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Research Article

Antimicrobial and synergistic potential of *Ocimum gratissimum* leaves and *Petiveria alliacea* bark against some selected microorganisms

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Abstract

Background: This study was carried out to investigate the antimicrobial and synergistic potential of the leaves of *Ocimum gratissimum* and bark of *Petiveria alliacea* against some tested bacterial and fungal isolates. Fresh and matured leaves of *Ocimum gratissimum* and bark of *Petiveria alliacea* were collected from the Institute of Agriculture, Research and Training, Ibadan, Nigeria. The specimens were identified at the Herbarium unit of the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. The pathogenic organisms used include bacteria namely, *Providencia stuartii, Bacillus cereus, Staphylococcus aureus, Corynebacterium Pyogenes, Streptococcus faecalis, Klebsiella oxytoca, Klebsiella pneumonia, Escherichia coli, Pseudomonas fluorescence, Serratia rubidae, Proteus mirabilis, Salmonella pullorum;* and fungi namely, *Trychophyton tonsurans, Candidia albicans, Trychophyton rubrum, Penicillium expansium, Alternaria* sp, *Fusarium* sp, *Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus,* and *Penicillium camenberti.*

Methods: Pure isolates of the tested microorganisms were obtained from the department of microbiology, University of Ibadan, Ibadan, Nigeria. The bacterial isolates were maintained on nutrient agar slant and the fungal isolates were on Sabouraud Dextrose Agar (SDA). Antimicrobial sensitivity test (AST) followed by Clinical and Laboratory Standard Institute. Minimal bactericidal and fungicidal concentrations were determined following established protocols.

Results: Fungal isolates of *Aspergillus flavus, Penicillium expansiumm, Trychophyton rubrum,* and bacterial isolates *Klebsiella oxytoca, Klebsiella pneumonia, Escherichia coli, Proteus mirabilis, and Salmonella pullorum* were all resistant to the plant extract. Findings from this study opined that ethanolic extract of *Ocimum gratissimum leaves* is more potent than the methanolic and aqueous extracts of *Petiveria alliacea.*

Conclusion: The plant extracts showed greater antimicrobial activity against bacterial- with respect to fungal isolates suggesting a broader spectrum of activity with ethanolic extract on the gram-positive and the gram-negative bacteria.

Introduction

The various therapeutic roles of plants that fight various diseases have led to an increase in the search for alternatives to conventional medicine. *Ocimum gratissimum* is widely used for medicinal purposes in the treatment of intestinal diseases [1]. It is often used in Nigeria as a condiment in preparing various dishes that gain prominence due to their nutritional power [2]. Medicinal plants are a rich source of anti-bacterial activity. There are many reports established on the effectiveness of traditional medicine against germs. Therefore, plants are still recognized as a pivot of modern medicine for the treatment of infectious diseases [3]. The traditional use of herbs as primary treatment because of their medicinal value is very common in western countries [4]. *Petiveria alliacea* is commonly referred to as "Anamu" or "Yes Aja" in southwestern Nigeria. It is traditionally used to improve memory and treatment of respiratory tract infections. It is also used to strengthen infection, pain, and treatment for a variety of chronic diseases including certain cancers [5]. There are many plants with antimicrobial and biological activity [6] currently used in the food industry as antibacterial and antifungal agents [7].

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Although the main purpose of spices is to provide flavor and therapeutic properties, antibacterial and antifungal properties have also been used [8]. Studies on medicinal plants should include both the phytochemicals and the biological properties of these plants. Numerous studies have been conducted to determine the different antimicrobial and phytochemical components of various medicinal plants that are widely used in the treatment of various microbial infections as alternative therapies for synthetic drugs when many infectious viruses develop resistance [9]. Recent scientific discoveries in the rational use of medicinal plants and the many therapeutic approaches of local communities can lead to the beneficial identification of useful techniques, and the conservation and sustainable use of local biodiversity [10]. The combination of various antimicrobial agents may result in interaction or opposition in the workplace. Where there is a combination, the antimicrobial effect is achieved at a lower concentration than when applied once [11].

Materials and methods

Preparation of plant samples

Fresh and mature *Occimum gratissimum* leaves and *Petiveria alliacea* bark were obtained from the Institute of Agriculture, Research and Training, Ibadan, Nigeria. Models courtesy of IFE Herbarium. 10 g of crushed *Ocimum gratissimum* leaves and *Petiveria alliacea* bark are carefully weighed and immersed in 1,000 mL of soluble extract for 72 hours and stirred continuously. The remains were filtered using Muslin cloth to filter the filtrate and were later filtered through Whatman No 1 filter paper under aseptic conditions. Filters evaporated using a rotating evaporator, then stored in the refrigerator at 4 ºC until needed for future use Ladipo, et al. [12]

Sources and preparation of test organism

Pure isolates of bacteria and fungi used in this study were isolated locally from wounds and the environment and obtained from the Department of Microbiology, OAU, Ile-Ife, Nigeria. Distinguishing bacteria are stored in the agar medium and fungal isolates in the SDA. These alone were placed in a test tube attached to a separate cotton container containing 10ml of Mueller-Hinton broth inoculated at 37ºC for 24 hours. Bacteria used include *Providencia stuartii, Bacillus cereus, Staphylococcus aureus, Corynebacterium Pyogenes, Streptococcus faecalis, Klebsiella oxytoca, Klebsiella pneumonia, Escherichia Poliumsee, Escherichia Salmonsuess, Pseudomonas, Serreptococcus flour, Pseudomonas, Pseudomonas, Pseudomonas, Candida albicans, Trichophyton rubrum, Penicillium expansium, Alternaria sp, Fusarium sp, Aspergillus niger, Aspergillus and Aspergillus andger, Aspergillus camenberti.* Synergistic antimicrobial effects of a variety of fungal and bacterial extracts were determined using a method developed by Oluduro and Omoboye [13]. To demonstrate interaction, an equal amount of plant mixture was measured and melted in the right amount of ingredients to give a concentration of 100 mg/ml used for antimicrobial testing Oluduro and Omoboye [13]

Sensitivity testing

Antibacterial and Antifungal tests are performed according to established criteria by Daoud, et al. [14]. One ml of a new culture of bacteria and mold was piped into an empty Petri container. Molten-cooled Muller Hi (PDA) mold was then poured into a Petri container containing the inoculum and blended. After hardening, the springs were drilled using a sterile cork borer (6 mm wide) into agar plates containing inoculum. Thereafter, 100 µl of each extract (20% w/v) was added to the appropriate sources. The plates are transferred to the refrigerator for 30 minutes so that they are evenly distributed. Then, the plates were placed at 37°C for 18 hours. Antimicrobial activity was obtained by measuring the area of the block after the incubation period.

Inoculum suspension

Inocula were prepared from stock cultures stored in nutritious agar at 40C and planted slowly in nutrient solution using a wide wire loop. The density of the suspension inserted in the media to test the trend was determined in relation to the standard 0.5 McFarland Barium sulphate solution Cheesbrough [15].

Minimal bactericidal and fungicidal concentration (MBC and MFC)

Minimal bactericidal concentration (MBC) was determined by incorporating 1ml of direct MIC tubes into the nutrient agar to obtain the bacteriostatic and/or bactericidal effect of the extracts. The MBC plant extraction was determined by a modification of the Spencer and Spencer method [16].

Phytochemical analysis

Phytochemical analysis of samples was performed in accordance with established conventions.

Saponin: Twenty-five grams (25g) of each powdered sample is placed in a bucket and boiled in 25ml of distilled water in a water bath controlled at 100ºC and filtered. 2.5ml of each filtrate is mixed with 5ml of distilled water and stirred vigorously to form a stable foam. The broth was mixed and then stirred vigorously and concentrated to form an emulsion of Obdoni and Ochuko [17].

Steroids: 2 ml (2ml) acetic anhydride added to 0.5g extracted ethanol for each sample and 2ml of tetra oxosulphate (VI) acid added. The color change from violet to blue or green indicated the presence of steroids Kolawole, et al. [15]

Flavonoids: Each portion of the powdered plant samples was diluted separately with 10ml of ethyl acetate in a water bath for 30 min. The mixture was filtered and 4ml of each filtrate was mixed and diluted with 1ml of dilute ammonia solution in a conical flask. The formation of a yellow color indicated the presence of flavonoids Harborne [16].

Tannins: A quantity of powdered sample (0.5g) boiled in 20ml clear water in a test tube and filtered in a concrete flask. A few (2-3) drops of 0.1% ferric chloride were added and marked with dark brown or blue-black color Trease and Evans [16].

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Phenols: Two grams (2g) of each sample diluted with 100ml of diethyl ether using the soxhlet apparatus for 2 hours. Two grams (2g) of the molten powder were boiled with 50ml of ether from the extract of the phenolic portion for 15min. 5ml of the extract was pipette into a 5ml volume conical flask and 10ml of distilled water was added 1ml of ammonium hydroxide solution and 5ml concentrated amyl alcohol was also added. Samples were left to react for 30 minutes to improve color. This was measured at 505nm using the UNICO 1100 RS spectrophotometer McDonald, et al. [17]

Terpenoids: Five milliliters (5ml) of liquid extract per plant sample mixed with 2ml (2ml) of chloroform in a test tube. Three milliliters (3ml) of tetraoxosulphate (VI) concentrated tetraoxosulphate (VI) acids are carefully added to the mixture to form a layer. A visible reddish-brown connective tissue is formed when a terpenoids component is present Edeoga, et al. [17].

Results

The biological effect of methanolic (M), Ethanolic (E), and Aqueous (A) extracts of Aspillia Ocimum gratissimum and Petiveria alliacea against some selected bacterial and fungal isolates

The zones of inhibition of the extracts ranged from 1-18mm for bacteria while fungal isolates ranged between 1 and 5mm (*Table 1*). The highest and lowest zones of inhibition of 18mm and 1mm respectively were obtained in *Staphyloccocus aureus* under the aqueous and methanolic fractions. *Bacillus cereus*, *Cornybacterium pyogenes*, *Klebsiella pneumonia*, and *Escherichia coli* showed a high degree of resistance to the plant extracts. The fungal isolates; *Candida albicans*, *Tryptophyton rubrum*, *Penicillium expansium*, and *Aspergillus flavus* were resistant to *Psidium guajava* extract since no significant activity was observed

Synergistic antimicrobial effects of the plant extracts on the tested bacterial and fungal isolates revealed that the diameter of zones of inhibition ranged from 1mm with combined aqueous extracts and ethanolic extracts to 35mm with ethanolic extracts of *P. alliacea* and *O. gratissimum* (*Table 2*). *E. coli* and *Proteus mirabilis* were resistant to the combined extract of *P. alliacea* and *O. gratissimum* among the bacterial isolates. *Aspergillus fumgatus* and *Aspergilus flavus* showed no antimicrobial activity to the combined extract among the fungal isolates.

The susceptibility of the test organisms to antibiotics and the antifungal drug showed that *Staphyloccocus aureus* and *Cornybacterium pyogens* primarily sensitive to the plant extracts were found to be resistant to some of the antibiotics used (*Table 3*). All the plant extracts showed strong antimicrobial activities against *Streptococcus faecalis and S. aureus*, whereas, these organisms were resistant to synthetic commercial products such as ofloxacin, sparfloxacin, chloramphenicol, amoxicillin, ciprofloxacin, and septrin. However, the antifungal drug (Ketoconazole) was more effective on the test fungi than the plant extracts.

Table 1: Diameter of zones of inhibition of the plant's extracts on selected bacterial and fungal isolates.

Diameter of zones of inhibition (mm)						
	Occimum grattisimum			*P. alliacea*		
Test Bacteria	A	E	M	A	E	M
Providencia stuartii	-	-	-	11	11	7
Bacillus cereus	2	5	-	5	7	2
Staphylococcus aureus	18	15	-	16	21	8
Corynebacterium pyogenes	3	5	-	-	-	-
Streptococcus faecalis	11	15	10	6	6	-
Klebsiella oxytoca	-	-	-	5	7	4
Klebsiella pneumonia	-	-	-	2	6	1
Escherichia coli	-	-	-	-	-	-
Pseudomonas fluorescens	-	-	-	13	15	4
Serratia rubidae	3	8	-	-	-	-
Proteus mirabilis	-	-	-	-	-	-
Salmonella pullorum	-	-	-	4	7	-
Test Fungi						
Trychophyton tonsurans	1	2	-	-	-	-
Candida albicans	-	-	1	2	2	-
Trychophyton rubrum	-	-	-	1	3	-
Penicillium expansium	-	-	-	-	-	-
Alternaria sp.	-	-	-	-	-	-
Fusarium sp.	3	5	2	7	5	1
Aspergillus niger	-	-	-	2	3	-
Aspergillus fumigates	-	-	-	-	-	-

Key: A=Aqueous extract, E = Ethanolic extract, M = Methanolic extract

Table 2: Synergistic antimicrobial effects of the plant extracts on tested microorganisms.

Diameter of zones of inhibition (mm)			
	Occimum grattisimum + P. alliacea		
Test Bacteria	A	E	M
Providencia stuartii	14	16	12
Bacillus cereus	10	17	4
Staphylococcus aureus	32	35	10
Corynebacterium pyogenes	6	8	2
Streptococcus faecalis	15	19	11
Klebsiella oxytoca	7	9	6
Klebsiella pneumonia	3	9	1
Escherichia coli	-	-	-
Pseudomonas fluorescens	10	13	5
Serratia rubidae	5	11	2
Proteus mirabilis	-	-	-
Salmonella pullorum	6	10	2
Test Fungi			
Trychophyton tonsurans	-	-	-
Candida albicans	-	-	-
Trychophyton rubrum	-	-	-
Penicillium expansium	-	-	-
Alternaria sp.	-	-	-
Fusarium sp.	-	-	-
Aspergillus niger	-	-	-
Aspergillus fumigates	-	-	-

Key: A=Aqueous extract, E = Ethanolic extract, M = Methanolic extract

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Table 3: Susceptibility of the test organisms to antibiotics and antifungal drug.

Test Bacteria							Diameter of Zones of Inhibition (µg)									
Gram-negative	OFL	SP	CHL	AUG	CPX	SXP	AMX	GEN	PEF	STR	APX		CT	ERY	N	CM
	(5)	(10)	(30)	(30)	(5)	(5)	(25)	(10)	(5)	(10)	(10)		(30)	(10)	()	()
Escherichia coli	18 (R)	15 (R)	14 (R)	13 (R)	20 (S)	13 (R)	15 (R)	16 (S)	18 (S)	12 (R)	-	-	-	-	-	-
Klebsiella pneumoniae	0 (R)	0 (R)	15 (S)	20 (S)	0 (R)	12 (R)	16 (R)	12 (R)	23 (S)	0 (R)	-	-	-	-	-	-
Klebsiella oxytoca	0 (R)	18 (R)	20 (S)	16 (R)	18 (S)	24 (S)	16 (R)	14 (R)	24 (S)	20 (S)	-	-	-	-	-	-
Pseudomonas fluorescens	0 (R)	13 (R)	20 (S)	12 (R)	18 (S)	14 (R)	20 (R)	16 (S)	12 (R)	20 (S)	-	-	-	-	-	-
Serratia rubidae	4 (R)	4 (R)	5 (R)	0 (R)	12 (R)	0 (R)	0 (R)	8 (R)	16 (S)	12 (R)	-	-	-	-	-	-
Proteus mirabilis	8 (R)	6 (R)	2 (R)	0 (R)	16 (R)	20 (S)	4 (R)	22 (S)	16 (S)	2 (R)	-	-	-	-	-	-
Salmonella pullorum	19 (S)	0 (R)	2 (R)	0 (R)	0 (R)	16 (S)	0 (R)	21 (S)	22 (S)	0 (R)	-	-	-	-	-	-
Providential stuartii	4 (R)	4 (R)	5 (R)	0 (R)	12 (R)	0 (R)	0 (R)	8 (R)	16 (S)	12 (R)	-	-	-	-	-	-
Bacillus cereus	-	-	-	-	-	-	0 (R)	24 (S)	26 (S)	20 (S)	0 (R)	24 (S)	0 (R)	4 (R)	8 (R)	10 (R)
Staphlococcus aureus	-	-	-	-	-	-	0 (R)	22 (S)	18 (S)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	19 (S)	0 (R)
Corynebacterium pyogenes	-	-	-	-	-	-	12 (R)	3 (R)	21 (S)	0 (R)	19 (S)	12 (R)	4 (R)	10 (R)	19 (S)	0 (R)
Streptococcus faecalis	-	-	-	-	-	-	12 (R)	17 (S)	7 (R)	0 (R)	8 (R)	10 (R)	10 (R)	15 (S)	6 (R)	0 (R)

Test Fungi	Concentrations of Ketofung (mg/ml)		
	05	10	20
Trychophyton tonsurans	18	34	37
Candida albicans	18	25	33
Penicillium expansium	16	22	28
Alternaria sp.	11	13	17
Fusarium sp.	21	26	32
Aspergillus niger	16	18	24
Aspergillus fumigates	18	25	30
Aspergillus flavus	20	22	28
Penicillum camenberti	14	18	22
Trychophyton rubrum	17	22	27

OFL= ofloxacin (5µg) , SP= Sparfloxacin (5µg) , CHL= Chloramphenicol (30µg) , AUG= Augmentin (30µg), CPX= Ciprofloxacin (5µg), SXP= Septrin (25µg), AMX= Amoxicillin (25µg), GEN= Gentamycin (10µg), PEF= Pefloxacin (5µg), STR= Streptomycin (10µg), APX= Ampiclox (10µg), Z= Zinnacef (µg), CT= Ceftriazone (30µg), ERY= Erythromycin (10µg), N= Nitrofurantoin (µg), CM= Clarithomycin (µg)

The Lowest Inhibitory Concentration (MIC) of the test microorganism revealed that the MIC ranged from 25 to 250µg/ml (*Table 4*). The lowest MIC (25µg/ml) was recorded in *P. stuartii*. Similarly, the aqueous and ethanolic extracts of *O. gratissimum* had a MIC of 25µg/ml on *S. aureus*. The highest MIC (250µg/ml) was recorded with methanolic extracts of *P. alliacea* on *S. faecalis*. The MIC of the various plant extracts against selected fungi ranged from 150-300µg/ml. The lowest MIC (150µg/ml) was obtained with aqueous and ethanolic extracts of *P. alliacea* on *C. albicans* and *Fusarium* sp. respectively. Similarly, MIC of 250µg/ml was recorded in aqueous and ethanolic extracts of *P. alliaceae* on *A. niger*.

The Lowest bactericidal concentration (MBC) and minimum fungicidal concentration of the various plant extracts ranged from 50-400µg/ml (*Table 5*). The lowest MBC (50µg/ml) was obtained with aqueous extracts of *P. alliacea* against *P. stuartii.* The highest MBC (400µg/ml) was obtained in methanolic extract of *P. alliacea* on *S. aureus*. Moreover, an MBC of 400µg/ml was noticeable in the methanolic extract of *O. gratissimum* against *C. pyogenes*. The lowest MFC was obtained with the aqueous and ethanolic extracts of *P. alliacea* against *C. albicans* and *Fusarium sp.* respectively. A minimum fungicidal concentration of 300µg/ml was recorded in ethanolic extracts of *P. alliacea* against *T. rubrum*. The highest MFC of 400µg/ml was obtained with the aqueous and ethanolic extracts of *P. alliacea* against *A. niger*.

Discussion

In this study, extracts from *P. Alliacea aqueous, ethanolic and methanolic* leaves were active against Gram-negative (*P. stuartii, K. pneumonia, S. pollurum, K. oxytoca and P. fluorescence*) species,

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Gram-positive (*B. cereus, S. aureus, and S. faecalis*) filtered and produced simple works on some of the fungus species (*C. albican, T. rubrum, Fusarium sp. And A. niger*) tested but failed at *E. coli, P. mirabilis, and S. rubidae.* Barnes, et al. [3] also reported bacteriostatic activity of *P. alliacea* in the Gram-positive bacterium of the pathogenic *S. aureus,* and was confirmed by Benevides, et al. (2003), Musah and Kubec (2009) reported antibacterial and antifungal activities of plant extract. Contrary to these reports was that of Adomi (2008), who reported that *P. alliacea* had no effect on *S. aureus, Salmonella sp., K. pneumonia, Pseudomonas sp., E. coli, Bacillus sp.,* and *Flavobacterium sp.* but a similar reaction was obtained in E. coli. Ocimum gratissimum L. (Lamiaceae) is used to treat various ailments, such as upper respiratory infections, diarrhea, headaches, fever, ophthalmic, dermatitis, and pneumonia (Onajobi, 1986; Ilori et al., 1996). In this study, *O. gratissimum* showed high inhibitory activity in *S. aureus,* and *S. faecalis,* and at least in *B. cereus, C. pyogenes* and *S. rubidae.* However, it did not show any inhibitory effect on other bacteria and all fungi tested. Koche, et al. (2012) also reported the antibacterial activity of chloroform solvent of the root extract of *O. gratissimum* L. to be high in *E. coli* has the extraction of the leaves of ethanol, chloroform, ethyl acetate, solvents, but root extraction in another solvent has been demonstrated. Central function against *S. aureus* and *K. Pneumonia.* Meanwhile, Ndounga and Quamba, (1997) in their study reported that the antibacterial activity of leaf extract

Table 4: Minimum inhibitory concentration (MIC) of the various extracts on the tested bacterial and fungal isolates (µg/ml).

	Occimum grattisimum			*P. alliacea*		
Test Bacteria	**A**	**E**	**M**	**A**	**E**	**M**
Providencia stuartii	-	-	-	100	50	50
Bacillus cereus	100	50	-	-	50	50
Staphylococcus aureus	25	50	-	200	50	50
Corynebacterium pyogenes	100	50	100	-	100	-
Streptococcus faecalis	-	-	-	-	100	-
Klebsiella oxytoca	-	-	-	-	200	200
Klebsiella pneumonia	-	-	-	-	-	-
Escherichia coli	-	-	-	-	150	150
Pseudomonas fluorescens	-	-	-	-	-	-
Serratia rubidae	100	100	300	-	-	-
Proteus mirabilis	150	100	-	-	-	-
Salmonella pullorum	-	-	-	-	-	-
Test Fungi						
Trychophyton tonsurans	-	-	-	-	-	-
Candida albicans	-	-	-	-	150	150
Trychophyton rubrum	-	-	-	-	250	200
Penicillium expansium	-	-	-	300	150	150
Alternaria sp.	-	-	-	-	250	250
Fusarium sp.	-	-	-	-	-	-
Aspergillus niger	-	-	-	-	-	-
Aspergillus fumigates	-	-	-	-	-	-
Aspergillus flavus	-	-	-	-	-	-

Key: A= Aqueous extract, E= Ethanolic extract, M= Methanolic extract

Table 5: Minimum bactericidal and fungicidal concentrations (MBC and MFC) of the various plant extracts on tested bacterial and fungal isolates (µg/ml).

MBC	*Occimum grattisimum*			*P. alliacea*		
Test Bacteria	**A**	**E**	**M**	**A**	**E**	**M**
Providencia stuartii	-	-	-	100	50	50
Bacillus cereus	200	100	-	-	50	150
Staphylococcus aureus	100	150	-	400	100	100
Corynebacterium pyogenes	100	100	250	-	150	150
Streptococcus faecalis	-	-	-	-	400	300
Klebsiella oxytoca	-	-	-	-	-	-
Klebsiella pneumonia	-	-	-	-	200	200
Escherichia coli	-	-	-	-	200	200
Pseudomonas fluorescens	-	-	-	-	-	-
Serratia rubidae	250	200	40	-	-	-
Proteus mirabilis	200	200	-	-	-	-
Salmonella pullorum **MFC**	-	-	-	-	-	-
Test Fungi						
Trychophyton tonsurans	-	-	-	-	-	-
Candida albicans	-	-	-	-	200	200
Trychophyton rubrum	-	-	-	-	300	300
Penicillium expansium	-	-	-	40	150	200
Alternaria sp.	-	-	-	-	250	250
Fusarium sp.	-	-	-	-	-	-
Aspergillus niger	-	-	-	-	400	400
Aspergillus fumigates	-	-	-	-	-	-
Aspergillus flavus	-	-	-	-	-	-

Key: A= Aqueous extract, E= Ethanolic extract, M= Methanolic extract

(ethanolic, chloroform, and ethyl acetate) of *O. gratissimum* L showed a certain level of activity against E isolate E. coli. The stem and leaf extract showed little antimicrobial activity in *S. aureus* and *K. pneumoniae* than in *E. coli*. While working on Annona muricata, Kingsley, et al, 2017, reported that root bark has very high active components, he said this is due to the Capillary action of plant transport vessels against gravity. Adebolu and Oladimeji (2005) suggested that only oils derived from *Occimum gratissimum* leaves had antibacterial activity against selected diarrheal infections. They reported that ethanol could improve the release of essential oils and may be responsible for the improved function of the aqueous ethanolic extract against tested bacteria compared to aqueous extract. The results of the current study do not match the report above. Ocimum oil has been reported to be effective against a number of pathogens (*S. aureus, L. monocytogenes, E. coli, Shigella sp., Salmonella sp., And Proteus sp.*) and fungi (*T. rubrum, T. mentagrophytes. C. . neoformans, Penicillum sp. and C. albicans* (Akinyemi, et al. 2004; Janine de Aquino Lemos, et al. 2005; Lopez, et al. 2005). Koche, et al. (2012) reported the effectiveness of -ethanolic extraction of O. *gratisimmum* in *E. coli* with a high inhibitory surface of 22mm at 250 mg/ml and 6.5mm at 50 mg/ml, and, in our study, no antimicrobial activity has been shown in *E. coli, Salmonella sp., Proteus sp. T.*

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rubrum, *Penicillum sp.* and *C. albicans.* 1969; Begum, et al. 1993; Nwosu and Okafor 1995; Akinyemi, et al. 2004; Janine de Aquino, et al. 2005; Lopez, et al. 2005). (21mm) was obtained by extracting ethanolic *P. alliacea* against *S. aureus*. This may be due to the fact that the bioactive compounds in *P. alliacea* is very soluble in ethanol as a solvent. In addition, ethanol itself has antimicrobial activity when used alone and has the ability to dissolve organic compounds better, thus releasing the active ingredient needed for plant antimicrobial activities (Elegalam, 2005). The results obtained from the minimum inhibitory concentration (MIC) of various extracted plants showed that the plant excretion was very strong against bacteria in the MIC of 25µg/ml. The high MIC obtained from the test mold confirmed their low tendency for almost all tested releases. MICs of fungal isolates vary between 150 and 300µg/ml. The easy entry of these fungi into these extracts may explain that most fungi interact with plants in the field and would naturally develop resistance.

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