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Research Article

Qualitative and Quantitative phytochemical screening and antimicrobial activities of *citrus sinensis* and *citrus aurantifolia* peel extracts

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Abstract

This study evaluated the qualitative and quantitative phytochemical screening and antimicrobial activities of *Citrus sinensis* and *Citrus aurantifolia* peel extracts using standard microbiological procedures. Fruits of the two citrus plants were purchased and the peels removed, dried, ground and extracted using soxhlet extraction technique. The test organisms used in the determination of the antimicrobial activities of the peel extracts were *Staphylococcus, Streptococcus, Pseudomonas, Escherichia* and *Candida* species. Qualitative phytochemical screening of the peel extracts revealed the presence of flavonoids, alkaloids, tannins, saponins, phenols, anthranoids and cardiac glycosides. Quantitative phytochemical screening of the *Citrus sinensis* peel extract revealed total phenol (4.24 mg GAE/g), flavonoid content (58.12 mg GAE/g), total tannin (7.24 mg GAE/g); total saponins (7.67mg GAE/g), alkaloids (10.30mg GAE/g); while that of *Citrus aurantifolia* revealed total phenol (20.10 mg GAE/g), flavonoid content (28.70 mg GAE/g), total tannin (4.24 mg GAE/g) total saponins (4.32mg GAE/g), alkaloids (7.40mg GAE/g). Antimicrobial susceptibility pattern showed zones of inhibition recorded ranged from 18mm to 26mm with *Citrus aurantifolia* peel extract and 22mm to 26mm with *Citrus sinensis* peel extract. Minimum inhibitory concentrations recorded ranged from 125mg/ml to 250mg/ml with *Citrus aurantifolia* peel extract and 250mg/ml to 500mg/ml with *Citrus sinensis* peel extract. Bactericidal activity was recorded at 250 mg/ml to 500mg/ml with *Citrus sinensis* peel extract. This study has revealed the medicinal properties of these plants through their antimicrobial activities on the selected pathogenic microorganisms. The medicinal and pharmacological properties of these medicinal plants should, therefore, be exploited maximally.

Keywords: Qualitative, Quantitative, Phytochemical, Antimicrobial, Extracts.

INTRODUCTION

Natural products, especially those derived from plants, have been used to help mankind sustain its health since the dawn of medicine (Turay et al*.,* 2020). There is great interest in the use of medicinal plants in developing countries because herbal remedies and their use in formulation of new drugs. Also, medicinal plants provide better and cheaper substitutes for synthetic drugs. The medicinal value of herbal/medicinal plants lies in some chemical substances (bioactives) that produce a definite physiological action on the human body (Bhinge et al*.,* 2017; Haroen et al., 2018).

Among the plants with proven medicinal properties are orange (*Citrus sinensis*) and lime (*Citrus aurantifolia*). There are many natural metabolites in citrus fruit that potentially provide advantage and are good for health. For example, essential oil and pectin of the fruits are used in the cosmetic and pharmaceutical industries. *Citrus aurantifolia* belongs to *Rutaceae*, it is a poly embroyonic plant cultivated in several parts of the world especially hot subtropical or tropical region such as India, USA, Nigeria, Mexico, West Indies and Egypt (Bakare et al*.,* 2012). The plant is used in traditional medicine for treatment of several diseases such as cold and stomach ailment. It can also be used as an antiseptic, mosquito repellant, antifungal, antibacterial and antiviral agent (Makni et al., 2018).

Citrus sinensis is included as one of the important fruits of the genus *Citrus* (Turay et al*.,* 2020). The fruit of *Citrus sinensis* is mostly recognized for its vitamin C content and is also an important source of other phytochemicals such phenolics and carotenoids which are reputed to have health benefits (Haroen et al., 2018). The citrus peel is seen as an important by-product in the citrus possessing industries where large amounts are produced and considered as waste.

The health benefits of *Citrus* plants are highly associated with the high amount of bioactive constituents they contain such as phenols, flavonoids, carotenoid, vitamins and minerals. Limes contain unique flavonoid compounds that have antioxidant and anti-cancer properties. The flavonoids help to inhibit cell division in many cancer cell lines in addition to its antimicrobial efficacy. The plant also demonstrated bioactive activities for cold, fever, sinusitis, sore throats, asthma and bronchitis (Khan et al*.,* 2012).

Antibacterial assessment of *Citrus aurantifolia* aqueous ethanol, acetone, chloroform, ethanol and petroleum ether leaf extracts conducted by Pathan et al*.* (2012) against various pathogens showed significant activity against *Staphylococcus aureus, Pseudomonas* species, *Klebsiella pneumoniae* along with antifungal activity against *Mucor* species, *Aspergillus fumigatus* and *Aspergillus niger*. Kandpal et al*.* (2012) isolated actinomycetes from *C. aurantifolia* and tested its antibacterial efficacy against different pathogens.

Several published reports had described the antimicrobial activity of various crude extracts of plants either singly or in combination. However, little work has been done with respect to*Citrus* peels which are regarded as waste in the society. This study evaluated the qualitative and quantitative phytochemical screening and antimicrobial activities of *Citrus sinensis* and *Citrus aurantifolia* peel extracts.

MATERIALS AND METHODS

Collection of samples

Peels of orange and lime were collected from fruit sellers within Owerri metropolis. They air-dried, ground in a sterile manual grinder and stored in air-tight containers in the refrigerator until required for extraction. Commercial antibiotic discs, for control, were bought from the market.

Materials and media preparation

The method described by Haroen et al*.* (2018) was adopted in the sterilization of the materials and media. All the glass wares that were used for the experiment were sterilized using the laboratory hot air oven at a temperature of 160^0C for 1 hour. Wire loop was sterilized over burning flame and allowed till it was red-hot, while glass spreader was sterilized by dipping into 70 % ethanol and passing over Bunsen flame. The media were prepared according to manufacturer's instructions and sterilized using the autoclave at a temperature of 121^0C at 15 psi for 15minutes.

Extraction of the active ingredients in the peel powders

The method described by Haroen et al*.* (2018) was adopted in the extraction of active ingredients from the peel powder. Twenty grams (20g) of ground peel powders were subjected to extraction using soaking method in ethanol. Twenty grams (20g) each of the powder was poured into 95% ethanol in a beaker. The contents of the beaker were allowed to soak overnight. The content was filtered using muslin cloth and stored in a sterile container.

Qualitative and quantitative phytochemical screening

The method described by Turay et al. (2020) was adopted in the determination of the phytochemical properties of the extracts. The phytochemicals determined were; phenols, flavonoids, tannins, anthranoids, saponins, alkaloids, phlobatannins, anthraquinones and cardiac glycosides.

Test organisms

The clinical isolates used for the determination of the antimicrobial activities of the peel extracts of the plants were; *Staphylococcus, Streptococcus, Pseudomonas, Escherichia* and *Candida* species.

Standardization of inocula

The method described by CLSI (2015) was adopted in the preparation of 0.5 M McFarland's standard solution of the test organisms used for the study. 1ml of concentrated sulfuric acid was added to 99ml of distilled water to make 1% solution sulfuric acid.

Similarly, 0.5g of dehydrated barium chloride ($BaCl_2$. $2H_2O$) was dissolved in 50ml of distilled water to make 1% w/v solution of barium chloride. Then 0.5ml barium chloride was added to 99.5ml sulfuric acid solution and mixed properly.

Antimicrobial screening of peel extracts

The agar well diffusion techniques as described by Haroen et al. (2018) was adopted for this study to evaluate the antimicrobial activity of the peel extracts. A sterile Pasteur pipette was used to drop 0.2 ml standardized inoculums equivalent to 0.5 McFarland's turbidity standards on the surface of already prepared and dry Mueller-Hinton agar. The inoculum was evenly spread using Hockey stick shaped glass rod. Two wells were carefully bored into each agar plate after standing for about 5 minutes with heat sterilized 6 mm diameter cork borer and labeled. The extracts were then poured into the wells and the plates were allowed to stand for about 30 minutes for proper diffusion of the solutions before being incubated at 37°C for 24 hours. After 24 hours, antimicrobial activity was evaluated by measuring the diameter of the zones of inhibition produced by the extracts against the test organisms in millimeters.

Tests for minimum inhibitory concentrations (MIC)

Tube dilution techniques described by CLSI (2015) was adopted in the determination of the minimum inhibitory concentrations of the peel extracts. Two milliliters (2ml) each of the extracts were added in four milliliters (4ml) of peptone water; this gives 500mg/ml. Thereafter, two fold serial dilutions was carried out from the 500 mg/ml concentration by transferring 2 ml of the 500 mg/ml concentration to 4 ml of peptone water contained in a test tube and homogenized properly. This procedure of transferring 2 ml of the tube to 2 ml of peptone water contained in the subsequent tube was continued until the last test tubes. The following concentrations thereafter were obtained, 500mg/ml, 250 mg/ml, 125 mg/ml, 62.50 mg/ml and 31.25 mg/ml. Having obtained the different concentrations and dilutions, three drops of broth cultures of the test organisms were inoculated into the dilutions in each case of the test organisms. The tubes were then incubated at 37^0C for 24 hours. The lowest concentration of the extracts that inhibited the growth of the test organisms was recorded as the MIC.

Test for minimum bactericidal/fungicidal concentrations (MBC/MFC)

Tubes showing no visible growth from the MIC test were subcultured onto sterile nutrient agar plates and incubated at 37^0C for 24 hours for bacterial isolates and 28^0C for 72 hours for fungal isolates. The lowest concentration of the extracts yielding no growth was recorded as the minimum bactericidal/fungicidal concentrations as the case may be.

RESULTS

Table 1 shows the qualitative and quantitative phytochemical screening of the peel extracts from *Citrus sinensis* and *Citrus aurantifolia.*
Table 2 shows the antimicrobial susceptibility pattern of the extracts against the test organisms. Zones of inhibition recorded ranged from 18mm to 26mm with *Citrus aurantifolia* peel extract and 22mm to 26mm with *Citrus sinensis* peel extract.
Table 3 shows the minimum inhibitory concentrations of the extracts against the test organisms. Inhibitory effect recorded ranged from 125mg/ml to 250mg/ml with *Citrus aurantifolia* peel extract and 250mg/ml to 500mg/ml with *Citrus sinensis* peel extract.
Table 4 shows the bactericidal/fungicidal activity was recorded at 250 mg/ml to 500mg/ml with *Citrus sinensis* peel extract.

Table 1. Qualitative and quantitative phytochemical screening of the peel extracts from *Citrus sinensis* and *Citrus aurantifolia*

Phytochemicals	**Qualitative analysis**		**Quantitative analysis (mg GAE/g)**	
	C. sinensis	***C. aurantifolia***	***C. sinensis***	***C. aurantifolia***
Phenols	+	+	4.24	20.10
Flavonoids	+	+	58.12	28.70
Tannins	+	+	7.24	4.24
Anthranoids	+	-	2.03	ND
Saponins	+	+	7.67	4.32
Alkaloids	+	+	10.30	7.40
Phlobatannins	-	-	ND	ND
Anthraquinones	-	-	ND	ND
Cardiac glycosides	+	-	0.17	ND

Key: + = Presence of phytochemicals - = Absence of phytochemicals ND = Not detected
mg GAE/g = Milligrams of gallic acid equivalents per gram

Table 2. Antimicrobial susceptibility pattern of the extracts against the test organisms

Test organisms	Plants/zones of inhibition (mm)	
	C. sinensis	***C. aurantifolia***
Staphylococcus species	26	26
Streptococcus species	22	18
Pseudomonas species	24	20
Escherichia species	24	22
Candida species	22	20

Key: mm = Millimeter

Table 3. Minimum inhibitory concentrations of the extracts against the test organisms

Test organisms	Plants/Concentrations (mg/ml)	
	C. sinensis	*C. aurantifolia*
Staphylococcus species	125	250
Streptococcus species	250	500
Pseudomonas species	125	500
Escherichia species	250	250
Candida species	250	500

Key: mg/ml = Milligram per milliliter

Table 4. Minimum bactericidal/fungicidal concentrations of the extracts against the test organisms

Test organisms	Plants/Concentrations (mg/ml)	
	C. sinensis	***C. aurantifolia***
Staphylococcus species	250	ND
Streptococcus species	ND	ND
Pseudomonas species	250	ND
Escherichia species	500	ND
Candida species	ND	ND

Key: mg/ml = Milligram per milliliter ND = Not detected

DISCUSSION

The results of this study are shown in Table 1 to Table 4 above. Table 1 shows the qualitative and quantitative phytochemical constituents of the peel extracts. Quantitative phytochemical screening of the *Citrus sinensis* peel extract revealed total phenol (4.24 mg GAE/g), flavonoid content (58.12 mg GAE/g), total tannin (7.24 mg GAE/g), anthranoid (2.03 mg GAE/g), saponins (7.67 mg GAE/g), cardiac glycosides (0.17 mg GAE/g) and alkaloid (10.30 mg GAE/g). Quantitative phytochemical screening of the *Citrus aurantifolia* peel extract revealed total phenol (20.1 mg GAE/g), flavonoid content (2.42 mg GAE/g), total tannin (4.24 mg GAE/g), saponins (4.32 mg GAE/g) and alkaloid (7.40 mg GAE/g).

From the results presented in Table 1, higher quantitative phytochemicals were detected with *Citrus sinensis* peel extracts compared to *Citrus aurantifolia* peel extract. In the qualitative screening results, the presence of anthranoids and cardiac glycosides were only recorded with *Citrus sinensis* peel extract.

Kabra et al. (2017) reported the presence of phenols, flavonoids, and absence of anthraquinone and phylobatannins in their study with *Citrus aurantifolia* and *Citrus sinensis* extracts. Similarly, Muhammad et al. (2018) reported the presence of alkaloid, glycoside, saponin, tannin, flavonoid, steroids, terpenoid and phenol with leaf extracts of lime (*Citrus aurantifolia*). Mohammed and Ayoub (2016) reported the presence of alkaloids, saponins, sterols and triterpenes, carotenoids, coumarins, tannins and carbohydrates from seed extracts of lime tree. Kabra et al. (2017) reported the presence of phenols, flavonoids, and absence of anthraquinone and phylobatannins in their study with *Citrus aurantifolia* and *Citrus sinensis* extracts. Oikeh et al. (2020) reported the presence of tannins, flavonoid, and phenol from orange peels. Similarly, Asowata-Ayodele et al. (2019) reported the presence of for alkaloid, oxalate, tannins, phytate and glycosides from *Citrus sinensis*(orange).

Secondary metabolites in citrus plants have been identified as therapeutic agents in the management of several diseases. Phytochemical analysis of *Citrus sinensis* has revealed the presence of flavonoids, glycosides, coumarin glycosides, volatile oils, organic acids, fats and fixed oils. Tannins, flavonoids, saponins, phenolic compounds and essential oils are believed to be the phytochemicals responsible for the antimicrobial effects of plants. Tannins form complexes with proline rich proteins that inhibit cell protein synthesis. Synergistic action of tannins, flavonoids, alkaloids and saponins are known to inhibit the growth of pathogens (Nwankwo et al., 2014). Tannins exert antimicrobial activities by iron deprivation, hydrogen bounding or specific interactions with vital proteins such as enzymes in microbial cells. Herbs

that have tannins as their component are astringent in nature and are used for the treatment of intestinal disorders such as diarrhoea and dysentery.

Table 2 shows the result of the antimicrobial susceptibility pattern against the test organisms. Zones of inhibition recorded ranged from 18mm to 26mm with *Citrus aurantifolia* peel extract and 22mm to 26mm with *Citrus sinensis* peel extract. *Citrus sinensis* peel extract showed better antimicrobial activity against the test organisms than that of *C. aurantifolia*. This could be attributed to the fact that *C. sinensis* extact has more phytochemicals, both qualitatively and quantitatively, as is shown in the result. Bioactive natural products from plants have proven to be very useful in the drug design and discovery process (Hafidh et al., 2011). Asowata-Ayodele et al., (2019) reported that ethanolic extract of *C. sinensis* and *C*. *aurantifolia* peels inhibited the growth of *B. cereus*, *E. coli* and *S. aureus, K. pneumoniae* was resistant to the extracts. Gupta et al. (2021) also reported the antibacterial and antifungal activities of *C. sinensis* and *C. reticulata* against *S. aureus, B. subtilis*, *S. typhi E. coli, C. albicans* and *A. niger*. Tauseef et al. (2023) reported that the application of citrus oil significantly ($p < 0.05$) controlled the growth of all bacterial strains (*E. coli, S. aureus, S. agalactiae*) used in their study. The result of the research by Azghar et al. (2023) also showed that essential oil from Citrus effectively inhibited SA ATCC 29213 and methicillin-resistant *Staphylococcus aureus*. Citrus wastes can be converted to pharmaceutically important products.

Kirbaslar et al. (2019) also reported antimicrobial activity of *Citrus aurantifolia* extracts. They reported zones of inhibition of 13mm for *Escherichia coli* and 12mm for *Candida albicans*. The better zone of inhibition recorded with *Citrus aurantifolia* could be as a result of wide variety of secondary metabolites present in the seeds. Seeds of *Citrus* contain a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids and flavonoids that are found to have effective as antimicrobial properties.

Citrus plant parts have also been noted to have antibacterial, and anti-inflammatory properties and they are also known to reduce the symptoms of disorders such as hyperthyroidism, diabetes, osteoarthritis and high blood pressure (Oben et al*.,* 2017). Seeds of citrus fruits showed a moderate inhibition to the bacterial isolates, this agreed with Kumar et al. (2017) who concluded that the aqueous extract of the *C. sinensis* seeds showed a moderate percentage with inhibition zone 9mm against *E. coli* and *S. aureus* and no effect against *S. typhi*and, *K. pneumoniae*. Additionally, Jwanny et al. (2017) concluded that aqueous extract of orange peels can produce an antibacterial activity against different gram positive, negative bacteria and fungi with zone of inhibition 7-12mm.

The results of this study have shown that different extracts from various *Citrus* plant parts can be used as effective antibacterial reagents even against multidrug resistant bacteria, and home-available, safe, cheap and with no side effect like the synthetic drugs. The results also revealed the presence of bioactive phytochemicals in the peel extracts in which evidences gathered in earlier studies have confirmed to be medicinally important. Therefore, the plant part could be used as a good source of antibacterial agent against pathogenic microorganisms affecting humans.

CONCLUSION

The result of this work has demonstrated that the ethanolic peel extracts of *Citrus sinensis* and *Citrus aurantifolia* could be potential antimicrobial agent against *Staphylococcus* species and other pathogenic organisms. However, combination of the peel extracts work in synergy with for better antimicrobial activities against the test organisms. The medicinal and pharmacological properties of these medicinal plants should therefore be exploited maximally.

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