EVALUATION OF ANTIMICROBIAL POTENTIAL AND PHYTOCHEMICAL SCREENING OF *CITRUS LEMON*

Amengialue, O. O.[1], Oviasogie, E. F.[2], Omoigberale, M. N. O.[3], Omoregie, B. O.[2] & Okoro, T. C.

[1] Department of Biological Sciences, College of Natural and Applied Sciences, Wellspring University, Benin City, Edo State, **NIGERIA**

[2] Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, **NIGERIA**

[3] Department of Microbiology, Faculty of Natural Sciences, Ambrose Alli University, Ekpoma Edo State, **NIGERIA**

ABSTRACT

With increasing rate of microbial resistance to synthetic antimicrobial agents/drugs, efforts are being made to explore natural plants for effective antimicrobial activity. Hence, the antimicrobial properties and the phytochemical composition of *citrus lemon* were evaluated in this study. The antimicrobial activity of cold distilled water, ethanol and methanol extracts of *citrus lemon* peels and seed were tested against some bacteria and fungi pathogens; *Staphylococcus aureus, Streptococcus pyogens, Bacillus subtillis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Penicillium, notatum, Aspergillus niger* and *Fuserium oxysporum*. Methanol extract of lemon seed generally exhibited maximum zone of inhibition with the highest (22.0mg/ml) against *Staphylococcus aureus* compared to lemon peel with maximum zone of inhibition (15.3mg/ml). Meanwhile, there was no zone of inhibition of any of the extracts against all the test fungi isolates. Both ethanol and methanol seed and extract had minimum inhibitory concentration range of 3.125mg/ml – 50.0mg/ml against all test bacteria isolates. Methanol peel and seed extract had a minimum bacteriocidal concentration range of 6.25mg/ml – 12.5mg/ml and 3.13mg/ml – 12.5mg/ml respectively for all bacteria isolates. The phytochemical screening of *citrus lemon* revealed a qualitative composition of saponins, tannin, flavonoid, glycoside and steroid in both lemon peel and seed. The result findings therefore revealed *citrus lemon* to have a better antibacterial potential than antifungal potential with the seed extract having a more efficient effect compared to the peel.

Keywords: Antimicrobial, extract, phytochemical, methanol, *Staphylococcus.*

INTRODUCTION

The medical use of antibiotics for the management of diseases and infections has undoubtedly recorded significant successes through their static and cidal effects. However, their uses have been associated with limitations like microbial‑ antibiotics resistance and antibiotics side effect. Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs which are utilized as therapeutic agents (Gislene *et. al*., 2000). In the quest of surmounting the challenges of microbial-antibiotics resistance and antibiotics effects, there has been high need for scientific research in seeking a more effective, efficient and less expensive antibiotics from natural sources over the commercially synthetic antibiotics.

For a very long period of times, plants have been a valuable source of natural products for maintaining human health. The use of plant extracts and phytochemicals, both with known

antimicrobial properties, can be of great significance in therapeutic treatments (Seenivasati *et. al*., 2006). Many plants have been used because of their antimicrobial traits which are due to compounds synthesized in the secondary metabolism of the plant. India has a rich tradition in use of medicinal plants to develop drugs.

According to World Health Organization (WHO), any plant which contain substances that can be used for therapeutic purpose or which are precursor of chemo pharmaceuticals, semi synthetic, new drugs is referred to as medicinal plant. Medicinal plant would be the best source to obtain variety of drugs as the phytochemicals are more specific. Phytochemical offer unique platform for structural diversity and biological functionality which is indispensable for drug discovery. These products are known by their active substances e.g. the phenobic compounds which are part of the essential oils, as well as tannin (Tyagi and Malik, 2010).

Citrus limon belongs to *Rutaceae* family; its common name is lemon and this originated from South East Asia, probably in India or Southern China. Lemon is a pale yellow, elliptically shaped berry fruit. Citrus fruit, in general contain sugar, polysaccharide, organic-acid, lipids, carotenoids, vitamins, minerals, flavonoids, bitter lemonoids and volatile compounds. Lemon is a good source of potassium, calcium & vitamin *C. Limon* or lime juice have been reported to exhibit antimicrobial activity against *Vibrio cholera* (Hiroyuki *et. al*., 2006). Consequently, this study was aimed at comparatively investigating the antimicrobial potentials of extracts of *Citrus lemon* peel and seed parts, and to screen for the phytochemical composition responsible for their antimicrobial potentials.

MATERIALS AND METHODS

Sample Collection

Fresh sample of *Citrus Limón* was purchased from a local market (Oba Market) in Benin City and transported in sterile polyethylene bag to the laboratory immediately to obtain the peels and seeds used in this study.

Test Microorganism

Bacterial and fungal cultures used in this study were obtained from University of Benin Teaching Hospital (UBTH). The bacterial cultures were *Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Escherichia coli* while the fungal cultures used were; *Aspergillus niger, Penicillium notatum* and *fusarium oxysporum.*

Preparation Of Plant Extracts

The peel and seed samples were sun-dry and grinded to powder form. Ten grams (10g) of each powder sample were measured in 90ml of distilled water, ethanol, and methanol in three different conical flasks separately, covered with cotton wrapped with aluminium foil and content was allowed to stay for 5 days before filtration using Whatmann No.1 filter paper. Filtrates were concentrated to paste-like form using the steam bath at 60^0C. Concentrates were dissolved with dimethyl sulfoxide and stored in sterile container and kept in the refrigerator at 4^0C until needed for use.

Antimicrobial Activity

Antimicrobial activity was performed to evaluate the antimicrobial properties of each extracts employing the agar well diffusion method as described by Ahmad and Beg, (2001). Nutrient agar plates were prepared for all extracts, 0.5ml inoculum of each selected bacterium was uniformly spreaded on agar plates with the help of glass spreader. After five minutes, three wells of 4mm in diameter were bored with the help of borer and the wells were filled with each extracts. The plates were incubated at 37°C for 24 hours and observed zone of inhibition. Similarly, potato dextrose agar plates were prepared for all extracts, 0.5ml inoculum of each selected fungus was uniformly spreaded on the PDA plates with the help of glass spreader. After five minutes, three wells of 4mm diameter were bored with the help of borer and the wells were filled with each extracts. The plates were incubated at room temperature for 24-48 hours and observed zone of inhibition.

Minimum Inhibitory Concentration (MIC)

The broth dilution method as earlier described by Bailey and Evelyn (1970) was employed in the determination of the minimum inhibitory concentration (MIC) of the extracts. To each test tube, 1.5ml of nutrient broth was dispensed. To the first test tube, was added 1.5ml of extract and serially diluted out to various concentrations ranging from 50 - 3.125mg/ml. A loop full of the test bacteria culture was inoculated into each of the test tubes and incubated at 37^0C for 24 hours and observed for growth in the form of turbidity. Similarly, this was also done for all fungal isolates using potato dextrose broth and incubated at room temperature for 24 – 48 hours.

Minimum Bactericidal And Fungicidal Concentration (MBC and MFC)

The bactericidal and fungicidal concentration of the extracts were determined with the absence of growth of bacteria and fungi colonies on plates. For MBC, 20µl of MIC tube content were cultured on nutrient agar plates and incubate at 37^0C for 24 hours. Thereafter, plates were observed for bacteria colonies. For MFC, 20µl of MIC tube content were cultured on potato dextrose agar plates and incubate at room temperature for 24 - 48 hours. Thereafter, plates were observed for fungi colonies.

Phytochemical Analysis

Steroids: 1ml of powder sample filtrate was added to 10% concentrated H_2SO_4 and was observed for green colour.
Tannins: About 0.5g each of dried powder sample was boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.
Glycosides*:* 4ml of sample extract was mixed with 2ml of lacial acetic acid containing 1-2 drops of 2% solution of $FeCl_2$. Mixture was then poured into another test tube containing 2ml of concentrated H_2SO_4. A brown ring at the interphase indicated the presence of glycoside.
Saponins: 2g of the powder sample was boiled in 20ml of distilled water. The formation of the stable foam was as an indication for the presence of saponin.
Flavonoids: Sample extract was mixed with 2ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids.

RESULTS

The results of this research work are shown in different tables. Table 1 shows the antimicrobial activities of seed extracts in 100mg/ml while table 2 also shows the statistical analysis of the antimicrobial activities of the peel extracts in 100mg/ml. Table 3, 4 and 5 shows the minimum inhibitory concentration of Aqueous, Ethanol and Methanol of seed extracts respectively, ranging from 50mg/ml to 3.125mg/ml. While table 6, 7 and 8 also shows the minimum inhibitory concentration of Aqueous, Ethanol and Methanol of peel extracts respectively, ranging from 50mg/ml to 3.125mg/ml. Table 9 shows the minimum Bactericidal and minimum Fungicidal concentration of seed extracts, (i.e. showing the level of concentration at which each of the isolates will have no growth in the seed extracts of the Citrus Limon). Table 10 shows the minimum Bactericidal and minimum Fungicidal concentration of peel extracts, (i.e. the level at which each of the isolates will a clear view in the test tubes containing the peel extracts of the Citrus Limon). Table 11 shows the qualitative phytochemical composition of seed and peel extracts respective.

Table 1: Antimicrobial Activity Of Seed Extracts At 100 mg/ml

Test Organisms	Zone of inhibition in mm							
	Aqueous			Ethanol			Methanol	
	MEAN	SE		MEAN	SE		MEAN	SE
Staphylococcus aureus	8.0	0.58		19.0	0.58		22.0	0.58
Streptococcus pyogenes	0.0	0.00		12.7	0.88		15.0	0.58
Bacillus subtilis	0.0	0.00		16.0	0.58		18.0	0.58
Escherichia coli	5.3	0.88		22.3	0.88		24.0	0.58
Pseudomonas aeruginosa	0.0	0.00		16.0	0.58		18.0	0.58
Klebsiella pneumoniae	0.0	0.00		11.7	0.88		15.0	0.58
Penicillium notatum	0.0	0.00		0.0	0.0		0.0	0.0
Aspergillus niger	0.0	0.00		0.0	0.0		0.0	0.0
Fusarium oxysporum	0.0	0.00		0.0	0.0		0.0	0.0

Table 2: Antimicrobial Activity Of Peel Extracts At 100mg/ml

Test Organisms	Zone of inhibition in mm								
	Aqueous			Ethanol			Methanol		
	MEAN	SE		MEAN	SE		MEAN	SE	
Staphylococcus aureus	0.0	0.0		12.7	0.88		15.3	0.88	
Streptococcus pyogenes	0.0	0.0		8.0	0.58		11.3	0.88	
Bacillus subtilis	0.0	0.0		11.0	0.58		12.3	0.88	
Escherichia coli	0.0	0.0		12.3	1.20		15.0	0.58	
Pseudomonas aeruginosa	0.0	0.0		9.0	0.58		11.0	0.58	
Klebsiella pneumoniae	0.0	0.0		6.0	0.58		7.7	0.88	
Penicillium notatum	0.0	0.0		0.0	0.0		0.0	0.0	
Aspergillus niger	0.0	0.0		0.0	0.0		0.0	0.0	
Fusarium oxysporum	0.0	0.0		0.0	0.0		0.0	0.0	

Table 3: Minimum Inhibitory Concentrations (MIC) Of Aqueous Seed Extract In mg/ml

Test Organisms	Aqueous seed extract				
	50.0 mg/ml	25.0mg/ml	12.5 mg/ml	6.25 mg/ml	3.125 mg/ml
Staphylococcus aureus	-	+	+	+	+
Streptococcus pyogenes	Nil	Nil	Nil	Nil	Nil
Bacillus subtilis	Nil	Nil	Nil	Nil	Nil
Escherichia coli	+	+	+	+	+
Pseudomonas aeruginosa	+	+	+	+	+
Klebsiella pneumoniae	+	+	+	+	+
Penicillium notatum	+	+	+	+	+
Aspergillus niger	+	+	+	+	+
Fusarium oxysporum	+	+	+	+	+

KEY: + = Presence of growth, - = Absence of growth

Table 4: Minimum Inhibitory Concentrations (MIC) Of Ethanol Seed Extract In mg/ml

Test Organisms	Ethanol seed extract				
	50.0 mg/ml	25.0mg/ml	12.5 mg/ml	6.25 mg/ml	3.125 mg/ml
Staphylococcus aureus	-	-	-	-	-
Streptococcus pyogenes	-	-	-	-	-
Bacillus subtilis	-	-	-	-	-
Escherichia coli	-	-	-	-	-
Pseudomonas aeruginosa	-	-	-	-	-
Klebsiella pneumoniae	-	-	-	-	-
Penicillium notatum	+	+	+	+	+
Aspergillus niger	+	+	+	+	+
Fusarium oxysporum	+	+	+	+	+

KEY: + = Presence of growth, - = Absence of growth

Table 5: Minimum Inhibitory Concentrations (MIC) Of Methanol Seed Extract In mg/ml

Test Organisms	Methanol seed extract				
	50.0 mg/ml	25.0mg/ml	12.5 mg/ml	6.25 mg/ml	3.125 mg/ml
Staphylococcus aureus	-	-	-	-	-
Streptococcus pyogenes	-	-	-	-	-
Bacillus subtilis	-	-	-	-	-
Escherichia coli	-	-	-	-	-
Pseudomonas aeruginosa	-	-	-	-	-
Klebsiella pneumoniae	-	-	-	-	-

Penicillium notatum	+	+	+	+	+
Aspergillus niger	+	+	+	+	+
Fusarium oxysporum	+	+	+	+	+

KEY: + = Presence of growth, - = Absence of growth

Table 6: Minimum Inhibitory Concentration (MIC) Of Aqueous Peel Extract In mg/ml

Test Organisms	**Aqueous peel extract**				
	50.0 mg/ml	25.0mg/ml	12.5 mg/ml	6.25 mg/ml	3.125 mg/ml
Staphylococcus aureus	+	+	+	+	+
Streptococcus pyogenes	+	+	+	+	+
Bacillus subtilis	+	+	+	+	+
Escherichia coli	+	+	+	+	+
Pseudomonas aeruginosa	+	+	+	+	+
Klebsiella pneumoniae	+	+	+	+	+
Penicillium notatum	+	+	+	+	+
Aspergillus niger	+	+	+	+	+
Fusarium oxysporum	+	+	+	+	+

KEY: + = Presence of growth, - = Absence of growth

Table 7: Minimum Inhibitory Concentration (MIC) Of Ethanol Peel Extract In mg/ml

Test Organisms	**Ethanol peel extract**				
	50.0 mg/ml	25.0mg/ml	12.5 mg/ml	6.25 mg/ml	3.125 mg/ml
Staphylococcus aureus	-	-	-	-	-
Streptococcus pyogenes	-	-	-	-	-
Bacillus subtilis	-	-	-	-	-
Escherichia coli	-	-	-	-	-
Pseudomonas aeruginosa	-	-	-	-	+
Klebsiella pneumonia	-	-	-	+	+
Penicillium notatum	+	+	+	+	+
Aspergillus niger	+	+	+	+	+
Fusarium oxysporum	+	+	+	+	+

KEY: + = Presence of growth, - = Absence of growth

Table 8: Minimum Inhibitory Concentration (MIC) Of Methanol Peel Extract In mg/ml

Test Organisms	Methanol seed extract				
	50.0 mg/ml	25.0mg/ml	12.5 mg/ml	6.25 mg/ml	3.125 mg/ml
Staphylococcus aureus	-	-	-	-	-
Streptococcus pyogenes	-	-	-	-	-
Bacillus subtilis	-	-	-	-	-
Escherichia coli	-	-	-	-	-
Pseudomonas aeruginosa	-	-	-	-	+
Klebsiella pneumonia	-	-	-	+	+
Penicillium notatum	+	+	+	+	+
Aspergillus niger	+	+	+	+	+
Fusarium oxysporum	+	+	+	+	+

KEY: + = Presence of growth, - = Absence of growth

Table 9: Minimum Bactericidal Concentration And Minimum Fungicidal Concentration Of Seed Extracts In mg/ml

Test organisms	Seed extracts		
	Aqueous (mg/ml)	Ethanol (mg/ml)	Methanol (mg/ml)
Staphylococcus aureus	50	6.25	3.125
Streptococcus pyogenes	Nil	6.25	6.25
Bacillus subtilis	Nil	6.25	6.25
Escherichia coli	Nil	6.25	3.125
Pseudomonas aeruginosa	Nil	12.5	12.5
Klebsiella pneumonia	Nil	12.5	12.5
Penicillium notatum	Nil	Nil	Nil
Aspergillus niger	Nil	Nil	Nil
Fusarium oxysporum	Nil	Nil	Nil

Table 10: Minimum Bactericidal Concentration And Minimum Fungicidal Concentration of Peel extracts in mg/ml

Test organisms	Peel extracts		
	Aqueous (mg/ml)	Ethanol (mg/ml)	Methanol (mg/ml)
Staphylococcus aureus	Nil	6.25	6.25
Streptococcus pyogenes	Nil	6.25	6.25
Bacillus subtilis	Nil	6.25	6.25
Escherichia coli	Nil	6.25	6.25
Pseudomonas aeruginosa	Nil	12.5	12.5
Klebsiella pneumonia	Nil	12.5	12.5
Penicillium notatum	Nil	Nil	Nil
Aspergillus niger	Nil	Nil	Nil
Fusarium oxysporum	Nil	Nil	Nil

Table 11: Qualitative Phytochemical Compositions

Parameters	Seed	Peel
Saponin	+	+
Tannin	+	+
Flavonoid	+	+
Glycoside	+	-
Steroid	+	+

KEY: + = Present, - = Absent

DISCUSSION

As plants remains a natural, more economical, efficient and readily available sources of drugs over the conventional synthetic drugs, their therapeutic properties is still very much under maximise. As such, there is a continuous need to fully explore the plant kingdom for their medicinal values through scientific research. Findings of this research has further showed the extent antimicrobial properties of *Citrus lemon* peel and seed as tested against three selected isolates each of gram positive bacteria, gram negative bacteria and fungi; and also the bioactive compounds of *Citrus lemon* peel and seed. The antibacterial activity of *Citrus lemon* seed extract was generally observed to be higher than that of the peel extract. This is possibly due to that fact that more of the bioactive compounds of the plant are more contained in the seed compared to the peel. Also, methanol extract of the *Citrus lemon* seed was seen to have the highest antibacterial activity of 24.0mm against *Escherichia coli*. This finding agrees with the work reported by Pandey *et. al*., (2011). While aqueous extract of both *Citrus lemon* seed and peel was generally seen to have recorded the least antibacterial activity against the test bacteria isolates, it could be said that water as a polar solvent, is a poor extracting solvent of bioactive phytochemical compounds. However, none of the extracting solvents used recorded any antifungal activity against the test fungi isolates.

Following the minimum inhibitory concentration of the various extract at 50.0mg/ml, 25.0mg/ml, 12.5mg/ml, 6.25mg/ml and 3.125mg/ml, it was observed that both ethanol and methanol seed extracts had MIC of 3.125mg/ml for all test bacteria isolates. Similarly, both ethanol and methanol peel extracts had MIC of 3.125mg/ml for all test bacteria isolates with the exception of *Pseudomonas aeruginosa* and *Klebsiella pneumonia* which had their MIC at 6.25mg/ml and 12.5mg/ml respectively. However, there was no MIC of the extracts of the seed and peel recorded against any of the fungi isolates.

While the minimum bactericidal concentration (MBC) of ethanol seed extract ranged from 6.25 – 12.5mg/ml, that of methanol seed extract ranged from 3.125 – 12.5mg/ml. Also, both ethanol and methanol extract of peel had MBC range of 6.25 – 12.5mg/ml. Meanwhile there was no minimum fungicidal concentration of the extracts recorded against the fungi isolates.
It has been widely observed and accepted that the medicinal value of plants lies in the bioactive phytocomponents present in the plants (Veermuthu *et. al.,* 2006). The qualitative phytochemical screening of the *Citrus lemon* peel and seed revealed the presence of saponin, tannin, flavonoid, glycoside and steroid composition in the seed and same (but with the absence of glycoside) in the peel of *Citrus lemon*. These phytochemical compounds are believed to be the bioactive ingredients of *Citrus lemon* responsible for its antibacterial activity.

REFERENCES

Ahmad, I. and Beg, A. J. (2001). Antimicrobial and phytochemical study on 45 Idian Medicinalplants against multidrug resistant human pathogens. *J. Enthopharmacol.* 73:113 - 123

Bailey, R. W., and Evelyn, B. S., (1970). The C. V. Moshy Coy. St. Louis. *Diagnostic Microbiology* Pp 120-160, 289-306.

Gislene *et. al.*, (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria. *Braz. J. Microbiol.* 31: 247-256.

Hiroyuki *et. al.*, (2006). Antimicrobial activity of citrus fruit juice against *Vibrio* species. *IJCSNS*. 52(2):157 – 160.

Pandey, A., Kaushik, A. and Tiwari, S. K. (2011). Evaluation of antimicrobial activity and phytochemical analysis of *Citrus limon*. *J. Pharmaceutical Biomed. Sci.* 13(17):1-7

Seenivasan, P., J. Manickkam and I. Savarimuthu, (2006). *In vitro* antibacterial activity of some plant essential oils. *BMC Complem. Altern. M.* 6: 39.

Tyagi, A.K. and A. Malik, 2010. Liquid and vapour-phase antifungal activities of selected essential oils against candida albicans. *BMC Complem. Altern. Med.* 10: 65.

Veermuthu D, Muniappan A, Savarimuthu I (2006). Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamilnadu, India. *BMC Complem. Altern. Med.*, 6: 35.