of extracts of both samples against *Pseudomonas aeruginosa*, a bacterium well known for its constitutive resistance to many antibiotics, was pronounced. The degree of sensitivity of both extracts was expressed as measure of the diameter of zone of inhibition in millimeters. It is notable that this study is in line with several studies conducted on the antimicrobial properties of herbs and spices (Khan *et al.*, 1998; Dorman and Deans, 2000; Hsieh *et al.*, 2001). However, not many researchers put the use of such multi-drug resistant or beta-lactamase producers into consideration.

In conclusion, water extracts of both samples investigated were more efficacious than the ethanol extracts in their bactericidal activities. The bactericidal activities of both extracts could be attributed to the presence of the biologically active constituents namely: tannin, flavonoid, saponnin, phlobatannin, and anthocyanin in the extracts. Since the extracts of both samples were very potent against both gram-positive and gram-negative bacteria, they could be processed for use as broad-spectrum antibiotics. However, pharmacological standardization and clinical evaluation of these plant extracts, together with the determination of their Minimal Inhibitory Concentration, isolation and characterization of their active constituents, may lead to the commercialization of the extracts for use in the treatment of diseases, and for other important chemotherapeutic uses. Better therapy for many bacterial diseases could be detected in the barks and leaves of some neglected plants. Therefore, growing and use of *O. gratissimum* and *V. amygdalina* should be encouraged.

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