Research Article

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Urinary Tract Pathogens: Analysis and Antimicrobial Effects of Ocimum Gratissimum Leaf

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Abstract

The search for alternative medicine for the treatment of pathogenic organisms associated with urinary tract infection (UTI) is on increase. The study was carried out to determine the antimicrobial activities of Ocimum gratissimum leaf extracts on three urinary tract pathogens; Escherichia coli, Staphylococcus aureus and Candida albicans, using their corresponding standard oganisms as controls. The phytochemical analysis of aqueous and alcoholic contents of Ocimum gratissimum leaf extracts revealed the presence of; alkaloids, carbohydrates, flavonoids, glycosides, phenolics, saponins, steroids, and tannins. The minimum inhibitory concentration (MIC), minimum bacteriostatic concentration (MBC) and minimum fungustatic concentration (MFC) of the extracts tested on the three uropathogens was 62.5 to 125 mg/ml. The ethanolic extracts at the concentration of 500mg/ml exhibited strong activity against the urinary pathogens with maximum zones of inhibitions of 23.5±1.5, 23.6±0.6 and 18.25±1.3mm while the aqueous extracts at the same concentration showed weak antimicrobial activity at 3.5±0.5mm as the highest zone of inhibition and 1.4±0.0mm as the lowest zone of inhibition. Notably, the inhibition zones increased with increase in extracts concentration and decreased with decrease in the concentration of extracts. Meanwhile, 12 fractions were eluted from O. gratissimum leave extract. The major phytochemical fractions were mainly alkaloids, flavonoid, and saponins. This study showed that the ethanolic extracts of Ocimum gratissimum is a good antimicrobial agent in-vitro and should not be neglected as an alternative medicine for treatment of pathogenic UTIs.

Keywords : UTI, Uropathogens, Ocimum gratissimum, Antimicrobial, Alternative medicine.

Introduction

The use of medicinal plants as traditional and alternative medicine is well known in rural areas of many developing countries and has been globally accepted and encouraged (WHO, 2016; Ezekwesili-Ofili & Okaka, 2019; Obeta et al., 2020). In Nigeria, Ocimium gratisimum (scent leave) have been used for making soup and stew (Obeta et al., 2021), eradication of Mosquitoes (Agbalaka et al., 2021; Anzaku et al., 2021) and for various medicinal uses (Bhavani et al., 2021; Lunyera et al., 2016). Nwinyi et al. (2009) investigated the antibacterial activity of the aqueous extracts of Ocimum gratissimum against Escherichia coli and Staphylococcus aureus. Dhanalakshmi et al. (2013) and Chijioke et al. (2014) also investigated the antibacterial activity of some medicinal plants used against Uropathogen pathogens and concluded that extracts of plants origin has remarkable anti microbial activity as compared to antibioticactivity. It was made known that organisms are gaining resistance day by day towards most of the anti biotics used for the treatment of UTI. As a result of this, so many natural products are being tried to overcome the antibiotic resistant action of these organisms. The present study has been undertaken to identify effective herbal medicines to control UTI since herbal medicines are available in our environment and believed to be safe when compared to antibiotics. If these medicinal plants continue to show antimicrobial activities against most pathogenic organismtht threatens human health, it will be an evidence that natural medicine can complement or take the place of antibiotics in future (Dhanalakshmi, 2013; Bazzaz et al., 2021) .

It has also been investigated that the essential oil of Ocimum

gratissimum contains eugenol and shows some evidence of antibacterial activity (Nweze & Eze, 2009). A polyherbal preparation of aqueous extract obtained from the leaves of Gongronema latifolia, Vernonia amygdalina and Ocimum gratissimum showed analgesic activity against many pathogenic organisms (Iroanya et al., 2009). The essential oil has potential for use as a food preservative (Nguefack et al., 2009) and is toxic to Leishmania. The oil of Ocimum gratissimum has been formulated into creams for clinical trials where favourable against certain dermatological disorders caused by pathogenic microbes (Sofowora, 1993). Many scientists have carried out researches on Ocimum gratissimum leaf extracts and discovered that the leaf possess antidiabetic properties and anti-hyperlipidemic effect against many pathogenic organisms (Owoyele et al., 2005), (Egesie et al., 2006), (Ayinla et al., 2011), (Shittu et al., 2016).

Lexa et al(2006) was able to demonstrate the anti fungal property of Ocimum gratissimum and discovered that the chloroformic fraction of the extract in hibited 23 isolates (92%) of Cryptococcus ne of or mans at a concentration of 62.5µg/ml (Silva et al., 2007) in vitro against humanpathogenic dermatophytes (Lexaetal., 2006). The essential oil of O. gratissimum has antimicrobial activities against pathogenic strains of Gram positive (S. aureus, Bacillus spp) and Gram negative (E. coli, P. aeruginosae, S. typhi, K. pneumonia, P.mirabilis) bacteria and pathogenic fungus C. albicans (Matasyoh et al., 2007). Ocimum gratissimum is rich in alkaloid, tannins, phytates, flavonoids,oligos accharides and has tolerable cyanogenic content (Nakaruma et al., 1999), (Lemos et al.,2005). The plant contains terpenoids, eugenol, thymol, saponins and alkaloids. The essential oil of O. gratissimum has antimicrobial activities against pathogenic strains of Gram positive (S. aureus, Bacillus sp) and Gram negative (E. coli, P. aeruginosae, S. typhi, K. pneumonia, P.mirabilis) bacteria and pathogenic fungus C. albicans (Matasyoh et al., 2007).

In traditional medicine, the leaves have been used in the treatment of UTIs, rubbed between palms to prevent cold, sniffed inside the nose to clear blocked nostrils, used as anti-diarrhea agent, and for the treatment of conjunctivitis by instilling directly into the eyes. Despite all thes, there is no evidence to back it up.

This study was carried out to evaluate the antimicrobial effects of ethanolic and aqueous extracts of Ocimium gratisimum L. (Lamiaceae) used in traditional medicine for the treatment of several ailments such as urinary tract infection on clinical isolates namely: Escherichia coli and Staphylococcus aureus and Candida albicans in comparison with a typed bacterium of E.coli (ATCC 11775), S. aureus (ATCC 6538) and typed fungal strain of Candida albicans (ATCC 10231).

The specific objectives was to carry out Ocimum gratissimum leaf extraction; to determine the in vitro activities on E .coli, S. aureus and C. albicans respectively through antimicrobial susceptibility assay; to determine the Minimum inhibitory Concentration (MIC) and Minimum Bacteriocidal Concentration (MBC) and to identify the bioactive compounds and fractions in the extracts that has the highest antimicrobial activity against the UTI isolates.

Materials and Methods

Collection and Preparation of Ocimum gratissimuml leaf

The Ocimum gratissimum leaves as shown in figure 1 used for this study was collected from Polo area of Jos in Jos North LGA, Plateau State within the month of July, 2020. It was identified in Habarium of Plant Science Department in University of Jos with voucher number UJH16000270. The leaves were washed and allowed to air dry and then grinded into fine powder in line with the method described by Nweze et al. (2009). The extract was sieved, labeled, weighed into sterile dry containers and stored at room temperature for use. The two solvents used for the extraction of the leaves were Water, and Alcohol.

Figure 1: Ocimum gratissimum Leaf

Extraction Method

Aqueous extraction was carried out using the cold maceration method accoding to the method described by Ncube et al. (2008) while the method described by Nweze et al. (2009) was used for the ethanolic extractions. The extracts were concentrated at reduced pressure to dryness at 40°C using rotary evaporator and a greenish black coloured sticky residue was obtained which was used to calculate the yield of each extract (formula 1). The extracts were then stored in refrigerator at 4^0C.

$$\text{Percentage yield of extracts} = \frac{W2 - W1}{W0 \times 100} \quad (1)$$

Where:
W2 is the weight of the extract and the container,
W1 is the weight of the container alone and
W0 is the weight of the initial dried sample

Phytochemical Analysis of Extracts

Phytochemical analysis of each extract was carried out using the description of Kolkate et al. (2003) to a certain the presence or absence of phenols, alkaloids, flavonoids, tannins, saponins, steroids and glycosides. Identification of Fractions was carried out using Thin-Layer chromatography (TLC) and column chromatography respectively.

The Preparation of the TLC Plates, choice of Solvent System (hexane, ethyl acetate and water (4:2:1), preparation of stock extracts to be spotted, development of Thin layer chromatography (TLC), procedure for column chromatography, detection of bands and calculation of retardation factor (Rf) was done using formular (2) according to method earlier described by Harwood et al. (1980) and Martins (2013).

$$Rf = \frac{\text{Distance traveled by the solute}}{\text{Distance traveled by the solvent front TLC plate}} \quad (2)$$

Distance traveled by the solvent front TLC plate

Antimicrobial activity of the fractions was carried out on the 5 fractions of the leaves against the 3 different organisms used. The zones of inhibitions were measured and recorded.

Collection of Standard Strain

Standard strains species which were used as controls were; Escherichia coli (ATCC 11775), Staphylococcus aureus (ATCC 6538) and Candida albicans (ATCC 10231) obtained from Microbiology laboratory of National Veterinary Research Institute (NVRI) Vom. The Staphylococcus aureus strains was cultured on Blood agar plates at 37°C and maintained on nutrient agar slants while the Candida albicans strain was grown on Sabroud dextrose agar (SDA) plate and maintained on SDA slant at 4^0C.

Collection and culturing of urine samples

Already confirmed urine samples of patients suffering from urinary tract infection were collected from the Laboratory of Bingham University Teaching Hospital Jos.

Identification of organisms

Colonies from the cultured plates were selected and characterized on the basis of morphological, cultural, physiological and biochemical characteristics.

Standardization of Test Organisms

Standardization of test organisms was done in Microbiology laboratory of Jos University. The suspension was incubated at 37°C for 24hours and the size was adjusted by diluting each suspension with sterile broth to the 0.5 MacFarland standard turbidity (CLSI, 1998) approximately 1.5×10^6 CFU/ml.

Antimicrobial susceptibility testing

The antimicrobial activities of the extracts were tested on the organisms using the agar-well diffusion method according to method described by Pottumarthy et al., 2006 using Mueller-Hinton agar plates.

Assessment of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration was determined according to national committee for clinical laboratory standard (NCCLS) (1998) using standard two-fold dilution broth methodology.

Determination of Minimum Bacteriocidal Concentration and Minimum Fungicidal Concentration (MBC and MFC)

5ul of each extract from each tube that exhibited no growth was taken and incubated at 37°C for 24h. With the aid of positive and negative cultures, the lowest concentration that shows no visible bacterial growth after sub-culturing was taken as MBC and MFC. Freshly prepared sterile nutrient agar was poured separately into sterile petridishes and allowed to set firmly, a loopful of mixture in the tube not showing growth was transferred to the agar in the plates and incubated for 24 hrs. At 37°C and observed for growth. The plate that did not show growth was recorded as MBC and MFC against the test organisms, respectively.

Statistical Analysis

One way analysis of variance (ANOVA) was used to statistically analyze all the data obtained from research using Statistical Package for Social Sciences (SPSS) Software version 22 and results were recorded.

(a) Dried, weighed extracts

(b) Soaking of extracts

(c) Crude extracts

(d) Drying of extract in rotaroy evaporator

(e) Drying of ethanolic extracts on hot plate

(f) Drying of aqueous extracts In hot water bath

(g) Phytochemical tests

(h) Macfarland standard

(i) Column packed with silica gel

(j) Elution of fractions

(k) Frictions elluteded from column

Figure 2: Extraction Processes of Ocimum gratissimum leaves

Results

The results of the analysis carried out on the Ocimum gratissimum leaves extract are presented as follows:

NAME OF PLANT	SOLVENT USED FOR EXTRACTION	WEIGTH OF POWDERED EXTRACT	W E I G H T OF CRUDE EXTRACTS
Ocimum gratissimum	Water	300g	147.0g
Ocimum gratissimum	Ethanol	300g	130.1g

Table 1: The Yield of Extracts of *Ocimum gratissimum*

Extraction Solvents	*Ocimum gratissimum*	
	AQUEOUS	ETHANOL
Phytochemicals		
Tannins	+	++
Alkaloids	+	+++
Flavonoids	++	+++
Saponins	+	+++
Glycosides	-	+
Steroids	-	+
Phenolics	++	+++
carbohydrates	+	-

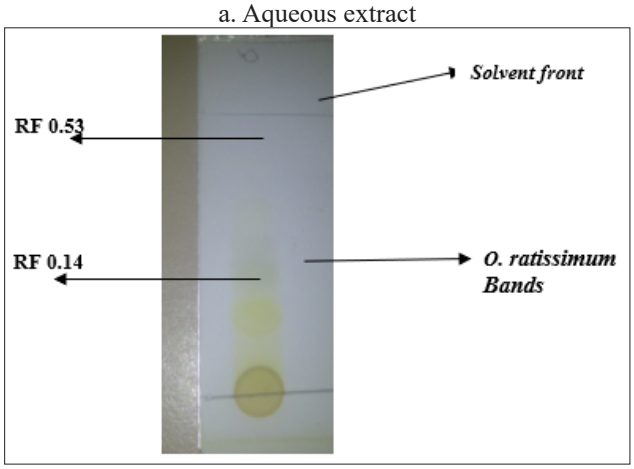
Key: (+++) = Highly present,
(++) = Moderately present,
(+) = Slightly present, (–) =Absent

Table 2: Phytochemical Composition of Aqueous and Ethanolic Extracts of *Ocimum gratissimum* Leaves

Thin Layer Chromatography (TLC)

Thin-layer chromatographic study revealed that the ethanolic and aqueous extracts of *O. gratissimum* has a maximum of 5 bands. The solvent front was 7.0cm with RF of between 0.14-0.53 as shown in figure bellow.

a. Aqueous extract



b. Ethanolic extract

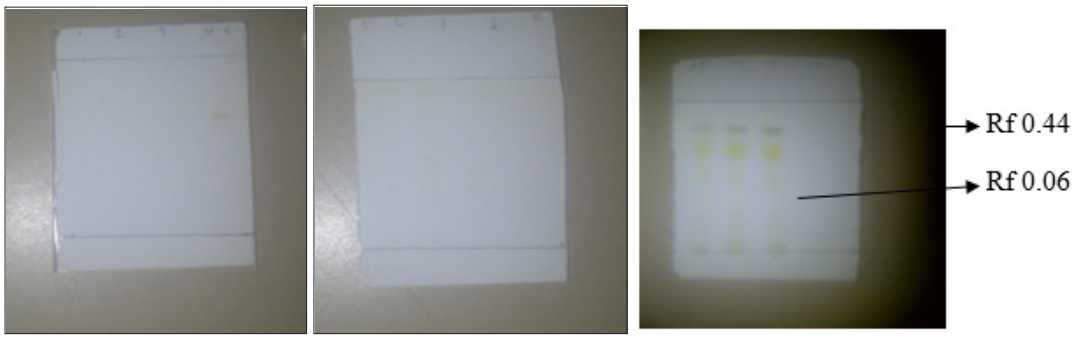
Figure 3: Thin-layer chromatographic bands of *Ocimum gratissimum* leaves extract

FRACTIONS OF OCIMUM GRATISSIMUM LEAVES EXTRACT ELUTED FROM COLUMN CHROMATOGRAPHY

During column chromatography, 12 fractions were eluted from O. gratissimum leaves extract.For the, bioactive compounds the distance moved by the solvent front was 8.0cm with RF value of between 0.06- 0.44 as shown in Tables 3 and Figures 4.

No. of Bands	Fraction	Rf values	Colour of bands	Spraying Reagent	Colour of bands appeared	Phytochemicals Detected
1	F1	0.31	Light green	Ammonia solution	Dark grey	Flavonoid
2	F2-3	0.43	Dark green	Conc. HCl	Dark brown	Saponins
3	F4-6	0.29	Orange	Dragendorffs reagent	Orange	Alkaloid
4	F7-9	0.14	Light yellow	Ammonia solution	Yellow	Flavonoid
5	F10-12	0.17	Light grey	$FeCl_3$	Red	Phenol

Table 3: ELUTION PROFILE OF COLUMN CHROMATOGRAPHY OF ETHANOLIC LEAF EXTRACTS OF *OCIMUM GRATISSIMUMIN* ON MOBILE PHASE OF N- HEXANE: ETHYL ACETATE: WATER (4:2:1)



(a) F1-4, (b) F5-8, (c) F9-12

Figure 4: TWELVE FRACTIONS OF *O.GRATISSIMUM* LEAVES EXTRACT ELUTED FROM COLUMN CHROMATOGRAPHY

SUCEPTIBLITY OF THE DIFFERENT FRACTIONS AGAINST TEST ORGANISMS

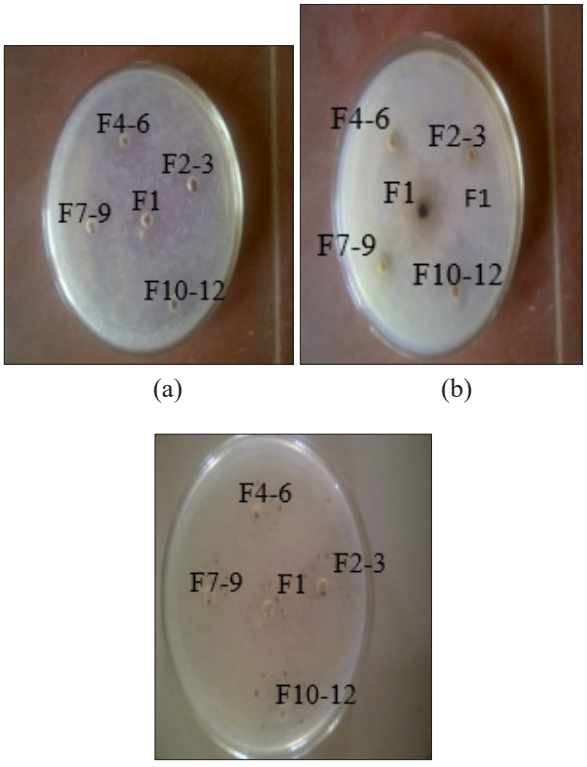
The antimicrobial activity of the extract of *Ocimum gratissimum* were located at RF values of between 0.06- 0.44. F1 has the highest activity on Escherichia coli with zone of inhibition of 20.00mm, 9.11mm on *Staphylococcus aureus* and 6.01mm against *Candida albicans*. F 2-3 shows the antimicrobial activity on *Escherichia coli* with zone of inhibition of 15.00mm, 8.01mmon Staphylococcus aureus and 7.00mm on *Candida albicans*, F4-6 also showed activity on *Escherichia coli* with zone of inhibition of 6.03mm, 4.02 against *S.aureus* and 2.00mm against *Candida albicans* while F 7-9 did not show activity against *Escherichia coli*, *Staphylococcus* aureus and *Candida albicans*, F10-12 also did not show any activity on any of the organisms.

Ocimum gratissimum

Fraction	*E. coli*	*Staph aureus*	*Candida albicans*
F1	*20.00*	*9.11*	*6.01*
F 2-3	15.00	8.01	7.00
F4-6	6.03	4.02	2.00
F 7-9	-	-	-
F10-12	-	-	-

Key:
(-) = No inhibition F = Fraction

Table 4: THE ANTIMICROBIAL ACTIVITY OF THE DIFFERENT FRACTIONS OF *O.gratissimum* LEAVE EXTRACT AGAINST THE THREE UROPATHOGENS



(a) (b)

(c)

Key: F = Fraction

Figure 5: Inhibition zones of the fractions from column chromatography on the uropathogens (*a. E.coli, b. S.aureus, c. C. albicans*) respectively

Identification of Test Organisms

Each organism was identified using their specific biochemical tests. Confirmatory tests carried out to identify the isolates were Gram staining reaction, indole test, catalase test, coagulase test and germ tube test. It was observed that E. coli was negative for Gram staining reaction, positive for indole test, negative for catalase, coagulase tests, and germ tube test. Staphylococcus aureus was catalase positive, positive for gram staining reaction, negative forindole test, and coagulase positive and germ tube test negative. C. albicans was only positive for germ tube test negative for Gram staining reaction, indole test, catalase production and coagulase test as shown in table 5.

Gs	In	Cat	Cao	Gtt	Identity of isolates
-	+	-	-	-	E. coli
+	-	+	+	-	Staph aureus
+	-	-	-	+	C.albicans

Key: Gs-Grams staining Reaction, In-indole; Cat- catalase; Coa-coagulase, Gtt-germ tube test, +: Positive -: Negative.

Table 5: IDENTIFICATION OF UTI ISOLATES

Antimicrbial Activity of Aqueous Extract of The Leaves on The Three Uropathogens

The aqueous extract of Ocimum gratissimum leaves extract showed weak antimicrobial activity on the test organisms with 3.5±0.5mm as the highest zone of inhibition at a concentration of 500mg/ml and 1.4±0.0mm as the lowest zone of inhibition at a concentration of 31.25mg/ml.

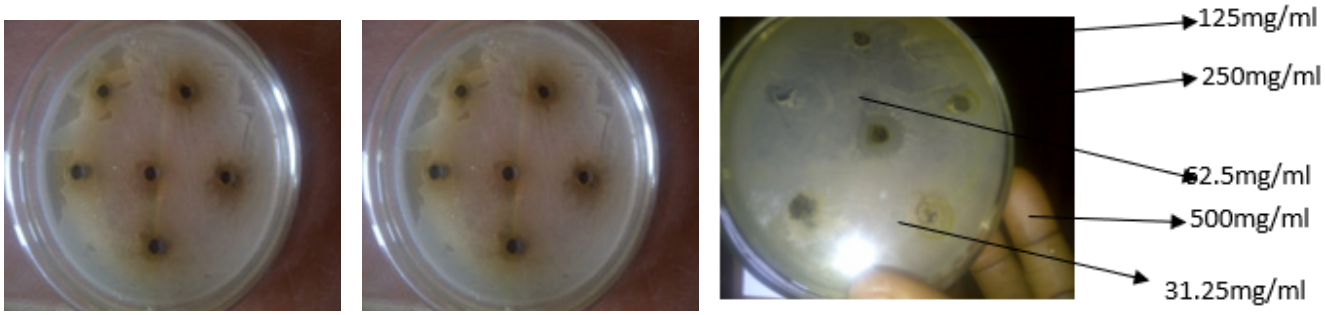
Table 6 shows the susceptibility of aqueous extract of Ocimum gratissimum leaves extract on the three UTI isolates and also on standard strains. It was observed that all the three isolates were inhibited at concentration of 500mg/ml with varying zones of inhibitions. Also at 250mg/ml and 125mg/ml all the three isolates were also inhibited at varying zones of inhibitions but at concentration of 62.5mglml, only S. aureus was inhibited with zone of inhibition of 0.5±0.7c while the other test organisms were not inhibited. At 31.25mg/ml none of the organisms was inhibited. The LSD was calculated against each organisms with S.aureus having the highest LSD of 1.80 and E.coli ATCC11775 having the least LSD of 0.74. Means tagged with different letter alphabet are significantly different at P=0.05 example 5.5±0.5a and 2.1±0.1b against E.coli while Means tagged with the same letter of alphabet are not significant at P=0.05 example 2.1±0.1b and 1.6±0.6b against E.coli.

conc(mg/ml)	E.coli (mm)	S.aureus (mm)	C.albicans (mm)	E.coli ATCC11775 (mm)	S.aureus ATCC 6538 (mm)	C.albicans ATCC10231 (mm)
500	3.5±0.5a	3.0±1.1a	2.0±0.0a	3.9±0.1a	3.1±0.1a	2.5±0.5a
250	2.1±0.1b	2.1±0.0b	1.8±0.1b	2.0±0.0b	2.0±0.0b	1.9±0.1a
125	1.6±0.6b	1.9±0.1b	1.4±0.1c	1.4±0.0b	1.8±0.1c	1.7±0.1a
62.5	-	0.5±0.7c	-	-	-	-
31.25	-	-	-	-	-	-
LSD	1.21	0.8	0.18	0.74	0.18	0.82

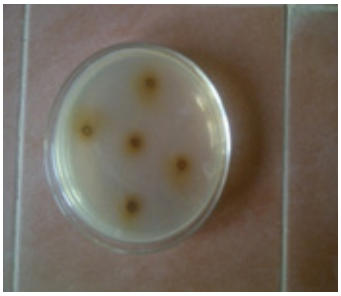
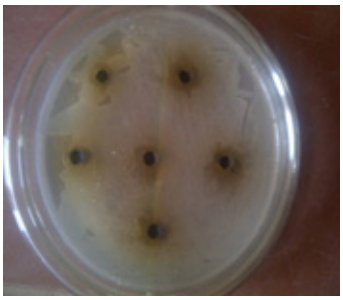
Footnote: Means tagged with different letter alphabet are significant at P=0.05

Key: - = No inhibition

Table 6: Suceptibility Of Aqueous Extract of *Ocimum Gratissimum* Leaf on The Three Uti Isolates and Standard Strains



a) O.gratissimum against E. coli b) O.gratissimum against S.aureus c) O.gratissimum against C.albicans


d) O.gratissimum against E.coli ATCC 11775 e) O.gratissimum against S.aureus ATCC 6538 f) O.gratissimum against C. albicans ATCC 10231

Figure 6: Suceptibilty of aqueous extract of O.gratissimum leaves on the UTI Pathogens with their corresponding standards

ETHANOLIC EXTRACTS OF Ocimum gratissimum LEAF

The ethanolic extract of Ocimum gratissimum leaves extract exhibited strong antimicrobial activity Escherichia coli, Staphylococcus aureus and Candida albicans. It also inhibited the organisms with maximum zones of inhibitions of 23.5±1.5, 23.6±0.6 and 18.25±1.3mm, respectively at contration of 500mg/ml.

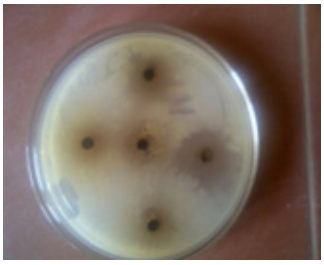
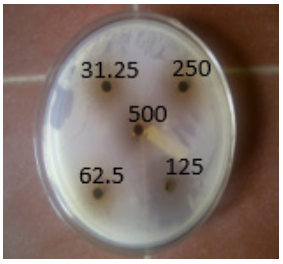
The susceptibility of ethanolic extract of Ocimum gratissimum leaves extract on the three UTI isolates and standard strains is shown in Table 7. It was observed that all the three isolates were inhibited at concentration of 500mg/ml with varying zones of inhibitions- 23.5±1.5a, 23.6±0.6a, 18.25±1.3a, 22.0±1.9a, 24.6±0.4a and 19.0±1.0 on Escherichia coli, ATCC11775, Staphylococcus aureus ATCC 6538 and Candida albicans ATCC10231 respectively. Also at 250mg/ml and 125mg/ml all the three isolates were inhibited at concentration of with varying zones of inhibitions but at concentration of 62.5mg/ml, only E.coli isolate, and E.coli strain- ATCC11775 were inhibited with zones of inhibition of 4.1±4.1b and 3.5±3.5b respectively while the other test organisms were not inhibited. At 31.25mg/ml, none of the organism was inhibited. The LSD were calculated against each organisms with C.albicans ATCC10231 having the highest LSD of 10.00 and S.aureus, having the least LSD of 3.30. Means tagged with different letter alphabet are significantly different at P=0.05 as in table 7.

Conc (mg/ml)	E.coli isolate (mm)	S.aureus isolate(mm)	C.albicans isolate (mm)	E.colistrain-ATCC11775 (mm)	S.aureus strain-ATCC 6538(mm)	C.albicans strain-ATCC10231 (mm)
500	23.5±1.5a	23.6±0.6a	18.25±1.3a	22.0±1.9a	24.6±0.4a	19.0±1.0 a
250	19.7±0.4a	19.2±1.3b	12.1±2.1b	19.3±0.1a	18.7±1.7b	7.5±5.5b
125	15.3±3.3a	8.5±1.5 c	6.6±0.6c	14.6±3.4a	9.2±1.3c	3.5±2.5b
62.5	4.1±4.1b	-	-	3.5±3.5b	-	-
31.25	-	-	-	-	-	-
LSD=	8.87	3.30	4.10	8.55	3.51	10.00

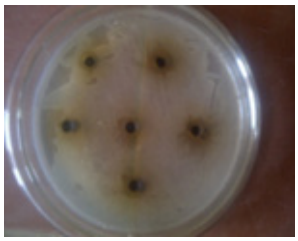
Footnote: Means tagged with different alphabet are significant at P=0.05

Key: (-) = No inhibition

Table 7: Antimicrobial Activity of Ethanolic Extract of Ocimum Gratissimum Leaf on The Three Uti Isolates And Standard Strains

a) O.gratissimum against E. coli b) O.gratissimum against S.aureus c) O.gratissimum against C.albicans



d) O.gratissimum against E.coli ATCC 11775 e) O.gratissimum against S.aureus ATCC 6538 f) O.gratissimum against C. albicans ATCC 10231

Figure 7: Susceptibility test of ethanolic extract of O.gratissimum leaves on the Uropathogens with their corresponding standards strains

Positive and Negative Controls

Positive (Nitrofurantoin) was used on E coli, Streptomycine was used on S. aureus while Fluconazole was used on C.albicans. The zones of inhibitions were 25.6±0.5, 20.0±0 and 18.0±1.0 on E. coli, S.aureus, and C.albicans respectively at the concentration of 500mg/ml. The zones of inhibitions for the standard strains were; 21.1±0.1, 20.5 ±2.5 and 18.0±1.0 at the concentration of 500mg/ml for E. coli ATCC 11775, S.aureus ATCC 6538, and C.albicans ATCC 10231 respectively. At the concentration of 250mg/ml and 125mg/ml all the three isolates were inhibited at concentration of with varying zones of inhibitions but at concentration of 62.5, only E.coli was inhibited with zones of inhibition of1.00mm. At 31.25 none was inhibited.

Comparing the zones of inhibition of the antibiotics to that of plant extracts, it was observed that the highest zone of inhibition of the control was 25.6±0.5mm while the highest zone of inhibition of the Plant extracts was 24.7±0.5mm. There is statistical difference as the difference between the zones of inhibition of the plant extract to that of the antibiotics was just as little as 0.9±0.0mm. The negative control (sterile distilled water) did not show any antimicrobial activity in both the aqueous and ethanolic leave extracts as presented in plates below.

Conc. of positive controls	E.coli	E.coli ATCC11775	S.aureus	S.aureus ATCC 6538	C.albicans	C.albicans ATCC10231
500	25.6±0.5	20.0±0	18.0±1.0	21.1±0.1	20.5±2.5	18.1±0.0
250	18.6±1.0	16.6±1.0	10.0±1.0	10.0±1.0	12.0±1.0	11.5±0.5
125	8.5±0.5	5.5±0.5	4.3±0.5	3.5±0.5	2.2±0.0	2.0±0.0
62.5	1.0±0.0	-	-	-	-	-
31.25	-	-	-	-	-	-
Negative Control (Sterile Distilled water)	-	-	-	-	-	-

Key: = No inhibition

Table 8: SUSCEPTIBILITY TEST ON POSITIVE AND NEGATIVE CONTROLS
Positive controlsNitrofurantion StreptomycinFluconazole

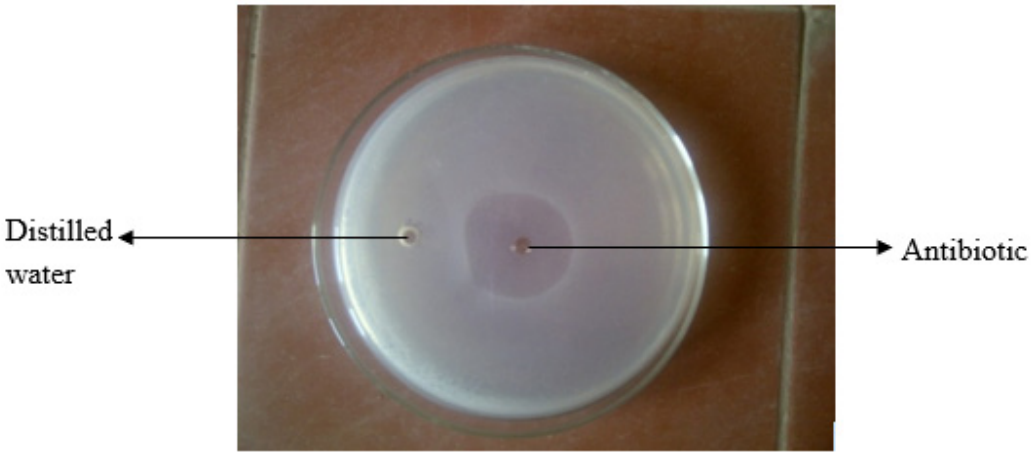


Figure 8: Positive and negative controls
NOTE: Positive control – Antibiotics Negative control - Distilled water

Minimum Inhibitory Concentrations (Mic) of The Extract on The Isolates

The Minimum Inhibitory Concentration (MIC) was determined using broth dilution method. The organisms were observed for their ability to produce visible growth on series of broth dilutions containing diluted antimicrobial agent. It was observed that each microorganism has a level of antimicrobial agent which inhibits their growth. The lowest concentration of antimicrobial agent in (mg/ml) that prevents the appearance of visible growth of a microorganism within 24hours was taken as the MIC.

All the microorganisms (E coli, S. aureus and C.albicans) used in this research were inhibited at varying concentrations of between 500(mg/ml) and 31.25(mg/ml). The minimum inhibitory concentrations of the ethanolic extract showed remarkable activities on the test organisms. It was equally observed that the extract has MIC of 62.5mg/ml on all the isolates except C.albicans which was inhibited at concentration of 125 mg/ml as shown in table 9.

Clinical/typed strains	500(mg/ml)	250 (mg/ml)	125(mg/ml)	62.5(mg/ml)	31.25(mg/ml)	ESC control	MIC
S. aureus ATCC 6538	+	+	+	-	-	NG	62.5
S.auerus	+	+	+	-	-	NG	62.5
E.coli ATCC 11775	+	+	+	-	-	NG	62.5
E.coli	+	+	+	-	-	NG	62.5
C. albicans ATCC 10231	+	+	-	-	-	NG	125
C.albicans	+	+	-	-	-	NG	125

Key: + = Growth -= No growth ESC = Extract sterility control

Table 9: Mic of Ethanolic Extracts of Ocimum Gratissimum Leaves on The Isolates

Mean Diameter of zone of inhibitions shown by different concentration of the extract (mm)

Minimum Bacteriostatic Concentration (Mbc) and Minimum Fungistatic Concentration (Mfc) of The Extracts

It was observed that the extracts have MBC of 62.5mg/ml on both the isolates and their standard strains.

Clinical/typed strains	500(mg/ml)	250 (mg/ml)	125(mg/ml)	62.5(mg/ml)	31.25(mg/ml)	ESC	MIC
S.aureus strain-ATCC 6538	+	+	+	-	-	-	62.5
S.auerus isolate	+	+	+	-	-	-	62.5
E.coli strain-ATCC 11775	+	+	+	-	-	-	62.5
E.coli isolate	+	+	+	-	-	-	62.5
C.albicans strain-ATCC 10231	+	+	+	-	-	-	62.5
C.albicans isolate	+	+	+	-	-	-	62.5

Key: + = Growth -= No growth ESC = Extract sterility control

Table 10: The Mbc and Mfc Results of The Ethanolic Extract on The Organisms

Mean Diameter of zone of inhibitions shown by different concentration of the extract (mm)

Discussion

Several phytochemical compounds which exhibited antimicrobial activities were extracted from the leaves of O.gratissimum and this suggest that urinary tract infections can be reduced or treated with natural products (Flores-Mireles et al., 2015) such as the leaves of O. gratissimum which may aid in the reduction of the prevalence of UTI if well harnessed. The phytochemical tests of the aqueous and ethanolic extracts showed that the leaves contains Tannins, Alkaloids, Flavonoids, Saponins, Glycosides, Steroids, Phenolics, and carbohydrates. These compounds have been documented by other researchers in previous studies (Sofowora, 1993). The presence of these phytochemicals in O. gratissimum leaves extract is an indication that the plant has pharmacological importance which may be used as drug in treating UTI pathogens (Adebolu & Salu, 2005).

This is in affirmation to the result obtained by Cowan (1999) who reported that plant extracts possess different phytochemicals. In this study it was observed that alkaloids, flavonoids, saponins and phenol were higher in concentration than the other phytochemical compounds. This is in accordance to the work of Nweze et al. (2009) who also observed the presence of alkaloids, flavonoids, saponins and phenol in higher concentration, this maybe as a result of using the same method

of extraction. Sterols was equally extracted from the leaves of O. gratissimum and present sterols have also been reported to have antibacterial properties in other studies (Cowan, 1999; Okwu, 2001). The presence of alkaloids and saponins in the present work is in agreement with the opinion of Kasolo et al. (2010) who noted that saponins and alkaloids were two active constituents in plants.

It was equally documented by other studies that alkaloids are nitrogenous compounds occurring naturally which are commonly found to have antimicrobial properties like in this study due to their ability to intercalate with DNA of microorganisms. However, the presence of saponin in this plant is in agreement with the previous findings of Yusha'a et al. (2011) who also extracted saponin in his study (Yusha'a et al., 2011). The activity of plant extracts against bacteria have been studied for years, but in more intensified way during the last three decades.Duringthisperiod, numerousantimicrobialscreening have been published based on different traditional regions like; Chinese, AfricanandAsiancountries who have used some of theseplant asdrugs (Suffredim et al., 2004).

The aqueous extracts of O.gratissimum leave extracts showed weak antimicrobial activities and this maybe as a result of the extraction solvents used, knowing fully well that water is more polar than other compounds, so non polar compounds may not have been extracted from the leaves. This means that a lot of bio-active compound may not have been extracted by water. This result is contrary to the result of Lapornik et al. (2005) who reported that water is a better extraction solvent as compared to ethanol because in this study, water was not the best solvent for extraction. Hower, it is in accordance with the findings of Das et al. (2010) who reported that water soluble flavonoids have no antimicrobial significance and water soluble phenolics are only important as antioxidant compounds. Though traditional healers use primarily water to extract plant but organic solvents have been found to give more consistent antimicrobial activity compared to water extract (Lapornik et al., 2005).

In this present study, the results of the antimicrobial properties of the plant extracts on different organisms varied depending on the organism tested and the concentration of the extracts, it was observed that the zone of inhibition decreased with decrease in concentration of the extracts and increase with increased in concentration of the extracts. This is comparable to to previous studies (Taylor et al., 2001; Farnsworth, 1993) which recorded that active compound may be present in insufficient quantities in extracts which may or may not show antimicrobial activity on pathogenic organisms. It is observed that the higher the concentration, the higher the antimicrobial activity, it is therefore suggested that in order to rule out the inability of plants extract to show antimicrobial activity, high concentration should be used (Farnsworth, 1993; Jager et al., 1993).

The ethanolic extracts exhibited stronger activity on all the UTI pathogens at varying concentrations. This may be attributed to the extraction solvent (ethanol) used or maybe the compounds in the leaves were less polar since ethanol is polar. It should be noted that ethanol was not inhibiting the uropathogens rather it was the bioactive compounds in the ethanolic extracts doing the inhibition because all the ethanol used in the extraction was evaporated via rotary evaporator. Ethanol was considered to be more efficient in cell walls and seeds degradation which have unipolar character and cause polyphenols to be released from cells (Cowan, 1999).

Conclusion

Having done thorough investigation beyond any reasonable doubt, the leaves extract of O. gratissimum have exerted strong, good and promising antimicrobial activities on Uropathogens. The antimicrobial activities it shows on both the gram positive and gram negative bacteria as well as on fungi shows that it has a broad spectrum effect which is very impressive. The differences observed in the antimicrobial activities between aqueous and ethanolic extracts shows that solvent used for extraction is very paramount.

Thispresentdemonstratedthatherbalmedicinescan also be effectiveasmodernmedicinestocombatsome pathogenic microorganisms if well harnessed. The actual bioactive phytochemicals in the Ocimium gratisimum leaves chromatographically identified are flavonoids, saponins and phenols.

It is recommendable that further study should be carried on Ocimium gratisimum leaves extract in order to convert the essential phytochemicals in the leaves to complimentary medicine in the treatment of urinary tract infections leading to the formulation of natural UTI drugs.

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Conflict of Interest

None

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None

Ethics Statement

None.

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