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Antibacterial potential of leave extract of different species of *Ocimum (O. gratisimum* & O. *guineas)* on bacterial isolates from urinary tract

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Abstract

Urinary tract infection is a threat to the world population and economic, and the situation is further worsen by the high costs and hazardous side-effects of synthetic drugs in mix of drug resistance. The importance of this study lies in evaluating the antimicrobial potentials of different solvents extracts of *Ocimum* spp against some human bacteria causing urinary tract infections. Following standard laboratory procedures, the leaves extracts of *Ocimum gratisimum* and *Ocimum guineas* were studied on Gram positive bacteria (*Staphylococcus aureus*) and two strains of Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). The results showed that the petroleum ether and 1%HCl extracts showed high antibiotic potentials that are similar to standard antimicrobial drugs. Worrisome is the observed insensitivity against the urinary tract infection herein studied with the water and ethanolic extracts of *Ocimum gratisimum* and *Ocimum guineas*. However, acetone extract in *Ocimum guineas* was sensitive to *S. aureus* only. Conclusively, petroleum ether and 1%HCl extracts may served as preferred extraction solvents for *Ocimum gratisimum* and *Ocimum guineas* leave considering the inhibitory activities against urinary tract microbes.

Keywords

Herbs, Therapeutic Potential, Antimicrobial, Urinary Tract Infection, Ocimum Species

1. Introduction

Urinary tract infections (UTIs) are the second most common infections in community practice, with about 150 million people diagnosed worldwide annually and costing the global economy excess of 6 billion US dollars ([1]). According to Gupta ([2]), *Escherichia coli* and *Staphylococcus saprophyticus* account for 75-90% and 5-15% of UTI isolates and cases of uncomplicated cystitis respectively. In the light of this fact, evolving changes in drug resistance in various communities have forced the importance to a re-evaluation of local empiric choices for managing UTI ([3], [4]). In the present scenario, there is an urgent and continuous need for exploration and development of cheaper, effective new plant based drugs with better bioactive potential and least side effects. Hence, recent attention has been paid to biologically active extracts and compounds from plant species used in herbal medicines ([5]). Medicinal herbs are plants which contain substances that can be used for therapeutic purpose, of which are precursors for the synthesis of drugs ([6]).

The use of plant, plant extract or plant-derived chemicals to treat diseases; topical, subcutaneous and systemic, has stood the test of time ([7]). Recently however, there has been a gradual revival of interest in the use of medicinal plants for the reason that herbal medicines have been reported safe and without adverse side effect especially when compared with synthetic drugs ([8]) couple with the little or no report of any form of microbial resistance during

the use and administration of herbal medicines ([9]).

One such plant of interest is the *Ocimum* spp; belonging to the *Lamiaceae* family and characterised by a pleasant smell. It is a perennial shrub which grows in several regions all over the world ([10], [11]). Although it is commonly called Scent leave, it is known by different local names; 'Nchu anwa' or 'Ahuji' by the Igbo speaking populations of South Eastern Nigeria, 'Effirin-nla' by the Yoruba speaking people of South Western Nigeria and known as 'Daidoya' by the Hausa Speaking populations of Northern Nigeria ([12], [13]).

While it serves as essential ingredient and seasoning in food because of its aromatic flavour; as in the local Cuisine popularly known as 'pepper soup', it is an important herbal medicinal plant not only in Nigeria but also in the sub-Saharan Africa ([14], [15]). For example, crude aqueous extract of Ocimu Spp is commonly used in the treatment of epilepsy, high fever and diarrhoea ([16]), mental illness ([17]), digestive disorders ([18]), fungal infections, fever, cold and catarrh as well as in the management of the baby's cord, to keep the wound surfaces sterile ([19]). Furthermore, in different part of the world, the leaves are rubbed between the palms and sniffed as a treatment for blocked nostrils (Kokwaro, 1993) as well as in the treatment of abdominal pains, sore eyes, ear infections, coughs, barrenness, fever, convulsions, tooth gargle, regulation of menstruation and as a cure for prolapsed of the rectum ([20], [21]). The whole plant has been used for the treatment of sunstroke, headache, and influenza, as a diaphoretic, antipyretic and for its anti-inflammatory activity ([22]).

Considering these numerous reported health implications, this study evaluates the antimicrobial activity of two different species of Ocimum (*O. gratisimum* and O. *guineas*) leaves extracts on human urinary tract bacteria isolates.

2. Materials and Methods

2.1. Processing of Plant Samples

Plant materials (leaves of *Ocimum gratisimum* and *Ocimum guineas*) were collected from in and around Ekpoma, Edo State, Nigeria and authenticated by a Botanist in the Department of Botany, Ambrose Alli University, Ekpoma. The leaves were subsequently washed in tap water, rinsed in sterile distilled water and dried for 5 days at 60 ^{o}C in Lab 1 of the Department of Microbiology, Ambrose Alli University, Ekpoma. The dried leaves were separately blended to powder with a clean kitchen blender (Sonik, Japan) and stored in air tight glass containers kept in laboratory cupboard, until required for preparation.

2.2. Preparation of Extracts

5grams of each *Ocimum spp.* leave was weighed into 100ml reagent bottle and 95ml of extraction solvent (water, ethanol, 1% HCl, acetone and petroleum ether) was added and left to extract on a mechanical shaker overnight at room temperature. This was done using all the five extraction solvents on the different *Ocimum spp.*

The extract solution was filtered aseptically into another 100ml reagent bottle using a watt-man No 1 filter paper. All the filtrate were screened for purity by inoculation unto MacConkey agar and nutrient agar plates and incubated at 37^{0}C for 48 hours. Filtrates yielding growth of any organism was re-filtered and rescreened for purity until a sterile extract solution was obtained, following the methods outlined in Orhue ([23]).

2.3. Micro Organism Preparation/Growth

The test organisms used were all human pathogenic organisms of clinical origin and were isolated from urinary tract of infected patients attending the University of Benin Teaching Hospital, Benin City, Nigeria. They include one strain of Gram positive bacteria (*Staphylococcus aureus*) and two strains of Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). They were stored in the Department of Microbiology, Faculty of Natural Sciences, Ambrose Alli University, Ekpoma-Nigeria, where they were kept as stock cultures at 4°C. Biochemical analysis was carried out on each of the test organisms for confirmation.

2.4. Determination of Minimum Inhibitory Concentration (MIC)

Using a 50ml specific gravity bottle, the density of the extract solution was determined. In a similar manner, the density of the plain solvent was also determined. To determine the concentration of the extract, the density of the plain was subtracted from that of the extract solution ([30]). This was done for all 5 extraction solvents. With the known leave extract concentrations and the three clinical isolates of *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa*, the MIC of the extract solutions and standard drugs (Peflacine and Cefuroxine) were determined. The experiments were performed in 3 repetitions for each of the extraction solvents and *Ocium spp* leaves and the average was calculated ([30]).

2.5. Data Analysis

Data were keyed into SPSS (version 16) and the average of each determined MIC was then presented in suitable table for simple descriptive statistics. The MICs of the different *Ocimum spp* in the different extraction solutions were compared with the values of the standard antibiotic drugs.

3. Results

As shown in table 1 and 2, there were difference in the minimum inhibitory concentrations (MIC) recorded for the different leaves extract of *Ocimum spp*. Furthermore, there were significant differences ($p<0.05$) in MIC of the extraction solvent used compared to the standard drugs. Also, differences were observed in the patterns *S. aureus, E.*

coli and *P. aeruginosa*, become sensitive to the leaves extracts of *Ocimum spp.*

Distinctively, leave extract of *Ocimum Gratisimum* in water, ethanol and acetone showed no antimicrobial potential to the gram positive or negative bacterial. On the other hand, petroleum ether and 1%HCl showed some degree of antimicrobial potential with that of petroleum ether been more powerful with minimum inhibitory concentrations of 25.0 mg/ml for the three bacterial isolates (table 1). Extraction in 1% HCl did not show any antibacterial potential against *P. aeruginosa*, but showed antimicrobial potential against *S. aureus* (11,000.0 mg/ml) and *E. coli* (1000.0 mg/ml) (table 1).

Similarly, leave extract of *Ocimum guineas* in water and ethanol did not show any antimicrobial potential against the gram positive or negative bacterial studied. Leave extract of *Ocimum guineas* in petroleum ether presented the most antimicrobial potentials against the bacterial isolates studied compared to acetone and 1%HCl. While *P. aeruginosa* was not sensitive to leave extract in 1% HCl, *P. aeruginosa* and *E.coli* were not sensitive to leave extract in acetone (see table 2).

Comparatively, petroleum ether extract of *Ocimum Gratisimum* was more potent while 1% HCl extract of *Ocimum guineas* was more potent against the urinary tract bacterial isolates. Also, the standard antibiotic drugs; perflacine and cefuroxime, showed minimum inhibitory concentrations of 4.0 mg/ml and 6.0 mg/ml respectively to the bacterial organisms studied, and by implication were better antibiotics than the leaves extracts of *Ocimum spp.* Furthermore, there were statistical difference ($P<0.05$) in the MIC of the standard drugs compared to the leave extract of *Ocimum Gratisimum* and *Ocimum guineas.* In addition, the leave extracts of *Ocimum Gratisimum* and *Ocimum guineas* that showed antimicrobial activities were significantly different ($p<0.05$) from extracts without antimicrobial activities.

Table 1. *MIC of different extraction solvents of Ocimum Gratisimum (leaves) compared with standard antibiotics*

Organisms Isolated	Standard anti-biotic drugs		Extraction Solutions from *Ocimum Gratisimum* (leaves) VNB: *Ebehi*				
	Perflacine	Cefuroxime	Water	Ethanol	1%HCl	Acetone	Petroleum ether
S. aureus,	4.0*	6.0*	0.0	0.0	11000	0.0	25.0**
E.coli,	4.0*	6.0*	0.0	0.0	1000	0.0	25.0**
P. aeruginosa	4.0*	6.0*	0.0	0.0	0.0	0.0	25.0**

* signifies statistical significant in antimicrobial activities between standard drugs and *Ocimum Gratisimum* extraction solutions. ** signifies statistical significant between the different extraction solutions.

Table 2. *MIC of different extraction solvents of Ocimum guineas (leaves) compared with standard antibiotics*

Organisms Isolated	Standard anti-biotic drugs		Extraction Solutions from *Ocimum guineas (leaves)* VNB*: Ebamwonkho*				
	Perflacine	Cefuroxime	Water	Ethanol	1%HCl	Acetone	Petroleum ether
S. aureus,	4.0*	6.0*	0.0	0.0	200.0	40.0**	40.0**
E.coli,	4.0*	6.0*	0.0	0.0	200.0	0.0	40.0**
P. aeruginosa	4.0*	6.0*	0.0	0.0	0.0	0.0	40.0**

* signifies statistical significant in antimicrobial activities between standard drugs and *Ocimum guineas* extraction solutions. ** signifies statistical significant between the different extraction solutions.

4. Discussion

Urinary tract infection is considered threat to the world population and economic ([1]), the situation is worsen considering dissatisfaction from high costs and hazardous side-effects of synthetic drugs couple with the evolving changes in drug resistance. The importance of this study lies in evaluating the antimicrobial potentials of *Ocimum* spp against most common bacteria causing urinary tract infections. In the present study, leaves of *Ocimum gratisimum* and *Ocimum guineas* showed antimicrobial activities against *S. aureus, E.coli,* and *P. Aeruginosa* with the petroleum ether and 1%HCl extracts. Specifically, the petroleum ether extracts were more potent to the gram positive and negative bacterial herein studied and that of *Ocimum gratisimum* was more potent compare to *Ocimum guineas.* The antimicrobial findings in this study is in line with the study by Lexa and Co. ([20]), who reported *Ocimum gratisimum* from 13 different populations to have pronounced antibacterial and anti fungal activities again gram positive (*S. Aureus and Bacillus spp*) and gram negative (*E.coli, P.aeguginosa, S.typhi, K.pneumoniae and P.mirabilis*). Indeed, numerous publications have presented data on essential oils of variety Ocimum gratissimum and found them to possess appreciable antibacterial activity against a wide range of organisms ([24]). Also, Williamson ([25]) has evaluated the antimicrobial activities and found it to be effective in varying degrees in the animal models. It is also regarded as highly antiseptic and has been applied in the prevention of postpartum infections ([26]).

Worrisome is the observed insensitivity against the urinary tract infection herein studied with the water and ethanolic extracts of *Ocimum gratisimum* and *Ocimum guineas*. However, acetone extract in *Ocimum guineas* was sensitive to *S. aureus*. By implication, petroleum ether and 1%HCl extracts may served as preferred extraction solvents for *Ocimum gratisimum* and *Ocimum guineas* leave considering the inhibitory activities presented compared to

the standard synthetic antibacterial drugs. Indeed, different solvents have various degrees of solubility for different phyto-constituents ([27]).

The antimicrobial mechanism of action showed by the petroleum ether and 1%HCl leaves extracts of *Ocimum gratisimum* and *Ocimum guineas* may therefore be for the reason that these plants contain phytochemicals which are bactericidal. Plants are known to contain food and mixture of extraordinary chemicals, some of which act beneficially to human and others may have detrimental effects ([28]). Plant produces these chemicals to protect itself, but recent research demonstrates that many phytochemicals can protect humans against diseases too ([29]). Hence, the phytochemicals in extracts of *Ocimum gratisimum* and *Ocimum guineas* can be protective or have disease preventive properties especially those that are of bacterial origins.

Conclusively, petroleum ether and 1%HCl leave extracts of *Ocimum gratisimum* and *Ocimum guineas* may be used as a source of antibacterial for infections of urinary tract origin. Further studies are however needed to determine their antimicrobial impact against different infections and human diseases.

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