

Original Research Article

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Antibacterial Activities of Selected Fruit Peels against Organisms Associated with Urinary Tract Infection

Ayuba Sunday Buru^{1,2*}, Ikya Homior Paschal¹, Ifeanyi Tony Ojizeh¹,
Oluboyo Bernard Oluwapelumi¹, Akele Yomi Richard¹, Akinseye Janet Fumilayo¹
and Adewumi Ajoke Funmi¹

¹Department of Medical Laboratory Science, College of Medicine and Health Sciences,
Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria

²Department of Molecular Microbiology, College of Medicine and Health Sciences,
Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria

*Corresponding author

ABSTRACT

The use of natural products as anti-infective agents, have given rise to new chemical diversity and are preferred in modern world. Among all these natural sources, plants and its products is more reliable because of its renewability. Urinary tract infections (UTIs) are among the most common infectious diseases occurring in either the community or healthcare setting. Orange and Plantain peels were used to determine the antibacterial activities. A measure of 50 grams of powder was filled in the thimble and extracted with 150 ml of ethanol successively up to 48 hours. The solvent extracts were concentrated separately under reduced pressure, 2g of each concentrated solvent extracts were dissolved in 20 ml of 20% dimethyl sulphoxide and used for antibacterial assays using agar well diffusion and broth method. The phytochemical analysis of sweet orange peel shows that carbohydrate, reducing sugars, tannins and flavonoids were present, The zone of inhibition effect of the extracts on selected organism was concentration dependent (25<50<75<100). Higher concentration of extract recorded larger/wider effect on each test organism. *Musa paradisiacal* peel showed no effect on test organism except at 100mg/ml that recorded 4.7±0.47mm against *E. coli* growth. The MIC and MBC of both orange peel and plantain peels suggest potency against test organisms. Orange peel has advantage over plantain peel; *S. aureus*, *E. coli* and *P. aeruginosa* were susceptible to the extract exempting *K. pneumonia*, which showed no susceptibility to the duo extracts. The peels of *Citrus sinensis* and *Musa paradisiacal* exhibited inhibitory activity against certain bacteria, which can be attributed to the presence of certain secondary metabolites. But when compared with standard antibiotics, the ethanoic extracts of the duo was less efficient as there was a smaller size of zone of inhibition against the luxurious growth of tested organisms.

Keywords

Antibacterial,
Urinary tract
infections (UTIs),
Citrus sinensis
and *Musa*
paradisiacal.

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Introduction

According to World Health Organization (WHO), medicinal plants are defined as any herbal preparations made by incorporating

plant materials to its extraction, fractionation, purification, concentration, or any biological, physical processes which

may be produced as herbal product for the immediate consumption (Alo *et al.*, 2012).

The use of natural products as anti-microbial agents, have given rise to new chemical diversity and are preferred in modern world. The sources of these products are plants, animals and microorganisms. Among all these natural sources, plants and its products is more reliable because of its renewability. Plants are also rich in certain nutrients hence are used as sources of food and also used as pain relieve due to certain compounds which they have. From earliest times, the lack of detailed knowledge about natural product and the compounds present, their mode of action was a challenge for plants to be used for the treatment of diseases. Around the world, over the centuries different societies have developed their own traditional sense of medicinal plants and their applications.

The use of natural product for human health has been reported for a very long time, the phytochemicals from the plant extracts with known antimicrobial properties can be of great significance in the treatment of illnesses (Seenivasan *et al.*, 2006). Secondary metabolites such as phenol compounds, tannin are well documented as part of essential oil and have shown great antimicrobial activities, these compounds can be synthesized once their active substances have elucidated (Tyagi and Malik, 2010). In controlling biofilms, the use of essential oils is highly recommended, owing to high effective diffusion and mode of contact (Al-Shuneigat *et al.*, 2005). Therefore, the essential oils and other promising plants extracts have aroused interest as sources of natural products. Their exploration as potential alternative remedies for the treatment of various infectious diseases cannot be underscore (Tepe *et al.*, 2004; Dorman and Deans, 2000).

Urinary tract infections (UTIs) account for most of the common infectious diseases found within the community or healthcare setting (Nicolle *et al.*, 2005).

In empirical antimicrobial treatment in both primary and secondary care setting, UTIs are the commonest clinical indication, and urine samples usually constitute the single largest category of specimens examined in most medical microbiology laboratories (Morgan, 1993). Uncomplicated UTIs typically occur in healthy adult non-pregnant woman, while complicated UTIs (cUTIs) may occur in all gender and age groups and are frequently associated with either structural or functional urinary tract abnormalities.

Examples include foreign bodies such as calculi (stones), indwelling catheters or other drainage devices, obstruction, immunosuppression, renal failure, renal transplantation and pregnancy (Lichtenberger and Hooton, 2008). UTI in the elderly is almost always complicated in men with prostatic hypertrophy and in post-menopausal women who may have an increased post-void residual volume (Nicolle, 2001). The likelihood of treatment failure and serious complications, particularly the development of antimicrobial resistance, is more common in complicated UTI.

Although a broad range of pathogens can cause complicated UTI, *E. coli* remains the most common; however, even this organism is becoming resistant to the agents that are normally prescribed (Nicolle, 1997). This study is aimed at investigating the antibacterial activities of some natural plant extracts against organisms associated with urinary tract infection.

Materials and Methodology

Collection of Plant Materials

Plantain and sweet orange were obtained from Sabo market in Ado-Ekiti. The plant materials were washed with water thoroughly, air dry and peeled. The peels were shade dried in medical laboratory department for about three weeks and a further three days drying in the oven at a temperature of 37°C and were grounded to fine powder and store away from sunlight.

Test Microorganisms

Pure cultures of pathogenic bacteria that are associated with urinary tract infection (UTI) were obtained from the Department of Medical Microbiology and Parasitology, Ekiti State University Teaching Hospital. The bacteria isolates include *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and a gram positive bacteria, *Staphylococcus aureus*. The test organisms were sub cultured into nutrient agar slant and stored at 4°C until when required for use.

Preparation of ethanolic extract

A measure of 50 grams of powder was filled in the thimble and extracted with 150 ml of ethanol successively up to 48 hours. The solvent extracts were concentrated separately under reduced pressure using method described by Harborne (1992). Extract from this method was weighed and stored at 4°C in a refrigerator until required. After complete solvent evaporation, 2 gram of each concentrated solvent extracts were dissolved in 20 ml of 20% dimethyl sulphoxide and used for antibacterial assays.

Test for Phytochemicals

Test for carbohydrate (Molisch's test), Reducing sugars (Fehling's test), Tannins

(Gelatin test), Flavonoid, Terpenes (Salkowski test) and Saponin were carried out using standard method as described by Aboh *et al.*, (2014).

Preparation of Test Isolates and Standardization

The preserved test organisms were sub-cultured on nutrient agar and incubated overnight to obtain discrete colonies. A colony were picked and inoculated in peptone water and incubated for 18 to 24 hours. The overnight culture broth was then compared and was adjusted to match that of 0.5McFarland standard (10^5 CFU/mL) by diluting it with distill water.

Testing Assay

Preparation of Nutrient Agar and Well Diffusion Susceptibility Test

In the preparation, appropriate quantity was weighed following the manufacturer's instruction, dissolved in distilled water, mixed thoroughly and sterilized at 121°C (15 lbs of pressure) for 15 minutes. It was then allowed to cool to a temperature of 50°C, poured into petri dishes, allowed to solidify Well diffusion, several punched holes were made on the agar plate, seeded the test isolate on the medium and holes were filled with varying concentrations of the test extract. It was then incubated at 37°C for 18 hours and inhibition zones measured in millimeters (Cheesbrough, 2000)

Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

Minimum inhibitory concentration was carried out using standard methods as described by Cheesbrough (2000).

Results and Discussion

The phytochemical analysis of sweet orange peel shows that carbohydrate, reducing sugars, tannins and flavonoids were present while terpenes and saponins were completely absent. Plantain peels, yield carbohydrate, reducing sugars and tannins that are present (table 1).

The effects of *C. sinensis* on zone of inhibition of selected organisms are shown on table 2. The effect was concentration dependent (25<50<75<100). Higher concentration of extract recorded larger/wider (zone of inhibition (mm) on each test organisms. *Musa paradisiacal* peel showed no effect on test organism except 100mg/ml of extract that recorded 4.7±0.47mm against *E. coli* growth (Table 3). The MIC and MBC of both orange peel and plantain peels are shown on table 4. Orange peel has more inhibitory effect over plantain peels: *S. aureus*, *E. coli* and *P. aeruginosa* were susceptible to the extract except *K. pneumonia*, which showed no susceptibility to the duo extracts.

Medicinal plant and aromatic compounds had been found to be sources of bioactive component that are responsible for inhibiting bacterial pathogens (Bruneton, 1995). Enormous research has been carried out to determine the substances that have the capacity to inhibit pathogens growth without favoring the toxic effect towards the host cell. Many studies have reported the potential antibacterial activities of medicinal plant including tropical fruits peels such as sweet orange, banana and a host of others (Karuppiah and Mustaffa, 2013). Although some of the antibacterial activities of medicinal plant are well-documented, their antibacterial capacity invitro may have a wide degree of variation depending on several factors such as test medium, the

method employed, test organism and the difference in nature of the plants and plant products (Fagbemi *et al.*, 2009). Ekwenye and Edeha (2010) reported antibacterial activity of the ethanol and aqueous extract of *Citrus sinensis* leaf against *E.coli*. But when compared with the ethanoic extract from the orange peel, the peel was more efficient against test organisms, the variance may not be unconnected to difference in the phytochemical composition in various part of the plant, the extraction methods used, environmental factors and difference in the genotypes of the citrus plant used.

Antimicrobial properties of extract are primarily related to its phenol compounds. Whatever phenol substances in the extract is much higher, their antimicrobial properties is more (Kim *et al.*, 1995). The phytochemical analysis of the peel of citrus revealed that secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids that are known to have antimicrobial properties. The structure and functional properties of lipid fraction of the plasma membranes of bacteria and yeasts are affected by monoterpenes thereby causing leakage of intercellular material and expulsion of critical molecules and ions leading to death of microbes. Terpenoids inhibits microbial respiratory oxygen enzymes uptake and oxidative phosphorylation (Cox *et al.*, 2000 and Trombette *et al.*, 2005). Charis *et al.*, (2004) in a research showed that different citrus have different effects on different bacteria. Antimicrobial properties of oils extracted from the citrus peel it is directly affected by its constituents. Essential fatty acids and alkaloids, lactone, polyacetylene are compounds effective on different bacteria. The antimicrobial effect of citrus depends on so many factors which include soil separation, harvesting season, the extraction process and the type of bacteria.

Orange peel extract showed inhibitory activity against all the pathogens tested with exception of *K. pneumoniae* while the plantain peel extract had effect only on *E. coli*. This observation is in tandem with the report of Nannapaneni *et al.*, (2008) who showed that natural compounds of *Citrus sinensis* peel had inhibitory effects on different strains of *Escherichia coli*, *Salmonella* and some food pathogenic bacteria. *Citrus sinensis* peel extracts had significant antimicrobial effects on gram positive and gram negative bacteria. The ethanolic extraction from orange peel had inhibitory effect on Gram positive and gram negative bacteria exempting *K. pneumoniae*. This report corroborate with the earlier report of Omodamiro and Umekwe (2013) and Khushwaha *et al.*, (2012), respectively. It was also observed that the degree of susceptibility was concentration dependent, the higher the concentration the larger/wider the size of zone of inhibition likewise the lower concentrations. However, Madhuri *et*

al., (2014) documented that methanol extract showed inhibitory effect on tested pathogens including *K. pneumoniae* which is at variance with the result obtained in this study. The extract from *Musa paradisiacal* show no significant inhibitory activity on most of the tested organisms, except *E. coli* which might be as a result of low concentration of hydrocarbons, monoterpene and oxygenated monoterpenes and other valuable phytochemicals that seems to be tolerable to the tested organisms. The observation from this study is in agreement with Chabuck *et al.*, (2013) who also reported insignificant inhibitory activities of *Musa paradisiaca* on *E. coli*, but contradict the work of Igadharo (2012), Okechukwu (2012), Ehiowemwenguan *et al.*, (2014) and Akhraiye *et al.*, (2016) who in their various reports indicated that Plantain peel had significant inhibitory activities on test organisms of both gram positive and gram negative bacteria.

Table.1 Phytochemical Analysis of Ethanol Extract of Sweet orange And Plantain Peels

Phytochemical	Orange	Plantain
Carbohydrate	+	+
Reducing sugars	+	+
Tannins	+	+
Flavonoids	+	—
Terpenes	—	—
Saponin	—	—

Key: (-)= Negative (+) = Positive

Table.2 Antibacterial Activity of Ethanol Extract of *Citrus sinensis* Using Agar Well Diffusion Method

Organism	Concentration (mg/ml)				Control Antibiotics		
	25	50	75	100	Augumentin	Gentamycin	Cotrimodaxole
<i>S.aureus</i>	7.7±1.60	13.7±4.64	18±4.24	22±4.97	25±4.87	28±4.47	25±4.87
<i>E.coli</i>	NS	NS	6.7±2.91	10.7±2.70	NS	22±4.97	33±5.97
<i>K.pneumoniae</i>	NS	NS	NS	NS	24±4.37	19±4.77	NS
<i>P.aeruginosa</i>	NS	2.3±2.05	7±1.41	10.7±1.63	22±4.97	NS	10±4.47

Key: NS= Not Sensitive, Mean ±SD, Values (Mean ±SD) of 3 Samples Test

Table.3 Antibacterial Activity of *Musa Paradisiacal* Peel Extract Using Agar Well Diffusion Method

Organism	Concentration (mg/ml)				Control Antibiotics		
	25	50	75	100	Augmentin	Gentamycin	Cotrimodaxole
<i>S. aureus</i>	NS	NS	NS	NS	25±4.97	28±4.87	25±4.97
<i>E.coli</i>	NS	NS	NS	4.7±0.47	NS	22±4.97	33±5.67
<i>K.pneumonia</i>	NS	NS	NS	NS	24±4.88	19±4.67	NS
<i>P.aeruginosa</i>	NS	NS	NS	NS	22±4.97	NS	10±4.37

Key: NS= Not Sensitive, Mean ±SD, Values (Mean ±SD) Of 3 Samples Test

Table.4 Showing Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of Orange and Plantain Peel Extract

Organism	Orange (mg/ml)		Plantain(mg/ml)	
	MIC	MBC	MIC	MBC
<i>S. aureus</i>	7±2.97	25±4.87	NS	NS
<i>E. coli</i>	14±4.25	50±5.67	25±4.87	50±5.67
<i>P. aeruginosa</i>	50±5.67	14.5±4.35	NS	NS

Key: NS =Not Sensitive, MIC =Minimum Inhibitory Concentration, MBC =Minimum Bactericidal Concentration

The variation in these reports could be as a result of harvesting, storage and processing methods used. The genotypes of the test organisms could also be a serious factor to be considered. In general, the result obtained from *C. sinensis* peels correlates with the reports of earlier scientists, though differences with test isolate of *K. pneumonia* which might probably be due to the quantity of the constituent that seems non-toxic to this organism. Meanwhile, these extracts exhibit potent antibacterial activity on the tested organisms but, when compared with standard antibiotics, the ethanolic extracts of the duo were less efficient as there was a smaller size of zone of inhibition against the luxuriant growth of tested organisms.

In conclusion, the peels of most fruits are considered to be a waste by processing industries. The peels of *Citrus sinensis* and *Musa paradisiacal* exhibited inhibitory activity against certain bacteria, which can be attributed to the presence of certain secondary metabolites. It is however suggestive that these fruit peels can be of medicinal value. Although when the efficacies of these peels are compared, sweet orange peels exhibited a better inhibitory activity against bacteria associated with Urinary tract infection.

Conflict of interest

No conflict of interest expressed

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