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Determination of the Antibacterial Effects of Prunus amygdalinus (Almond Leaf) and Ocimum Gratissimum (Basil Leaf) on Selected Bacteria

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ABSTRACT

The antimicrobial acts of leaf extracts of *Ocimumgratissimum* and *Prunusamygdalinus* were checked on *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi* using agar cup plate method. The outcomes demonstrated that the chosen organisms were susceptible to the plant extracts at concentrations of 500mg, 50mg and 5mg respectively with diameter zone of inhibition (DZI) of about 12.00± 2.00, 13.00± 2.00, 11.60±3.40, and 12.30±3.30. Phytochemical screening of the leaf extract of *Ocimumgratissimum* manifested the existence of alkaloid, phenols, glycosides, saponin, steroids and triterpenes while the leaf extract of *Prunusamygadalinus* demonstrated the existence of saponin, flavonoid and triterpenes. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assayed reveals MIC of 5mg and MBC of 50mg respectively. *Ocimumgratissimum* and *Prunusamygdalinus* extracts may be applied to treat disorder caused by these organisms.

Key words: Antimicrobial, Ocimumgratissimum, Prunusamygdalinus, Diameter Zone of Inhibition

1. INTRODUCTION

There is growing interest in exploiting plants for medicinal purposes especially in Africa. The stems from the fact that microorganisms are developing resistance to many drugs and as such created circumstance where some of the frequent and less valuable antimicrobial factors are failing efficiency. Herbal medicine which applies medicinal plants initially is an alternative to such situation (1). These therapeutic plants have immensely helped to the development of human health and welfare. Concomitantly, there is an expansion in data and enormous patronage to herbal products round the world (2). Medicinal plants such *Ocimumgratissimum* and *Prunusamygdalinus* have been asserted to provide various culinary and medicinal properties exert bactericidal effects on some bacteria. Thses consequences have been applied to the alkaloid, glycosides, phenols, sapanins, steroids, phlobatanins, triterpenes additionally flavonoids which are major elements in these plants (3). *Ocimumgratissimum* belong to the family *Lamiaceae.* Ii is naturally used in the treatment of different diseases which includes: upper respiratory tract infections, diarrhea, and headache, conjunctivitis, skin disease, and pneumonia, and tooth and gum disorder, fever and as mosquito repellants. *Prunusamygdalinus* has been applied as cure for cancers, tumors, ulcers, diabetes mellitus since it includes functionally no carbohydrates as well as dermatitis. Thus, this study seeks to investigate the scientific basis for the traditional use of these medicinal plants for treating ailments associated with *Esherichia coli* and *Staphylococcus aureus, almonellatyphi* and *Pseudomonas aeruginosa* infections (4). *Staphylococcus aureus, Escharichia coli, Pseudomonas aeruginosa* and *Salmonella typhi* are bacterial pathogens recognized to drive critical health problems in developiong countries, composing Nigeria. Health Authorities are dealt with the problem of getting sufficient manage amount against these disorders. These pathogens have expanded resistance

against some of the antibiotics frequently applied for their treatment also this has directed to increase in morbidity additionally mortality. There is due to the need to develop alternative treatment which these pathogens will not be resistant to one of such alternatives is the use of medicinal plants. This study focus on the effects of the crude extracts of *Ocimumgratissimum* and *Prunusamygdalinus* on these pathogens.

2. MATERIALS AND METHODS

2.1. Collection of plant materials

The therapeutic plants applied in this assay were *Ocimumgratissimum* and *Prunusamygdalinus*. The leaves of these plants were collated within Bosso, Niger State, Nigeria and were determined by a Renown Botanist in the Department of Biological Sciences, Federal university of Technology, Minna, Nigeria.

2.2. Extraction procedure

The leaves of the plants were air-dried, grinded into powder from applying mortar also pestle. 150g each of the plant powder was broken in 750ml, 75% ethanol for 72 hours in a conical flask. The content of each plant powder was agitated daily after which was filtered using Whatman's (No 11) filter paper. The filtrate was evaporated to dryness using steam evaporator and the extract was stored at 4^{o}C until required (5).

2.3. Screening of test organisms

Stock cultures of *Staphylococcus aureus, Escharichia coli, Pseudomonas aeruginosa* and *Salmonella typhi* were isolated from specimens obtained from patients attending General Hospital, Minna. These cultures were examined for viability additionally essentiality applying gram-stain responses also different biochemical assays. The organisms were kept on agar slant and stored at 4^{o}C until when desired.

2.4. Standardization of the test organisms

The test organisms were standardized to 10^6 using the McFarland dilution technique and stored as an 18 hour culture at 4^{o}C.

2.5. Screening for Antibacterial Activity

Susceptibility Test

Each of the organisms was subjected to the action of the extracts using the agar cup plate techniques as described by (5). Using corkborer no 4 three holes were bored on the surface of the agar medium equidistant from one to another the base of each was sealed with molten agar to avoid seepage. When solidified, each of the cups or holes created was filled with recognized volume also concentration of the pre-prepared extract solution and allowed to fully diffuse. The surface of the agar was streaked for confluent growth with an 18 hours culture of the test organism which has been previously standardized to 10^6 and incubated at the temperature of 37^{o}C in the incubator for 24 hours. Disc of commercially developed antibiotics frequently were applied against the analysis organisms to compare the action of the plant extract with the commercially developed antibiotics.

2.6. Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) was calculated applying the tube dilution methods. Changing measure of the extract in diverse concentration was composed 9ml of extract and 0.1ml of the overnight culture of the analysis organisms diluted at 106 cell/ml was concluded to all analysis tubes and was incubated at 37oC for 24 hours. The least concentration of plant extract that did not allow any clear amplification of the inoculated analysis organisms in broth culture was acquired as minimum inhibitory concentration (MIC) (6).

2.7. Determination of Minimum Bactericidal Concentration (MBC)

This was calculated by plating out the tubes manifesting no growth from the MIC on nutrient agar plates also incubated at 37oC for 24 hours also lack of growth on incubation was caught as the minimum bactericidal concentration (MBC).

2.8. Phytochemical analysis of plant extracts for active components

Phytochemical screening of the extract was carried out according to the methods described by (7, 8) for the detection of active component like saponins, tannins, alkaloids, phlobatannins, glycosidese.t.c. Alkaloids: 1cm^3 of 1% HCL was added to 3cm^3 of the extract in a test tube. The mixture was then warmed for 20 minutes, cooled furthermore filtered. About 2 drops of Mayer's reagent was summed to 1cm3 of the extract. A creamy precipitate was an icon of the existence of alkaloids.

a. Tannins – 1cm^3 of freshly prepared 10% KOH was added to 1cm^3 of the extract. Absence of dirty white precipitate showed the absence of tannins.
b. Phenolics: Two drops of 5% FeCl3 of the extract in a test tube. Existence of greenish residue pointed the existence of phenolics.
c. Glycosides: 10cm3 of 50% H2SO4 was accompanied to 1cm3 of the extract also the combination warmed in boiling water for about 15 minutes. 10 cm3 of Fehling's solution was that time accompanied and the combination boiled. A brick-red residue was confirmatory for the presence of glycosides.
d. Saponins: (i) Frothing test: 2cm^3 of the extract was vigorously shaken in the test tube for 2 minutes. Frothing was viewed. (ii) Emulsion analysis: 5 drops of olive oil was assessed to 3cm^3 of the extract in the analysis tube additionally actively shaken. Existence of stable emulsion created indicates the presence of saponins.
e. Flavonoid: 1 cm^3 of 10% NaOH was added to

3cm^3 of the extract. There was yellow colouration which is indicative the presences of flavonoids.

f. Steriods: Salkowski experiment: 5 drops of concentrated H2SO4 was totaled to 1 cm3 of the extract in a test tube. Red colouration was viewed which is suggestive for the existence of steroids.Phlobatanins: 1 cm^3 of the extract was added to 1% HCl. No red precipitate observed which means negative result.

g. Triterpenes: 1 cm3 of the extract was summed to 5 drops of acetic anhydride also a drop of concentrated H2SO4 summed. The combination was that time steamed for 1 hour additionally neutralized with NaOH followed by addition of chloroform. Presence of blue-green colour indicated the presence of triterpenes (8).

3. RESULTS AND DISCUSSION

3.1. Sensitivity Testing

Table 1 displayed the average diameter of areas of restriction when commercially antibiotics dics were applied against *E. coli, P. aeruginosa, S. aureus* and *S. typhi.*

Table 1. shows the mean diameter of zones of inhibition in (mm) of plant extract at different concentration (mg/ml)

Organism	*Ocimumgratissium*			*Prunusamygdalinus*		
	500	**50**	**5**	**500**	**50**	**5**
E. coli	12.00±2.00	11.00±2.00	8.00±4.00	11.60±3.40	8.00±4.00	6.30±3.30
P. aeruginosa	13.00±2.00	10.60±3.30	-	14.00±2.00	11.00±2.00	8.60±3.40
S. aureus	11.60±3.40	8.30±3.30	5.00±2.00	12.00±2.00	8.60±3.40	7.30±4.60
S. typhi	12.30±3.30	9.00±2.00	6.00±2.00	16.60±0.40	13.30±3.20	11.60±3.40

Table 2 displayed the average diameter of areas of restriction when commercially antibiotics dics were applied against *E. coli, P. aeruginosa, S. aureus* and *S. typhi.*

Table 2. Susceptibility testing using commercial antibiotic disc zones of inhibition (mm)

Organism	*Ocimumgratissium*			*Prunusamygdalinus*		
	AMX	**APX**	**RD**	**AMX**	**APX**	**RD**
E. coli	8.30±3.30	-	-	8.00±4.00	8. 60±3.40	-
P. aeruginosa	7.30±3.30	7.60±3.20	-	14.00±6.00	-	-
S. aureus	-	10.00±4.00	-	10.00±4.00	-	-
S. typhi	-	8.60±3.40	10.00±4.00	8.30±3.30	-	7.30±3.30

Table 3 shows the mean diameter of zones of inhibition when commercially antibiotics discs were used against *E. coli, P. aeruginosa, S. aureus and S. typhi*

Table 3. Potentiality-assaying applying commercial antibiotic discs areas of restriction (mm)

Positive Control						
Organism	SX	CPX	PN	AU	CN	PN
E. coli	-	-	10.00±4.00	-	10. 00±4.00	-
P. aeruginosa	7.30 + 3.30	7.60±3. 20	-	14. 00±6.00	-	-
S. aureus	-	10.00±4.00	-	10. 00±4.00	-	-
S. typhi	-	8.60±3.40	10.00±4.00	8.30±3.30	10.00±4.00	7. 30±3.30

Table 4 shows the mean diameter of zones of inhibition when commercial antibiotic discs were used against *E. coli, P. aeruginosa, S. aureus* and *S. typhi.*

Table 4. Susceptibility testing using commercial antibiotic discs zones of inhibition (mm)

Negative control

Negative Control								
Organism	SXT	CPX	PN	AU	CN	PN	AU	CN
E. coli	-	-	10.00±4.00	10.00±4.00	-	10.00±4.00	-	-
P. aeruginosa	-	-	-	8.60±3.40	7.30±3.3	7.30±3.30	10.30±3.30	-
S. aureus	-	10.30±3.30	-	8.30±3.30	-	8.60±3.40	8.30±3.30	-
S. typhi	8.30±3.30	-	-	7. 30±3.30	-	8.30±3.30	10.00±4.00	7.30±3.30

KEY:

RD	-	Rifampin
AMX	-	Amoxacillin
APX	-	Ampiclox
CPX	-	Ciprofloxacin
CN	-	Gentamycin
SXT	-	Septrin
AU	-	Augmentin
PN:	-	Ampicillin

3.2. Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

Table 5 Displays the concentration at which each of the test organisms was restricted in growth also was entirely killed following being incubated with each of the leaf extracts. The growth of all- inclusive the assay organisms were restricted at 5mg/ml as well as the assay organisms were entirely killed at 50mg/ml.

Table 5. minimum inhibitory concentration and minimum bactericidal concentration (mg) of plant extract on test organisms

Organisms	*Ocimum gratissium*		*Prunus amygdalinus*	
	MIC	MBC	MIC	MBC
E. coli	5	50	5	50
P. aeruginosa	5	50	5	50
S. aureus	5	50	5	50
S. typhi	5	50	5	50

The *Ocimumgratissimum* extract includes additional phytochemical elements than the *Prunusamygdalinus* extract. Alkaloid, phenolics, and glycosides additionally steroids were existent in the *Ocimumgratissimum* extract but absent in *Prunusamygdalinus* extract. Both extracts contained saponins, flavonoids and triterpenes (Table 6).

Table 6. Phytochemical components of *Ocimumgratissimum* and *Prunusamygdalinus* leaf extracts

Plant Phytochemicals	*Ocimumgratissium extract*	*Prunusamygdalinus extract*
Alkaloids	+	-
Tannis	-	-
Phenolics	+	-
Glycosides	+	-
Saponins	+	+
Flavonoids	+	+
Steriods	+	-
Phlobatanin	-	-
Triterpenes	+	+

KEY: + = Present, - = Absent (not present)

The consequences of sensitivity analysis of zones of inhibitions created displayed that the tests organisms were susceptible to the leaf extracts of *O. gratissimum* also *P. amygdalinus* at concentrations of 500mg/ml, 50mg/ml and 5mg/ml respectively. In the leaf extract of *O. gratissimum, E. coli* and *S. typhi* showed susceptibility with clear zones of 8 ± 4.00 and 6 2.00 mean diameter of 5 2.00. *S.aureus* at a concnentration of 5mg/ml which had a mean diameter of 5 2.00.*P. aeruginosa* was not susceptible at this peptidoglycan layer of the cell wall is relatively thin which permitted easy diffusion of extracts being a gram negative The leaf extracts demonstrated higher clear zones of inhibition than the commercial antibiotic discs creating more susceptible pathogens to be injured due to as the distance elevated, the concentration dropped. The growth concentration (MBC) was 50mg/ml which defines that the extracts killed the organisms at the concentration. A cidal (9). From the results of the phytochemical screening of the leaf extracts, *O. gratissimum* contained alkaloids, , glycosides, saponins, steroids, flavonoids and triterpenes while *P.amygdalinus* contained saponins, flavonoids additionally triterpenes. The existence of these active elements could be responsible for the antimicrobial action of the extracts converse the test organisms, for instance saponins are better and they protect the plant against concentration. *S. typhi* displayed the best potentiality to the extract of *P. aeruginosa* than the difference organisms with an average diameter of 16.60 3.40 at a concentration of 500mg/ml. from the consequences superior, it was viewed that the leaf extracts of *O. gratissimum* also *P. amygdalinus* were most strong against *S. typhi* than the other organisms. This could be as an effect of the cell structure; the

bacterium.

of entire the experiment organisms was restricted by the leaf extracts *of O. gratissimum* as well as *P.amygdalinus* at a minimum inhibitory concentration (MIC) of 5mg/ml while the minimum bactericidal factor kills a pathogen at levels only 2-4 MIC whereas a static factor kills at much higher concentration phenolics

microbes and fungi (10).

4. CONCLUSION

The results of this study revealed that the leaf extracts of *Ocimumgratissium* and *Prunusamygdalinus* have inhibitory properties on all the test organisms. The leaf

extracts displayed the existence of lively factors which could be responsible for the antibacterial movement of the extracts converse the test organisms. The leaves extracts have bactericidal causes on complete the test organisms. The leaf extracts of the therapeutic plants applied in this study could be a source of critical chemical factors to be applied to treat bacterial disorders also infections caused by *E. coli, S. aureus, S. typhi* and *P. aeruginosa.*

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CONFLICT OF INTEREST

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