WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 8, 132-141. Research Article ISSN 2277– 7105

IN-VITRO ANTIBACTERIAL ACTIVITY OF CRUDE CITRUS EXTRACTS ON STAPHYLOCOCCUS AUREUS AND ESCHERICHIA COLI ISOLATED FROM THE HUMAN SKIN AND STOOL RESPECTIVELY.

***[1]Ezeadila Joachim O., [1]Orubu Valerie A., [2]Ejike Onyibo S. and [1]Udemezue Onyekachukwu I.**

[1]Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, P.M.B. 5025, Awka, Anambra State, Nigeria.

[2]Department of Pathology, Federal Teaching Hospital Abakaliki, P.M.B. 102, Abakaliki, Ebonyi State, Nigeria.

Article Received on
03 June 2016,

Revised on 24 June 2016,
Accepted on 15 July 2016

DOI: 10.20959/wjpr20168-6668

***Corresponding Author**
Ezeadila Joachim O.
Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, P.M.B. 5025, Awka, Anambra State, Nigeria.

ABSTRACT

Medicinal plants play very important roles in herbal medicine. As antibiotics resistant bacteria are frequently encountered in the last two decades or more, medicinal plants are being exploited for products that could combat these superbugs. This study focuses on the in-vitro antibacterial activity of crude Citrus extracts on *Staphylococcus aureus* and *Escherichia coli* isolated from human skin and stool respectively. A total of 50 skin swab and rectal swab samples were randomly collected from some students of Nnamdi Azikiwe University, Awka and processed by culturing them on suitable media. The antibacterial activities of crude extracts of different Citrus fruits were determined using the disc diffusion method. The results obtained confirmed antibacterial activity of extracts of Citrus fruits with inhibition zone diameters comparing favourably to those of the commercial antibiotic discs. The highest zone of inhibition (19mm) was observed with the crude extract of *Citrus limon* and followed by *Citrus aurantifolia* (17mm) on *Staphylococcus aureus*, with a minimal effect on *Escherichia coli* producing zone of 11mm and 13mm respectively. The crude extract of *Citrus paradisi* produced a zone of inhibition of 11mm on *Staphylococcus aureus*, whereas *Escherichia coli* was resistant to it. The two organisms were both resistant to the crude extracts of *Citrus sinensis*. This study suggests that Citrus extracts especially those of *Citrus limon* and *Citrus*

aurantifolia show great antibiotic potential against *Staphylococcus aureus* and *Escherichia coli* and thus promise an efficacious substitute of synthetic antibiotics, or evaluated for possible synergism when both are co-administered.

KEY WORDS: *Escherichia coli*, *Staphylococcus aureus*, *Citrus* spp, antibacterial activity.

INTRODUCTION

The increase in bacterial antibiotic resistance is largely due to the injudicious use of antibiotics in medicine, agriculture and improper disposal of antibiotics-laden waste from pharmaceutical industries. While the former is understood as abuse or misuse of prescription antibiotics as in undertreatment or overtreatment of infections, the latter denotes inappropriate disposal of industrial waste rich in antibiotics into streams and water bodies. From these sources these pharmacologic agents end up in human eventually selecting a population of superbugs whose progenies grow luxuriantly in the presence of therapeutic concentrations of these antibiotics. The problem is further worsened by paucity of new antibiotics in the fight to contain the ever expanding pool of resistant bacteria strains. Increasing treatment failures in hitherto minor infections and the emergence of multi-drug resistant bacteria necessitated the search for novel phyto-pharmacologic agents with reproducible efficacy in handling such infections.

Escherichia coli, Salmonella paratyphi B, and *Shigella sonnei* are the major bacterial aetiologic agents of gastrointestinal infections. Enteric bacteria belong to the family enterobacteriaceae, which are large group of Gram negative, peritricously flagellated straight rods with simple nutritional requirements, and they grow best under aerobic conditions but also ferment carbohydrates by an anaerobic pathway. Enteric pathogenic bacteria have developed resistance against existing antibiotics due to indiscriminate misuse and abuse of antimicrobial agents. The emergence of *Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus* and many other β -lactamase producers has become major therapeutic problem.[1] *E. coli* and other facultative anaerobes constitute about 0.1% of gut flora[2] and fecal-oral transmission is the major route through which pathogenic strains of the bacterium cause disease.

The problem of resistance of microorganisms to antimicrobial drugs is one of the world's current challenges. On the one hand, plant-based antimicrobials are attractive as they are often devoid of the many side effects associated with synthetic antimicrobials. On the other

hand, such antimicrobials usually have a narrow spectrum of activity. The search for new antimicrobial from natural sources is still ongoing.[3]

Antibacterial properties of plant extract have been a hot topic for researchers. Besides plants, fruits also have been studied by researchers for the presence of pharmacoactive compounds commonly referred as phytochemicals which includes, but not limited to tannins, carotenoids, polyphenols and anthocyanins. The fruit extracts exhibit significant antibacterial effect, the pharmacologic activity being associated with mineral content and biologically active constituents.[4]

An estimated 100 million tons of citrus fruits are produced annually; thereby making the citrus family the largest contributor to the world's fruit production.[5] Citrus is one of the largest plant species known, consisting of 40 species that are distributed around the world. Among the most commonly consumed citrus fruit are tangerine, lime, lemon, and grape.[6]

Citrus juices are consumed majorly because of their nutritional value and special flavor. Fruit juice consumption is beneficial for the maintenance of good health and prevention of diseases. The positive health benefits of juices have been ascribed in part to vitamin C (ascorbic acid), the major vitamin found in fruits and vegetables.[7] Citrus fruit intake is associated with a reduced risk of stomach cancer.[8] Citrus fruits are also known to contain bioactive compounds such as phenolics, flavonoids, vitamins, and essential oils which are believed to be responsible for a range of protective health benefits including antioxidative, anti-inflammatory, antitumor, and antimicrobial activities.[9];[6]

The aim of this research is to determine the in-vitro antibacterial activity of some citrus fruit juices on *Staphylococcus aureus* and *Escherichia coli* isolated from the human skin and stool respectively.

MATERIALS AND METHOD

Sample Collection

Fresh and healthy sweet oranges, lime, lemon and grapes were sourced from the local market (Eke-Awka) in Awka, Anambra state, Nigeria. The fruits were identified by a botanist Mr. Chijioke, Maxwell at the Department of Botany, Nnamdi Azikiwe University, Awka. Confirmation of taxonomic identity of fruits was achieved by comparison with voucher specimens kept at the herbarium of the Department of Botany, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

Extraction of Orange extract

The fresh and healthy oranges were washed with water and the surfaces disinfected with 70% alcohol. They were peeled aseptically with sterile knife, cut into halves and squeezed to get the crude extract. The extract was then filtered into a sterile conical flask.

Source of Test Organisms

The test organisms (*Staphylococcus aureus* and *Escherichia coli*) were isolated from skin swab and rectal swab samples of some students of Nnamdi Azikiwe University, Awka, Anambra State, Nigeria. A total of fifty (50) skin swab and rectal swab samples were collected from the students and labeled appropriately. The samples were placed in nylon bags and transported to the Departmental laboratory of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka for analysis.

Plating Technique for the Isolation of Organisms

In the laboratory, the swab sticks were smeared separately over the surface of already prepared plates of Nutrient agar and MacConkey agar and incubated for 24 hours at 37^{o}C. After incubation, the colonies were observed and subcultured onto secondary plates and also incubated to obtain pure cultures, after which their morphology was recorded. The pure cultures were then stored on agar slants in Bijou bottles to preserve the isolates for further use.

Characterization and Identification of the Isolates

The identification of the isolates was done by Gram staining and the use of other biochemical tests.[10] Growth on eosin methylene blue agar (for the production of green metallic sheen) and Mannitol salt agar (to determine if the organism will utilize mannitol and produce yellow colonies) was also used to respectively identify *Escherichia coli* and *Staphylococcus aureus*.

Preparation of Discs for Sensitivity Testing

The whatman no 1 filter paper was punched out to get the discs (5mm in diameter). The discs were transferred into Bijou bottles and sterilized in the autoclave at 121^{0}C for 15 minutes. The crude citrus extracts were extracted into different sterile conical flasks. The discs were then transferred into the flask and were left in the conical flask to absorb the juice for about 20 minutes. They were taken out and placed in a glass container. Later the discs were dried in the incubator for 2 hours at 35^{0} C and kept for use.

Turbidity Standard for Inoculum Preparation

To standardize the inoculum density for the susceptibility test, barium sulphate turbidity standard, equivalent to 0.5 McFarland standards was used. One percent v/v solution of sulphuric acid was prepared by adding 1 ml of concentrated sulphuric acid to 99 ml of water and mixed well. A 1.175% w/v solution of barium chloride was prepared by dissolving 2.35g of dehydrated barium chloride in 200ml of distilled water. To make the turbidity standard, 0.5ml of the barium chloride solution was added to 1% 99.5ml sulphuric acid solution and mixed well. A portion (5ml) of the resultant turbid solution was transferred into sterile test tube and plugged with cotton wool. A 0.5ml of McFarland standard equals 1×10^{8} colony forming unit per ml (CFU/ml). Using a sterile wire loop, discrete colonies each of 24 hours pure culture of the test organisms was emulsified in 5ml of peptone water in a test tube which was also plugged with cotton wool. The turbidity of the suspension was then matched with the turbidity of the standard.

Determination of the Antibacterial Activity of Crude Citrus Extracts on Isolated Organisms.

Antimicrobial sensitivity was determined using disc diffusion technique as demonstrated by[11] Each test organism was seeded onto already prepared plates of Mueller Hinton agar. The discs impregnated with the crude citrus juice were then placed aseptically on the plates using sterile forceps. Conventional antibiotic discs (Amoxil, Ofloxacin, Rocephine, Ciprofloxacin, Gentamycin, Streptomycin, Zinnacef, Erythromycin, Perfloxacin and Septrin) for the Gram positive bacterium and those (Amoxil, Augmentin, Rocephine, Chloramphenicol, Sparflox, Ofloxacin, Septrin, Perfloxacin, Streptomycin and Tarivid) for the Gram negative bacterium was also used for the purpose of comparison. The plates were incubated at room temperature for 24 hours after which zones of inhibition were checked and measured with a meter rule.

RESULTS AND DISCUSSION

In this study, disc diffusion method was used to determine the antibacterial activity of the crude extracts of some citrus fruits against *Escherichia coli* and *Staphylococcus aureus* isolated respectively from rectal swab and skin swab samples. Some conventional antibiotics were used for comparison. The antibacterial activity of the crude extracts of the different citrus fruits on the test organisms is presented in table 1. It is observed that the crude juice of *Citrus limon (*lemon) has the highest antibacterial activity (with an inhibition zone diameter

of 19mm) followed by that of *Citrus aurantifolia (*lime) (17mm) against *Staphylococcus aureus* (Figs.1 and 2). The inhibition zone diameter of *C. limon* and *C. aurantifolia* was 11mm and 13mm against *Escherichia coli* respectively. This agrees with the work of [12] in which extracts of lemon and lime exhibited the highest antibacterial activity against tested pathogenic bacteria including *Escherichia coli* and *Staphylococcus aureus.* In this study, *Escherichia coli* and *Staphylococcus aureus* were both resistant to the extracts of *Citrus sinensis* (sweet orange). This also supports the findings of [12] where the extracts of *Citrus sinensis* showed inhibition zone diameters of 2mm and 4mm against *E. coli* and *S. aureus* respectively. Similar results have been recorded for juices of *Citrus aurantifolia* and *Citrus sinensis* against *Escherichia coli*.[13] Also, *Escherichia coli* was resistant to the crude juice of *Citrus paradisi* which agrees with the findings of.[14]

Table 1: In-vitro Antibacterial activity of crude extracts of the different *Citrus* species on *Escherichia coli* and *Staphylococcus aureus*.

Test Organisms	Inhibition Zone Diameter (mm)			
	C. sinensis	C. aurantifolia	C. limon	C. paradisi
Staphylococcus aureus	-	17	19	11
Escherichia coli	-	13	11	-

Key

- = No zone of inhibition

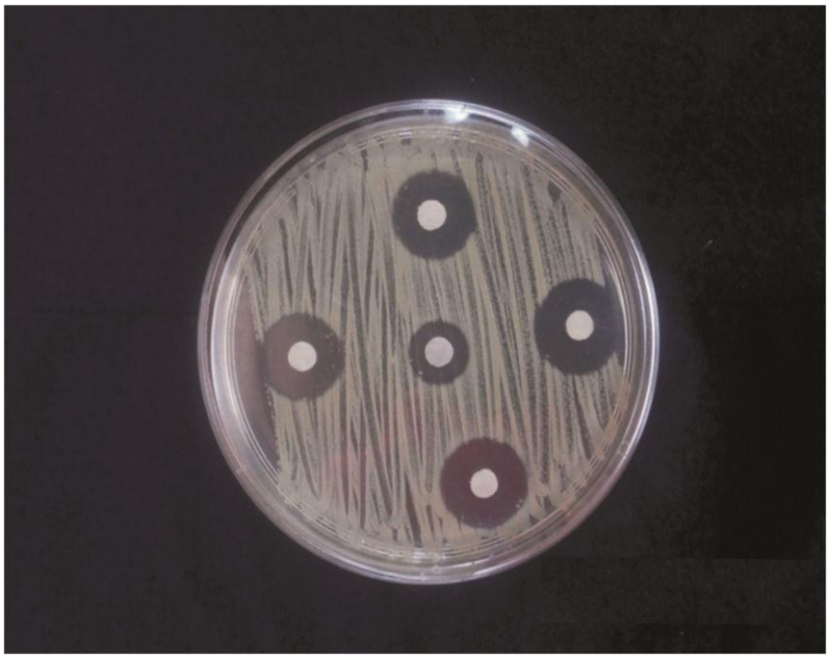

Fig 1: Zones of Inhibition Produced by juice of Citrus limon on Staphylococcus aureus

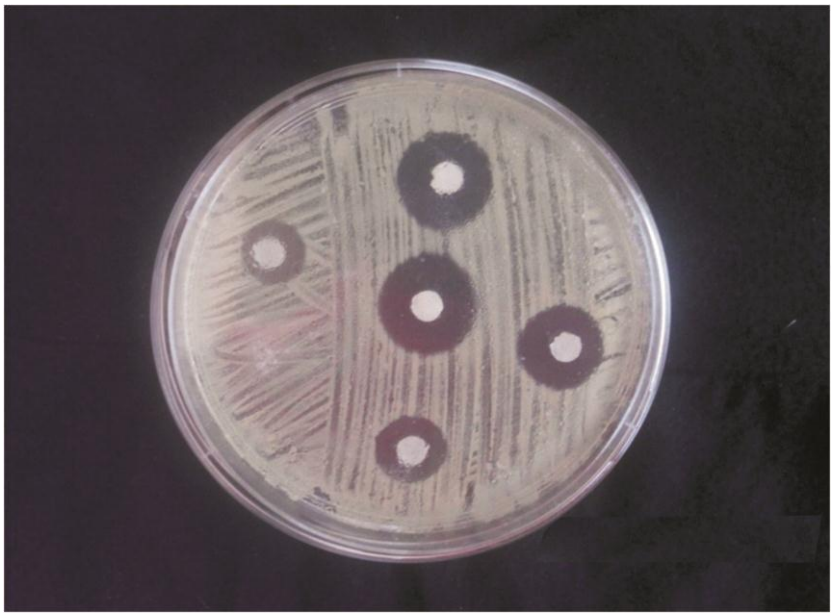

Fig 2: Zones of Inhibition Produced by juice of *Citrus aurantifolia* on *Staphylococcus aureus.*

In table 2, is presented the result of the susceptibility of the isolates against the conventional antibiotics used for comparison. From this study, it can be seen that gentamicin showed a marked antibacterial activity (20mm) against *Escherichia coli* followed by ciprofloxacin with inhibition zone diameter of 15mm against *Escherichia coli*. Similar observations have been made in which ciprofloxacin and gentamicin showed pronounced antibacterial activity against both *Escherichia coli* and *Staphylococcus aureus*.[15] Also, the findings of [16] showed 76.9% and 69.2% antibacterial effectiveness of gentamicin against Gram positive and Gram negative UTI isolates respectively. Septrin was the least effective against *Escherichia coli* followed by augmentin with inhibition zone diameters of 4mm and 6mm respectively (table 2). This doesn't agree with the work of [17] in which ciprofloxacin showed the highest inhibition zone diameter (24.5mm) followed by augmentin (22.5mm) against *E. coli*. This difference might be as a result of the fact that the strain of *E. coli* isolated in the present work is different.[18]

Septrin and pefloxacin showed the greatest activity against the Gram positive bacterium (*Staphylococcus aureus*) with inhibition zone diameter of 25mm each. This microorganism was resistant to ampiclox and amoxicillin while the inhibition zone diameters of zinnacef, rocephin, ciprofloxacin, chloramphenicol and streptomycin against this microorganism ranged between 15-21mm. This agrees with the findings of[19] in which septrin used as a

positive control drug showed an inhibition zone diameter of 25.2mm against *Staphylococcus aureus*.

Table 2: Susceptibility of the Isolates Against the Conventional Antibiotics

Isolates	Inhibition zone diameter of Antibacterial Drugs (mm)									
Gram Negative	SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX	S
Escherichia coli	4	12	9	15	10	6	20	8	14	9
Gram Positive	APX	Z	RO	CPX	AM	SXT	CN	PEF	E	S
Staphylococcus aureus	-	17	15	17	-	25	15	25	13	21

Key: SXT= Septrin, CH= Chloramphenicol, SP = Sparfloxacin, CPX = Ciprofloxacin, Am = Amoxicillin, Au = Augmentin, CN = Gentamicin, PEF = Pefloxacin, OFX = Tarivid, S = Streptomycin, APX= Ampiclox, Z= Zinnacef, RO = Rocephin, E = Erythromycin, - = No zone of inhibition.

CONCLUSION

It has been shown from the present study that the crude extract of *Citrus limon (*lemon) has the highest antibacterial activity (with an inhibition zone diameter of 19mm) followed by that of *Citrus aurantifolia (*lime) (17mm) against *Staphylococcus aureus.* The inhibition zone diameter of *C. limon* and *C. aurantifolia* was 11mm and 13mm against *Escherichia coli* respectively. Both *Escherichia coli* and *Staphylococcus aureus* were resistant to the extract of *Citrus sinensis* (sweet orange). Gentamicin and ciprofloxacin were most active against *Escherichia coli* while septrin and pefloxacin were most active against *Staphylococcus aureus.* This microorganism, however, was resistant to ampiclox and amoxicillin. The marked antibacterial activities of extracts of *Citrus limon* and *Citrus aurantifolia* against the test organisms (especially *Staphylococcus aureus*) compared favourably with those of the conventional antibiotics (septrin, pefloxacin, gentamicin and ciprofloxacin. This study reveals that Citrus extracts especially those of *Citrus limon* and *Citrus aurantifolia* have great potential antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* and thus recommends that more research be carried out with a view to extracting the pure pharmacologic agent (s) in these extracts so as to fully optimize their therapeutic use as implicated in the study.

REFERENCES

1. Bansode DS, Chavan MD. Studies on antimicrobial activity and phytochemical analysis of citrus fruit juices against selected enteric pathogens. *Int Res J Pharm*, 2012; 3(11): 122-126.

2. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, Gill SR, Nelson KE, Relman DA. Diversity of the human intestinal microbial flora. *Science*, 2005; 308 (5728): 1635–1638.
3. Parekh J, Jadeja D, Chanda S. Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity. *Turk J Biol*., 2005; 29: 203–210.
4. Khushwaha A, Singh RP, Gupta V, Singh M. Antimicrobial properties of peels of citrus fruits. *Int J Pharm Life Sc*., 2012; 2(2): 24-36.
5. Jwanny EW, El-Sayed ST, Salem AM, Mabrouk NA, Shehata AN. Fractionation, identification and biological activities of Egyptian citrus peel extracts. *Aust J Basic Appl Sc.*, 2012; 6: 34–40.
6. Karimi E, Oskoueian E, Hendra R, Oskoueian A, Jaafar HZE. Phenolic compounds characterization and biological activities of *Citrus aurantium* bloom. *Molecules*, 2012; 17: 1203–1218.
7. Rekha C, Poornima G, Manasa M, Abhipsa V, Pavithra Devi J, Vijay Kumar HT, *et al*. ascorbic acid, total phenol content and antioxidant activity of fresh juices of four ripe and unripe citrus fruits. *Chem Sc Trans*., 2012; 1: 303–310.
8. Gonzalez CA, Sala N, Rokkas T. Gastric cancer: epidemiologic asects. *Helicobacter*, 2013; 18(1): 34-38.
9. Aruoma OI, Landes B, Ramful-Baboolall D, Bourdond E, Neergheen-Bhujune V, Wagnerf K, *et al*. Functional benefits of citrus fruits in the management of diabetes. *Prev Med*., 2012; 54: S12–S16.
10. Cheesbrough M. District Laboratory Practice in Tropical Countries, Part 2, Cambridge University Press, Canbridge, UK, 2010; 40-197.
11. Cakir A, Kordali S, Zengin H, Izumi S, Hirata T. Composition and antifungal activity of essential oils isolated from *Hypericum hussopifolium* and *Hypericum heterophyllum. Flavr and Fragr J*., 2004; 19: 62-68.
12. Sameh HY, Abeer E, Sayed HA, Hanaiya AE. Chemical analyses, antibacterial activity and genetic diversity assessment of some Egyptian *Citrus* spp cultivars. *Afr J Biotech*., 2004; 13(26): 2626-2636.
13. Anwesha C, Anu C, Gopalkrishna KB. Does citrus fruit juice have antibacterial effect against enteric pathogenic bacteria? *Indian J Appl Res*., 2015; 5(5): 23-25.
14. Cvetnic Z. Antimicrobial activity of grape fruit seed and pulp ethanolic extract. *Acta Pharmceutica*, 2004; 54: 243-250.

15. Mbajiuka CS, Enya E. Asymptomatic bacteriuria among students in Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State, Nigeria. *World J Pharm Res*., 2015; 4(1): 159-167.
16. Nadia G, Talat YM, Samia A. Isolation, identification and antibiotic resistance profile of Indigenous bacterial isolates from urinary infections patients. *Pakistan J Biol Sc*., 2004; 7(21): 2051-2054.
17. Ezeadila JO, Echetabu IE, Ogu GI, Aneke FA. Isolation, identification and antibiotic sensitivity pattern of bacteria from urine samples of female students living in the hostels of Chukwuemeka Odumegwu Ojukwu University, Uli campus, Anambra State, Nigeria. *Int J Curr Microbiol and Appl Sc*., 2015; 4(12): 255-262.
18. Lukjancenko O, Wassenaar TM, Ussery DW. Comparison of 61 sequenced *Escherichia coli* genomes *Microbial Ecology*, 2010; 60(4): 708–720.
19. Ezeadila JO, Nwande MO, Ogu GI, Aneke FA. Antibacterial activity of ethyl acetate and n-hexane extracts of *Vernonia amygdalina* and *Moringa oleifera* leaves on *Staphylococcus aureus* and *Escherichia coli* isolated from urine samples. *Adv Appl Sc Res*., 2015; 6(11): 23-28.