# Antibacterial Evaluation of Nigerian *Ocimum Sanctum* Leaf Extracts against Bacterial Isolates Associated With Urinary Tract Infection

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## ABSTRACT

With the high socioeconomic burden associated with urinary tract infections (UTI), global increase in multidrug resistant (MDR) pathogens, and the need for alternate sources of antimicrobial agents, there is an increasing focus on possible role of plants as a source of antimicrobials. The effect of one such plant (*Ocimum sanctum*) has not been widely studied in Nigeria. This study set out to explore the antimicrobial activity of extracts of Nigerian *Ocimum sanctum* against MDR isolates associated with UTI. The antibacterial activity of three *Ocimum sanctum* leaf extracts (aqueous, ethyl acetate and ethanol) were tested at varying concentrations (50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml and 3.125 mg/ml) against 5 MDR clinical isolates using the agar well diffusion test. These yielded zones of inhibition ranging from 0 mm to 24 mm. Of the three extracts, the ethanol extract was the most effective with antibacterial activity noted against MDR *Proteus mirabilis* and *Pseudomonas aeruginosa* at concentrations as low as 12.5 mg/ml. However though, none of the three extracts showed antibacterial activity against both *Staphylococcus aureus* and *Escherichia coli*. Ethanol extracts of Nigerian *Ocimum sanctum*, showed promising results against some MDR UTI bacterial isolates. This study provides the first report on antibacterial activity of extracts of *Ocimum sanctum* against MDR isolates, perhaps pointing at a future possible use of this plant extract or its active ingredient in therapy.

**KEYWORDS**: *Ocimum sanctum*, antibacterial activity, MDR, UTI, Nigeria

## INTRODUCTION

Urinary tract infections (UTI) have been found to be one of the most commonly occurring human bacterial infections, with up to 150 million people estimated to be diagnosed with UTI each year globally (Fasugba *et al*., 2015). This poses high negative socioeconomic implications. In the United States specifically, pediatric UTIs alone have been noted to account for over 1.5 million visits to the clinician annually, with a related US$180 million burden on the health care system each year (Schmidt and Copp 2015). The total socioeconomic costs to the US, comprising of both health care costs and missed work time, have however been estimated to be up to US$3.5 billion annually (Flores-Mireles *et al*., 2015). For developing countries, which have been observed to have higher frequency rates than the rest of the world (Tandogdu and Wagenlehner 2016), this negative socioeconomic burden would even be higher. Prompt treatment of UTIs is essential to both reduce the length of morbidity and decrease the socioeconomic burden these infections place on society. Recent years have however seen an increase in antimicrobial resistance in pathogens associated with UTIs (McQuiston Haslaun *et al*., 2013, Blaettler et al., 2009, Tiruneh *et al*., 2014) and this could further negatively impact on the socioeconomic burden. More and more research has therefore been focused on exploring possible role of natural medicinal plant products as antimicrobial agents.

*Ocimum sanctum*, a member of the basil family (Pattanayak *et al*., 2010), is one such plant that has been studied. *Ocimum sanctum* is one of several species belonging to the *Ocimum* genus and fabled for their therapeutic role. This plant has traditionally been regarded as a 'wonder', 'cure all' drug with antiemetic, antiseptic, antipyretic, antidiabetic and anticancer properties (Khosla 1995, Prakash and Gupta 2005), leading to the name Holy basil. The antimicrobial activity of this plant has been thought to result from the synergistic activity of its components, with linoleic acid and eugenol thought to play crucial roles (Singh *et al*., 2005, Shokeen *et al*., 2008).

A number of studies have explored the antimicrobial efficacy of extracts of this plant against several bacterial agents. Majority of these have however focused mainly on the efficacy against periodontal associated bacterial agents (Agarwal *et al*., 2010, Subbiya *et al*., 2013, Mallikarjun *et al*., 2016, Eswar *et al*., 2016), with one report on UTI pathogens (Sharma *et al*., 2009) and no reports were found of studies carried out in Nigeria. This study therefore set out to evaluate and compare the antimicrobial activity of various extracts of *Ocimum sanctum* against bacterial agents isolated from suspected cases of UTI in Nigeria.

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## MATERIALS AND METHODS

### Preparation of leaf extracts

The leaves of *Ocimum sanctum* were first washed with sterile distilled water and air-dried at 28□C for 1 week, and aseptically homogenized into a powder using a sterile mortar and pestle. Following this, extraction was carried out using three different solvents (ethyl acetate, 100% ethanol and water), for 48 hours using a magnetic stirrer in a maceration jar. The extracts were then filtered and solvents eliminated using a rotary evaporator leaving dry extract residues (Okore *et al*., 2014).

### Phytochemical Analysis of *Ocimum sanctum*

Following the preparation of *Ocimum sanctum* leaf extracts, a qualitative phytochemical analysis was carried out on the aqueous leaf extract (Dey *et al*., 2012), to determine the presence of flavonoid, diterpenes, phlobatannin, carbohydrate, protein, tannin, glycoside, alkaloid, phenol, cholesterol and terpenoid.

### Characterization of Test Isolates

Five UTI bacterial isolates (*Staphylococcus aureus, Escherichia coli, Proteus mirabilis. Pseudomonas aeruginosa, Klebsiella* sp.) were obtained from the Medical Microbiology diagnostic laboratory of the University of Port Harcourt Teaching Hospital (UPTH). The identities of these isolates were confirmed using standard biochemical tests and Gram staining (Cheesbrough 2000) and their antimicrobial resistance profile determined using the disc diffusion Kirby-Bauer method (Bauer et al., 1966).

### Antibacterial activity bioassay

The antibacterial activities of the three leaf extracts were assessed using the agar well diffusion method and the minimum inhibitory concentration (MIC) dilution method used to assess the antibacterial effect of a standard antibiotic (gentamicin) against the test isolates (Magaldi *et al*., 2004; Valgas *et al*., 2007; CLSI, 2012). Both methods involved the use of a 0.5 McFarland standard inoculum on Mueller Hinton agar.

### MIC dilution method

MIC involved the preparation of a series of culture tubes containing a liquid medium and varying concentrations of gentamicin (0.5 mg/ml, 0.25 mg/ml and 0.125mg/ml). The tubes are then inoculated with 0.1 ml of clinical bacteria cell suspension corresponding to 0.5 McFarland standard and incubated for 24 hours at 35°C. The last tube served as a control, with no antimicrobial agent added into it. After the incubation period, the tubes are examined for turbidity and the lowest concentration of gentamicin which prevented bacterial growth was ascertained as the MIC.

### Agar well diffusion method

For the agar well diffusion method, six 4mm wells were made on solidified Mueller Hinton agar plates using a sterile cork borer. Following seeding of each plate with the relevant test organism, 100µl of 5 different concentrations (50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml and 3.125 mg/ml) of extract was introduced into each the various wells. After a 1 hour pre-incubation time to allow for diffusion of the extract into the medium, each plate was incubated at 37°C for 24 hours and the zone of inhibition noted. The last well served as the control.

## RESULTS

### Antibiotic Resistance Pattern

An assessment of the test isolates revealed a high degree of multidrug resistance (Table 1), with 3 of the 5 isolates resistant to all antibiotics assayed for. Of all 5 isolates, *S. aureus* was the most sensitive with a MAR index of 0.375.

### Phytochemical Screening

An analysis of the possible bioactive substances present in our test plant revealed the presence of only flavonoids, carbohydrates, protein, tannin and phenol (Table 2).

### Antibacterial activity of *Ocimum sanctum* leaf extracts

An analysis of the antibacterial activity of the leaf extracts showed varying results. All three extracts were totally ineffective against the *S. aureus* and *E. coli* isolates, even at higher concentrations (Table 3). Of all three extracts, only the ethyl acetate extract showed slight activity against the Klebsiella isolate at the highest concentration tested, while the aqueous extract (50mg/ml) was active against the *P.mirabilis* only. The most effective extract appeared to be the ethanol extract. This was active against both the P. mirabilis and *Pseudomonas aeruginosa* at three concentrations (50, 25 and 12.5 mg/ml). Analyzing the clinical bacteria isolates, the *P. mirabilis* was the most susceptible to the extracts, followed by the *Pseudomonas aeruginosa* and then the *Klebsiella* sp.

## DISCUSSION

In recent times, in a bid to solve the global epidemic of drug resistance and find an alternative to conventional antibiotics, research focused on the antibacterial activity of natural medicinal plants and a search for the specific active agent has been on the rise. One such plant, which has been studied, is the Holy Basil (*Ocimum sanctum*). This plant has been shown to possess antibacterial activity against several bacterial isolates (*Streptococcus mutans, Enterococcus faecalis, Salmonella typhi, E. coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*), but by studies predominantly from South East Asia (Agarwal *et al*., 2010, Subbiya *et al*., 2013, Sharma *et al*., 2009, Mandal *et al*., 2012). With respect to Nigeria however, there is a dearth of research on *Ocimum sanctum* and none specifically on *Ocimum sanctum* and bacterial isolates associated with UTI. Rather, a related herb (*Ocimum gratissimum*, scent leaf) has

Table 1: Antibiotic resistance pattern of test isolates

| Antibiotics | *S. aureus* (n = 8) | *Proteus mirabilis* (n = 8) | *Klebsiella* sp (n = 8) | *E. coli* (n = 8) | *Pseudomonas aeruginosa* (n = 8) |
|---|---|---|---|---|---|
| Streptomycin (S) | R | - | - | - | - |
| Chloramphenicol (CH) | S | - | - | - | - |
| Ciprofloxacin (CPX) | I | R | R | R | R |
| Erythromycin (E) | I | - | - | - | - |
| Levofloxacin (LEV) | S | - | - | - | - |
| Gentamycin (CN) | R | R | S | R | R |
| Rifampicin (RD) | S | - | - | - | - |
| Amoxycillin (AMX) | R | R | R | R | R |
| Nitrofurantoin (NIT) | - | R | R | R | R |
| Ampicillin (AMP) | - | R | R | R | R |
| Ceftazidime (CAZ) | - | R | R | R | R |
| Cefuroxime (CRX) | - | R | R | R | R |
| Ofloxacin (OFL) | - | R | S | R | R |
| MAR Index | 0.375 | 1 | 0.75 | 1 | 1 |

R = Resistant, S = Sensitive, MAR = Multiple Antibiotic Resistance

Table 2: Phytochemical composition of aqueous extract of *Ocimum sanctum* leaves

| Phytochemicals | Test Result |
|---|---|
| Flavonoid | + |
| Diterpenes (Phytosterols) | - |
| Phlobatanins | - |
| Carbohydrate | + |
| Protein | + |
| Tannin | + |
| Glycoside | - |
| Alkaloid | - |
| Phenol | + |
| Cholesterol | - |
| Terpenoid | - |

Table 3: Antibacterial activity of various *Ocimum sanctum* leaf extracts against five clinical isolates as determined by agar diffusion test

| Extract Type | Conc (mg/ml) | Zone of Inhibition (mm) | | | | |
|---|---|---|---|---|---|---|
| | | *S. aureus* | *Proteus mirabilis* | *Klebsiella* sp | *E. coli* | *Pseudomonas aeruginosa* |
| Ethanol | 50 | 0 | 24 | 0 | 0 | 14 |
| | 25 | 0 | 10 | 0 | 0 | 8 |
| | 12.5 | 0 | 4 | 0 | 0 | 5 |
| | 6.25 | 0 | 0 | 0 | 0 | 0 |
| | 3.125 | 0 | 0 | 0 | 0 | 0 |
| Aqueous | 50 | 0 | 16 | 0 | 0 | 0 |
| | 25 | 0 | 0 | 0 | 0 | 0 |
| | 12.5 | 0 | 0 | 0 | 0 | 0 |
| | 6.25 | 0 | 0 | 0 | 0 | 0 |
| | 3.125 | 0 | 0 | 0 | 0 | 0 |
| Ethyl acetate | 50 | 0 | 0 | 6 | 0 | 0 |
| | 25 | 0 | 0 | 0 | 0 | 0 |
| | 12.5 | 0 | 0 | 0 | 0 | 0 |
| | 6.25 | 0 | 0 | 0 | 0 | 0 |
| | 3.125 | 0 | 0 | 0 | 0 | 0 |
| Gentamicin | 0.5 | + | - | - | - | - |
| | 0.25 | + | - | - | + | - |
| | 0.125 | + | - | + | + | - |

received more attention (Iwalokun *et al*., 2001, Adebolu and Oladimeji 2005, Akinyemi *et al*., 2005, Chah *et al*., 2006, Junaid *et al*., 2006). These studies have shown the antimicrobial activity of extracts of this sister leaf, particularly to diarrheal agents. Preliminary screening of Nigerian *Ocimum sanctum* to ascertain its phytochemical composition (Table 2), showed slight similarities to several previously published studies, particularly in the presence of flavonoids and tannin, and the absence of phlobatannin (Devendran and Balasubramanian 2011, Al-Temimi and Al-Mashhedy 2015). Odumosu and colleagues in a recent study noted that their methanolic extracts of *Nymphae lotus* which had higher concentrations of flavonoids showed a better antimicrobial activity than *Spondias mombin* extracts with less concentration (Odumosu *et al*., 2016). Al-Temimi and Al-Mashhedy have previously reported that flavonoids act as antimicrobial agents, while tannin has been used in the treatment of UTI. These flavonoids have a 2-pheny-benzo[$\alpha$]pyrane or flavane nucleus basic structure and have been reported to be ubiquitous in green plant cells The antibacterial effects of flavonoids have been linked with inhibition of nucleic acid synthesis, cytoplasmic membrane function and energy metabolism (Cushnie and Lamb, 2005). The antibacterial activities of tannins on the other hand, have been shown to be linked with it astringency, action on membranes and metal ion deprivation (Scalbert 1991) and even reported to cause an inhibition of cell wall synthesis due to the formation of an irreversible complex with proline rich proteins (Shimoda 2006).

There was however variability in the results of the phytochemical screening of the plant extracts in this study, as glycoside, alkaloid and terpenoids, were not detected phytochemically. The less than 100% correlation in phytochemical composition of this plant with that reported in other studies is in line with what has been so far published. The reasons for this could be varied. A 2014 study (Padmalochana and Rajan, 2014), reported variations in phytochemical composition of different extracts from the same plant, linked to solvent used in extraction. Priyadarshini and colleagues in 2015 went further to report variations in phytochemicals detected based on the test method used for their detection. Furthermore, a 2015 Iraq study (Al-Temimi and Al-Mashhedy 2015), noted a variation in quantity of phytochemicals present based also on the mode of extraction, with greater amounts of the phytochemicals found in the ethanolic extract as opposed to the cold aqueous extract. One study even went as far as suggesting a possible influence of both environmental conditions and specific plant development growth stage in the chemical composition of plant products (Saharkhiz *et al*., 2014). When the fact that these studies could report on either the plant extract or the whole plant extract, it highlights the need for a standardized methodology to enable comparison between different research works and properly ascertain the suitability of these plant extracts as antimicrobial agents.

This study showed varying antibacterial activities of the different extracts to the various bacterial isolates, with the ethanol extract showing the highest activity. A similar report was made in an Iraqi study in 2015, whereby the ethanol extract of *Ocimum sanctum* showed more activity than the aqueous extract (Al-Temimi and Al-Mashhedy 2015). Additionally, Sharma *et al*., 2009 reported higher antibacterial activities of ethanol extracts than acetone and aqueous extracts. This higher antibacterial activity related to ethanol extracts have also been reported with respect to other plants such as *V. amygdalina* and *Ocimum gratissimum* (Ibrahim et al., 2009). This is thought to be linked with the increased solubility of the phytochemicals in ethanol. One other study however noted that methanolic extracts of *Ocimum sanctum* had even higher antibacterial activity than the ethanol and aqueous extracts, specifically against *Streptococcus* mutans (Kayalvizhi *et al*., 2016), but more reports have not been made on this. Despite previous reports on antibacterial activity of *Ocimum sanctum* against both *S. aureus* and *E. coli* (Pattanayak *et al*., 2010, Mishra and Mishra 2011, Matthew 2014), results of this study showed a total lack of activity of all extract types against these two isolates obtained from urinary tract infections. These findings could result from multiple factors such as, the source of isolates and specific methodology employed. The 2009 study by Sharma and colleagues highlights this effect of methodology on study outcome. Unlike this current study, which employed the use of the agar well diffusion test, the 2009 Sharma study reporting on antibacterial activity of *Ocimum sanctum* against UTI bacterial isolates, made use of a disc diffusion test method (Sharma et al., 2009). In their study, these authors reported antibiotic effect of ethanol and acetone extracts of *Ocimum sanctum* against *E. coli* isolates associated with UTI, but no effect of the aqueous extracts.

This present study however showed very promising results with respect to the use of ethanol extracts in the possible therapy of MDR *Pseudomonas* sp and Proteus mirabilis UTI bacterial isolates. While other studies have reported on the activity of extracts of *Ocimum sanctum* against various

bacterial agents, this is the first reporting its activity at such low mg/ml concentrations and specifically against such MDR isolates.

## CONCLUSION

This study provides the first documented report on the effect of extracts of *Ocimum sanctum* against UTI bacterial isolates in Nigeria. While the study showed no effect against both S. aureus and E. coli, it showed very promising results of the possible use of the ethanol extract of *Ocimum sanctum* against MDR *Pseudomonas* sp and *Proteus mirabilis* even at low concentrations. Despite this, a significant bit of work still remains for the scientific community with respect to standardization methodologies in this specific area of research to enable global comparisons of results. Furthermore, the positive results observed in this study need to be explored on a wider scale and the specific active agent determined.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

### Authors' contributions

This work was carried out in equal collaboration between all authors. Both authors KO and BD designed the study, managed the literature searches and wrote the protocol. KO wrote the first draft of the manuscript. BD was primarily responsible for the lab work. All authors read and approved the final manuscript.

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