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Effects of *Citrus Aurantifolia* Fruit Extract on Some Bacteria and Fungi

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Abstract
Antimicrobial efficacy of Citrus aurantifolia (lime) was investigated against Staphlococcus aureus, Escherichia coli and Candida albicans. Phytochemical screening of C. aurantifolia plant revealed the presence of alkaloids, saponins, flavonoids, tannins and phlobatanins. Aqueous, ethanolic, and undiluted juice extracts of C. aurantifolia was used for estimating the antimicrobial activity. Assessment concentrations of 15, 10 and 5mg/ml were used against the two bacteria and fungus by agar well diffusion method. Ciprofloxacin and Ketoconazole 10 mg/ml were used as standards. The results indicated that aqueous extract of the peel at 15 mg/ml had the highest activity on S. aureus (3.21±0.61), while the undiluted juice extract at the same concentration had the lowest activity (2.21±0.22). The ethanolic extract had the highest activity when tested against E. coli (3.00±0.11) at 15 mg/ml and undiluted juice extracts showed no activity .There was no activity of the plant on the fungus (C. albicans) when tested at different concentrations. The extracts from both the peels and juice are recommended for use in the Phytomedicare and control of S. aureus and E. coli related issues

Keywords: *Extract, Citrus aurantifolia (Lime), Antibacterial, Phytomedicine.*

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I. Introduction

Antibacterial properties of plant extracts have been a very hot topic for the researchers of the field of natural medicine. Two million people who acquired bacterial infection in United States of America (U.S.A) hospitals annually 70% of the cases involved the strains that are resistant to at least one antibacterial agent (1). The emergence of antibiotic-resistant microorganisms had swiftly reversed the advances of previous fifty years of research on antibiotics (2) and also the side effect associated with the available antibiotics has been alarming too.

C. aurantifolia is a shrubby tree, up to 5 m (16ft) with many thorns. Dwarf varieties exist which can be grown indoors during winter months and in colder climates. The trunk rarely grows straight and has many branches (3), *Citrus* species are among the native plants of Iran and the history of their cultivation dates to 4000 years ago from which time they have been widely used in ethno medicine. These species with the wide range of bioactive ingredients exert anti-infection and anti-inflammatory properties (4).

Lime seed is a relatively less expensive commodity in Nigeria and it is not scarce, hence it is economical to get extract from the seed. The extract is biodegradable and renewable, ecologically acceptable and do not contain heavy metals or toxic compound (5). Giving the alarming incidence of antibiotic resistance in bacteria and fungi of medical importance (6) there is a constant need for new and effective therapeutic agents.

Escherichia coli:- are gram-negative, facultative anaerobe, rod-shaped bacterium of the genus *Escherichia* that is commonly found in the lower intestine of worm-blooded organisms. Most *E. coli* strains are harmless but some serotypes may cause serious food poisoning in their hosts (7). The bacteria *E. coli* and other facultative anaerobes constitute about 0.1% of gut flora and fecal-oral transmission is the major route through which pathogenic strains of the bacterium cause disease (8).

*Staphlococcus aureus***:-** *S. aureus* is a Gram-positive coccal bacterium that is a member of the Firmicutes, and frequently found in the human respiratory tract and on the skin. It is positive for catalase and nitrate reduction. The organism appears as grape-like clusters when viewed through a microscope, with large, round, golden-yellow colonies, often with hemolysis, when grown on blood agar plates (9). Reproduction is asexually by binary fission. The two resulting daughter cells do not fully separate and remain attached to one another so the cells are observed in clusters (10).

Candida albicans: is a diploid fungus that grows both as yeast and filamentous cells and a causal agent of opportunistic oral and genital infections in humans (11). It is a commensal and a constituent of the normal gut flora comprising microorganisms that live in the human mouth and gastrointestinal tract. It lives in

80% of the human population without causing harmful effects, although overgrowth of the fungus results in candidiasis (candidosis). Candidiasis is often observed in immunocompromised individuals such as HIV-infected patients. A common form of candidiasis restricted to the mucosal membranes in mouth or vagina is thrush, usually easily cured in people who are not immunocompromised. Higher prevalence of colonization of *C. albicans* was reported in young individuals with tongue-piercing, in comparison to un-pierced matched individuals (12). To infect host tissue, the usual unicellular yeast-like form of *C. albicans* reacts to environmental cues and switches to become an invasive, multicellular filamentous form, a phenomenon called dimorphism (13).

II. Materials And Methods

The bacterial strains used in this study were one Gram-negative (*E. coli*) and one Gram-positive (*S. aureus*) and the fungi used is (*C. albicans*). The test organisms were obtained from Microbiology laboratory, Usman Danfodio University Sokoto. They were re-identified using standard procedures and stored in a nutrient agar slant at 4^0 C (for further analysis). Fresh and healthy fruits of unripe lime (*C. aurantifolia*) were bought from nearby onchard in Jega Local Government Area of Kebbi State. They were identified according to the taxonomical classification (with Vouchers number 285. A) by a Botanist Dr. Dharmendra Sighn in the Department of Biological Science (Botany Unit) Kebbi State Universty of Science and Technology Aliero. Kebbi State, Nigeria.

Extract A (Aqueous extraction): 100ml of sterile distilled hot water was added to the conical flask A, the flask was then plugged with rubber cork and was allowed to soak at room temperature for 72 hours with agitation for every 24 hours using sterile glass rod, then filtered using sterile filter paper after the 72 hours

Extract B (Ethanolic Extraction): 100 ml of ethanol was added to the conical flask B, plugged with rubber cork and allowed to soak at room temperate for 72 hours with agitation at every 24 hours using sterile glass rod and filtered using filter paper after 72 hours.

Extract C (Juice extract): 25 set of unripe lime fruits were washed with sterile water then cut open with sterile knife and the juice was aseptically squeezed out into a sterile conical flask and then filtered using sterile filter paper to remove seed then plugged with rubber cork. All the extract A, B and C were stored at 4^0 C until required for use.

Preliminary phytochemical screening: The extracts were analyzed for the presence of various phytoconstituents like flavonoids, glycosides, reducing sugars, phlobatannins, saponins and tannins according to standard methods. Antibiogram analysis method was adopted to evaluate the antimicrobial properties of *C. aurantifolia* plant extracts with the help of well diffusion for bacteria and for fungi as described by (14;15).

Three nutrient agar plates for the bacterium (*E. coli*) were prepared for all the extracts (A, B and C) and were labeled A_1, B_1 and C_1. 50 µl inoculum of the selected bacterium (*E. coli*). Equal volume of antibiotic (ciprofloxacin), distilled water and extract A at different concentrations as 15, 10 and 5 mg/ml (Aqueous extraction) were poured into the wells. The plates were incubated at 37^0 C for 24 hours and observed for zone of inhibition. This procedure was repeated for plates B_1 containing extract B (Ethanolic extract) and plate C_1 containing extract C (undiluted Juice extract). Sub-culturing was done according to the method described by Cheesebrough (2002) (16) and that used by Aneja (20(18) (17). Susceptibility test was done on the isolates for each of the extracts and ketoconazole using agar well method/principle described by Aneja (2018) (17).

III. Results And Discussions

Phytochemical evaluation was performed with aqueous, ethanolic, undiluted juice extracts of *C. aurantifolia* (Table1). The aqueous extract was rich in reducing sugar, flavonoids, saponins while the ethanolic extract was found to have the presence of glycosides, reducing sugars and saponins. Whereas the juice extract was rich in tannins, glycosides, reducing sugars, flavonoid and phlobatanins. Antibacterial potential of aqueous, ethanolic and undiluted juice extracts can be attributed to the presence of these phytochemicals. Saponins and flavonoid were present in appreciable amounts in the aqueous extracts. Ethanol and undiluted Juice extracts gave positive tests for phlobatanins, glycoside and reducing sugar.

The occurrence of glycosides was most prominent in aqueous extracts of the peelwhile the juie had most presence in the aqueous extract o the juice. The peel extracts did not show any presence of tannins, the same with the ethanolic extracts where glycosides were absent. Also, ethanolic peel extract did not reveal any presence of flavonoids. The most appreciable presence of the phytoconstituents was observed for flavonoids, saponins and phlobatanins in aqueous peel. Flavonoids were also appreciably present in ethanolic extracts of the juice.

Table 1: Phytochemical properties of *Citrus aurantifolia* peel and juice extracts

Phytoconstituents	Peel extracts		Juice extracts	
	Aqueous	Ethanolic	Aqueous	Ethanolic
Tannins	-	-	+	++
Glycosides	+++	+	+	-
Reducing sugar	++	++	+++	++
Flavonoids	+++	-	++	+++
Saponins	+++	++	++	+
Phlobatanins Tannins	+++	++	+	++

Key: + = present ++ moderately present +++ = appreciably present - = Absent

Antibacterial activities of *C. aurantifolia* : The antibacterial spectra of the aqueous, ethanolic and undiluted juice extracts of *C.aurantifolia*, the inhibition zones formed by standard antibiotics showing the zone of inhibition in millimeters, for gram positive and gram negative bacteria are summarized in Tables 2 and 3. The inhibitory effect of 15 mg/ml of aqueous *C. aurantifolia* on *S. aureus* was comparatively less than that of standard antibiotics whereas the activity of the ethanolic extract at 15 mg/ml against *E. coli* showed a pronounced effect than all other tested extracts and was found to be comparable with the standard antibiotic Ciprofloxacin (10 µg). Juice extract showed little effect against *S. aureus* and no activity was seen against *E. coli*. The antibacterial potency of the *C. aurntifolia* extracts can be attributed to the presence of various phytochemical constituents. Plant extracts inhibited bacterial growth but their effectiveness varied.

The highest inhibition by extract was noticed in15 mg/l of ethanolic peels extractwith 11.00 mm on *E. coli*, followed by 10 mg/l of the same extract on the same organism. The lowest inhibition zones were recorded from the lowest concentrations (5 mg/l) of both the peel and juice extracts. The performance by the extracts from peel was also observed to be higher than that of the juice on both *S. aureus* and *E. coli*.

Table 2: Inhibitory effects of *Citrus aurantifolia* peel extracts on bacteria

Microorganisms	Extracts	Concentration mg/ml /zone of inhibition (mm)		
		15 mg/ml	10 mg/m	5 mg/ml
S.aureus	Aqueous	8.21±0.61	2.83±0.31	2.21±0.22
	Ethanolic	9.12±2.23	2.01±0.12	2.20±0.13
	Control	17.52±2.13	17.52±2.13	17.52±2.13
E. coli	Aqueous	10.63±0.31	0.88±0.13	0.38±0,21
	Ethanolic	11.00±0.11	2.13±0.32	0.95±0.02
	Control	15.33±0.21	15.33±0.21	15.33±0.21

Table 3: Inhibitory effects of *Citrus aurantifolia* juice extracts on bacteria

Microorganisms	Extracts	Concentration mg/ml /zone of inhibition (mm)		
		15 mg/ml	10 mg/m	5 mg/ml
S.aureus	Aqueous	3.21±2.48	0.00±0.00	0.00±0.00
	Ethanolic	6.12±0.23	3.61±0.42	0.00±0.00
	Control	17.52±2.13	17.52±2.13	17.52±2.13
E. coli	Aqueous	3.63±2.24	0.00±0.34	0.00±0,00
	Ethanolic	3.92±0.83	4.13±0.25	0.00±0.0o
	Control	15.33±0.21	15.33±0.21	15.33±0.21

Antifungal activities of *Citrus aurantifolia*

The antimicrobial susceptibility showed promising evidence for the fruit peels and juice extract against *S.aureus, E.coli* except for Fungi (*C.albicans*) which showed no activity, this could either be as a result of low concentrations (15 mg/ml, 10 mg/ml, 5mg/ml) of the extracts used or the environmental conditions. The effect of ketoconazole control was pronounced and better than those of the extracts in all the plates. There was no effect by both the peel and juice extracts on the organism under the 5 mg/l treatments, as the results showed 0.00 mm.only a slight 4.47 mm zone of inhibition was recorded by the ethanolic extract of peels in 10 mg/l while other extracts in the same concentration did not show any positive inhibition.generally, the inhibition of the fungus was observed to be low because the highest inhibition was 7.30 mm in the 15 mg/l ethanolic peel extract, followed by 6.02 mg/l in the same concentration of aqueous juice extract. These inhibitions were far lower than the control, which recorded 14.67 mm inhibition of *C. albicans*.

Table 4: Inhibitory effects of *C. aurantifolia* fruit extracts on *C. albicans*

Microorganisms	Fruit part	Extracts	Concentration mg/ml /zone of inhibition (mm)

			15mg/ml	10mg/m	5mg/ml
		Aqueous	5.21±0.63	0.00±0.00	000±0.00
C. albicans	Peel	Ethanolic	7.30±0.38	4.47±0.21	0.00±0.00
		Aqueous	6.02±0.56	0.00±0.00	0.00±0.00
	Juice	Ethanolic	5.45±2.42	0.00±0.00	0.00±0.00
		Control	14.67±2.10	14.67±2.10	14.67±2.10

IV. Conclusion And Recommendations

From the above experiment it is inferred that *C. aurantifolia* aqueous, ethanolic extract of the peels as well as juice extracts have significant activity against Gram-positive and Gram-negative bacteria. The activities of aqueous and ethanolic extract were less effective when compared with the standard antibiotic. Further research into the use of this plant for the prevention and treatment of bacterial infections caused by various pathogenic bacteria such as *B. cereus, S. aureus, E. coli* that have developed some resistance to antibiotics is recommended.

References

[1]. Ushnie G, Lamb M. Dietary flavonoids and risk of coronary heart disease. *Physiology Research*. (2005);**50**(6):529–35.
[2]. Maida A, Mohan B. S, Jaiprakash O, Patil K. N, Chidambara G. K, Jayaprakasha B. C Mahadev and Bhimanagouda., S.P. (2009). *Journal of Agriculture and. Food Chemistry*., ,**57**(22),10933.
[3]. Gupta C, Garg A. P, Tripathi S. C.(2011) Antimicrobial activity and phytochemical analysis of fruit peels. *Journal of medicinal plants research* **47**(3) 83-9
[4]. Farooq M. A, Iqbal U, Malla R, Joshi B, Afzal R., Rasool A (2010) Mycopathology. *Journal of Microbiology***8**(2)81-84
[5]. Asipita, S. A, Ismail M, Majid Z.A. Majid C.S. Abdullah and jMirza. (2014). Green *bambusa arundinacea* leaves extract as a sustainable Corrosion inhibitor in steel reinforced concrete.*Journal of cleaner production*, **67**: 139-146.
[6]. Aibinu F. N, Raina V. K, Srivastava S. K & Syamasunder, *Flavour and Frag Journal*., (2002,2003.), 17, 358.
[7]. WHO, (2018) https://www.who.int/news-room/fact-sheets/detail/e-coli. accessed June, 2020
[8]. Peter A. B. (2001).Molecular Biology Problem Solver: A Laboratory Guide. Edited by Alan S. Gerstein Wiley-Liss, Inc. 462-486 ISBNs: 0-471-37972-7 (Paper); 0-471-22390-5 (Electronic)Vogt and Dippoid, 2005
[9]. Ryan, KJ; Ray CG, eds. (2004). *Sherris Medical Microbiology* (4th ed.). McGraw Hill. ISBN 0-8385-8529-9
[10]. Olen G. O., Hanselman BA, Kruth SA, Rousseau J, Weese JS; Kruth; Rousseau; Weese (2001). "Coagulase positive staphylococcal colonization of humans and their household pets". *Canadian Vetinarian.***50** (9): 954–8.
[11]. Rooprai T, Tsai L, Daniels K, Soll D (2001). "EFG1 Null Mutants of *Candida albicans* Switch but Cannot Express the Complete Phenotype of White-Phase Budding Cells". *Journal of Bacteriology*.**182** (6): 1580–1591.
[12]. Andrzej V. H, Jim L, Olusegun, K. A. and James A.O,(2010). "The Effects of *Aloe Vera* extract on corrosion and kinetics ofcorrosion process of zinc in HCl solution" *Corrosion Science*, **52**: 661-664.
[13]. Derazne Estella,Uroh A. A, Okeoma K. B and Alozie G. A, (2009). The inhibitive effect of *Solanum melongenal* leaf extract on the corrosion of aluminium in tetraoxosulphate (vi) Acid. *African Journal of Pure and Applied Chemistry*, **4**(8):
[14]. Adom KK, Liu RH (2002). Antioxidant activity of grains. J Agric Food Chem 50(21), 6182–6187.Aibinu F. N, Raina V. K, Srivastava S. K & Syamasunder, *Flavour and Fragrance Journal*., (2002,2003.), 17, 358.
[15]. Zhang L, Liu R, Niu. and W. (2014) Phytochemical and Antiproliferative Activity of Proso Millet. PLoS ONE 9(8): e104058. https://doi.org/10.1371/journal.pone.0104058
[16]. Sagar S. D, Kulkarni Hegde Y. R. (2007) Evaluation of wood, bark and leaves extracts and their influence on the growth of some pathogenic bacteria. *International Journal of Plant Science* **2**(2) 155-158.
[17]. Aneja, K. R. (2018). *Experiments in Microbiology Plant Pathology Tissue culture and Microbial Biotechnology.* Fifth Edition. New Age International Publishers New Delhi 580pp

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