Phytochemical Screening and Antibacterial Activity of *Citrus Sinensis* Peel Extracts on Clinical Isolates of *Staphylococcus Aureus* and *Salmonella Typhi*

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Abstract

The study was conducted to determine the phytochemical composition, antibacterial activity and activity index orange (*Citrus sinensis* (L.) Osbeck) peel extracts against clinical isolates of *Staphylococcus aureus* and *Salmonella typhi*. The result of phytochemical screening of the peel extracts showed the presence of alkaloid, glycoside, saponin, tannin, flavonoid, terpenoid and phenol. The result of the antibacterial efficacy of the extracts against the isolates indicated the extracts were active against the isolates with higher activity in ethanol extract (with average zone of inhibition of 15 mm) when compared to aqueous extract (12.25 mm). The result of susceptibility of the isolates to the extracts showed *Staphylococcus aureus* was more sensitive to the extract with average zone of inhibition of 15.25 mm when compared to *Salmonella typhi* with average zone of inhibition of 12.00 mm. The minimum inhibitory concentration (MIC) of the extracts showed that dilutions of various concentrations of aqueous and ethanol of peel extracts can inhibit the growth or kill the isolates at a concentration of between 2.125 – 20 mg/ml. the average activity index of the extracts on the test isolates was found to be 0.64 which indicated that the plant extract can compete with the standard antibiotic used. Statistical analysis of the results indicated that there is no significant different in the activity of the extracts against the isolates used at p<0.05. Findings from this study support the use of orange peel extracts for medicinal purposes.

Keywords

Antibacterial activity, *Citrus sinensis*, Phytochemical, *Salmonella typhi*, *Staphylococcus aureus*.

Introduction

Natural products such as plant have been an integral part of ancient (Such as Chinese, Ayurvedic and Egyptian) traditional medicine systems [1]. Medicinal plant is any plant in which in one or more of its organs (stem, root, leaves, rhizomes, fruits, flower and seeds), contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi synthesis [2]. Such a plant (medicinal plant) will have such parts employed in the treatment or control of a disease condition and therefore contains biochemical components called phytochemicals that are of medical importance [3]. Phytochemicals are considered as bioactive substances of plant origin. They are regarded as secondary metabolites because they are of little need by the plant that manufactured them. The phytochemicals are naturally synthesized in all parts of plant such as bark, leaves stem, root, flower, fruits, seeds, etc [4]. Most of the drugs enlisted as orthodox medication were originally obtained from plant [5]. Many studies today confirmed that the herbs boost the immune system by stimulating the production of disease fighting white blood cells [6].

Sweet orange (*Citrus sinensis* (L.) Osbeck) is a small evergreen tree 7.5 m high and sometimes up to 15 m. Its origin is China and it has been cultivated over the years, but is grown commercially worldwide in tropics, semitropical and some warm temperate regions and has become the most widely planted tree fruit
in the world today according to Nicolosi et al. [7]. Citrus fruit products act as antimicrobial agents against the bacteria and fungus. The sweet orange product has an important and physiological role because of its commercial value in pharmaceutical and food industries of the entire world [8]. The antioxidant activity is also present in the plant materials due to the presence of many active phytochemicals such as flavonoids, vitamins, cumarins, terpenoids, carotenoids, saponin, lignin and plant sterols and so on [8]. The sweet orange fruits and their juices are an important source of bioactive methanol, the compound is important to human nutrition which include the antioxidant such as ascorbic acid, phenolic compound, flavonoids and pectins [9].

The present study was conducted to determine the phytochemical constituents, antibacterial activity and activity of aqueous and ethanol extracts of sweet orange peel on clinical isolates of Staphylococcus aureus and Salmonella typhi isolated from stool samples of typhoid fever patients attending Murtala Muhammad General Hospital Kano, Nigeria.

Collection, identification and Authentication of Orange Fruits

The plant used in this study (Citrus sinensis (L.) Osbeck) were obtained from fruit sellers at Dorayi quarters, Opposite Sar-kin Dutse Estate, Gwale Local Government Area in Kano state, Nigeria. After collection, the Orange fruits were identified and authenticated at the herbarium in the Department of Plants Science, Bayero University Kano with the following voucher number BUKHAN 0389, voucher specimens were deposited in the herbarium for future references. The fruits were washed, peeled and air dried at room temperature for 14 days. The dried orange peels were ground into fine powder using sterile pestle and mortar under laboratory condition and stored in container for further use.

Figure 1: Citrus plant with fruits

Bacteria isolates

Clinical isolates of Salmonella typhi and Staphylococcus aureus, were used in this study. The isolate was obtained from Microbi-ology laboratory of Murtala Muhammad General Hospital Kano, Nigeria. The bacteria were isolated from typhoid fever infected patient attending the Hospital. The isolates were diagnosed in the Hospital to the species level by using different laboratory procedures including; Gram’s stain, cultural characterization and Bi-ochemo tests include (Indole, Methyl red, Vogues Proskeaur, Catalase, Citrate utilization) for Salmonella typhi while catalase, coagulase and DNase test for Staphylococcus aureus. The isolates were maintained on Nutrient agar slants at 40C and transported to Laboratory of Science Laboratory Technology Department, School of Technology Kano for further use.

Extraction of Orange Peel

Aqueous and 80% ethanol solvents were used for extraction process of the phytochemical components of the orange peel. For aqueous extract, water extraction method as described by Ahmed and Beg [10] was employed. During the process, 100g of the ground peel was weighted and mixed with 500ml of distilled water in a sterile conical flask and kept for 4 days with intermittent shaking. The extract was filtered using Whatman filter paper and the filtrate was concentrated in water bath at 50°C. For ethanol, 100g of the powdered peel was extracted in 500ml of ethanol for 3 days. The mixture was filtered using Whatman No. 1 filter paper and the extract was evaporated to dryness using rotary evaporator at 40°C. The residue obtained was diluted using 10% Dimethylsulphoxide (DMSO) to produce 100 mg/ml of the extract from which various concentrations of 75, 50 and 25 mg/ml were produced.

Phytochemical Screening of the Extracts

Phytochemical screening was conducted using laboratory method as described by Soforowa [11]. This was done to determine the presence of alkaloid, saponin, steroid, glycoside, tannin, terpenoid, anthraquinone, flavonoid and reducing sugar in the aqueous and ethanol extracts of the orange peel.

Antibacterial Activity of the Extracts

Agar well diffusion method was adapted to determine the antibacterial activity of the orange peel extract against the test isolates in this study. During the process, 0.1ml of standardized organisms (0.5 MacFarland standard) were introduced onto the surface of Mueller Hinton agar in a sterile Petri dish and labelled accordingly. A sterile cork borer 5 mm was used to produce five wells at equal distance in the inoculated agar. The wells were filled with different concentrations of the extracts accordingly as 25, 50, 75 and 100mg/l while the last well contain 50mg/ml of standard antibiotic Gentamicin (Micro lab limited) which was used as positive control in the study. The agar plates were allowed to diffuse for a period of hour and incubated at 37°C for 24 hours. After then, the diameter of the zones of inhibition around each well was measured to the nearest millimetres [12].

Determination of Minimum Inhibitory Concentration (MIC) of the Extracts
The MIC of the extracts was determined using broth dilution technique. Two fold serial dilutions of the extracts were prepared by adding 2ml of 100mg/ml of the extract into a test tube containing 2ml of Nutrient broth, thus producing solution containing 50mg/ml of the extract. The process continued serially up to test tube No. 5, hence producing the following concentrations; 50, 25, 12.5, 6.25 3.125 mg/ml. Test tube No. 6 do not contain extracts and serve as negative control. Exactly 0.5 ml of 0.5 McFarland equivalent standards of test organisms were introduced into the test tubes and incubated at 370 C for 24 hours. After incubation the test tubes were observed for growth by checking for turbidity [13].

Determination of Minimum Bactericidal Concentration (MBC) of the Extracts

From the result of MIC, the test tubes that did not show visible growth were used for MBC determination. About 0.1 ml was aseptically transferred onto the surface of Mueller Hinton agar plates. The plates were incubated at 37°C for 24 hours. The MBC of the extracts was recorded as the lowest concentration of the extract that had less than 99% growth on Mueller Hinton agar plates [13].

Evaluation of activity index of the extracts

The activity index (AI) of the crude extracts was calculated as described by Vedpriya et al. [14]. The Activity index (AI) is expressed as follows;

\[
\text{Activity index (AI)} = \frac{\text{Mean zone of inhibition of the extracts (mm)}}{\text{Zone of inhibition obtained from standard drug (mm)}}
\]

Statistical Analysis

The data of average zone of inhibition produced by the isolates against the antibiotics used was analyzed using one way Analysis of Variance (ANOVA) with the aid of statistical program SPSS (Statistical package for Social Sciences) version 21.0. Significance level for the differences was set at p<0.05.

Results

Phytochemical Screening

Phytochemical screening of Citrus sinensis peel extracts in (Table 1) indicates the presence of alkaloid, tannin, saponin, glycoside, flavonoid, terpenoid, and Phenols while reducing sugar, steroid and anthraquinone were absent.

Antibacterial Activity of the Extracts

The antibacterial activity of aqueous and ethanolic extract of Citrus sinensis peel against Clinical isolates of Salmonella typhi and Staphylococcus aureus is presented in (Table 2). The result showed that the ethanol extract demonstrated higher activity of 19 mm at 100mg/ml. The zone of inhibition shown by the control (50 mg/ml Gentamicin) is found to be 24 mm

| S/N | Phytochemical                        | Aqueous extract | Ethanol extract |
|-----|-------------------------------------|-----------------|----------------|
| 1   | Alkaloids                            | +               | +              |
| 2   | Flavonoid                            | +               | +              |
| 3   | Glycosides                           | +               | +              |
| 4   | Reducing sugar                       | -               | -              |
| 5   | Saponin                              | +               | +              |
| 6   | Steroids                             | -               | -              |
| 7   | Phenols                              | +               | +              |
| 8   | Terpenoid                            | +               | +              |
| 9   | Anthraquinone                        | -               | -              |
| 10  | Tannin                               | +               | +              |

Key: + = presence of phytochemical, - = absent of phytochemical

| Extracts | Conc. (mg/ml) | Salmonella typhi | S. aureus |
|----------|--------------|------------------|-----------|
| PAE      | 25           | 07               | 10        |
|          | 50           | 08               | 12        |
|          | 75           | 13               | 15        |
|          | 100          | 17               | 16        |
|          | 25           | 10               | 12        |
| PEE      | 50           | 14               | 17        |
|          | 75           | 18               | 20        |
|          | 100          | 19               | 20        |
| Control  | 50           | 21               | 22        |

Key: PAE = Peel Aqueous Extract, PEE = Peel Ethanolic Extract.

Table 2: Antibacterial Activity of the Citrus sinensis Peel Extract against the isolate

MIC and MBC of the Extracts

The minimum inhibitory Concentration of aqueous and ethanol extract of orange peel is represented in Table 3. The result showed dilutions of various concentrations of aqueous and ethanol extracts can inhibit and/or kill the isolates. Lower MIC (3.125 mg/ml) was shown by ethanol extract than aqueous extract with 6.25 mg/ml. MBC of the extract ranges between 12.50 - 50mg/ml.
Activity index of the peel extracts

The activity index of the Orange (Citrus sinensis) peel extracts against standard antibiotic is presented in Table 4. The result showed that leaves ethanol extract has the highest activity index of 0.70 while the lowest activity is shown by leaves aqueous extract (0.57). The average activity index of the extracts is found to be 0.64.

| Extracts | Total ZOI | Average ZOI | Activity index |
|----------|-----------|-------------|----------------|
| PAE      | 98        | 12.25       | 0.57           |
| PEE      | 120       | 15.00       | 0.70           |
| Total    | 218       | 13.63       | 0.64           |

Key: PAE = Peel Aqueous Extract, PEE = Peel Ethanolic Extract, ZOI = zone of inhibition.

Table 4: Activity index of the extracts against standard antibiotic used

Discussion

The Phytochemical screening of the Citrus sinensis (Orange) peel extracts indicated the presence of alkaloid, tannin, saponin, glycoside, flavonoid, terpenoid, and phenols. The presence of the above phytochemicals in the plant parts was responsible for its antibacterial activity. Flavonoids have been shown to possess anti-inflammatory, anti-hepatotoxic and antimicrobial activities [15]. Saponins are known to possess antibacterial activities [16, 17] whilst tannins play an important role in wound healing and also possess some antimicrobial activities. According to this study, Alkaloid is present in the extracts. Alkaloid consists of large group of nitrogenous compound which are widely used as anticancer anesthetics and Central Nervous Stimulants. Alkaloids are known to play some metabolic roles and control development in living system. It also interferes with cell division, hence the presence of alkaloids in the Citrus sinensis (Orange) peel could account for their use as antimicrobial agents.

The antibacterial activity of the plant showed that the plant peel extracts demonstrated an antimicrobial effect against the test isolate with higher activity in ethanol extract compared to aqueous extract. The ethanolic peel extract had highest zone of inhibition of 20 mm at 100mg/ml against S. aureus while 19 mm for S. typhi, while aqueous extract had highest zone of inhibition of 17 mm at the same concentration for S. typhi while 16 mm against S. aureus at 100 mg/ml. This may be due to the better solubility of the active components in the organic solvent ( ethanol) than water which leads to better efficacy of the ethanol extracts. It suggests that the active component is more soluble in ethanol than in the other solvents. However, Doughari et al. [18] stated that the anti-microbial effect of the plant could be due to the bioactive compounds such as the Phytochemicals constituent present in the plant. The results showed that the potency of the orange peel extracts on the test isolates had different hierarchy of susceptibility among the organisms. The findings of this study indicated that Gram positive bacteria (S. aureus) was more sensitive to the extracts with average zone of inhibition of 15.25mm when compared to Gram negative (S. typhi) with average zone of inhibition of 12.00 mm. This could be attributed to presence of phospholipid layer in the Gram negative bacteria. Generally, against the isolated bacteria, higher concentration of the extract shows a greater zone of inhibition; this result is in agreement with the report of Bisno and Stevens [19] which states that the higher the concentration of antibacterial substance, the higher it shows an appreciable zone of inhibition. The antibacterial activity of aqueous extracts of peel, juice and leaves from fresh Citrus sinensis was evaluated against three Gram positive (Staphylococcus aureus, Streptococcus pyogenes and Enterococcus faecalis) and six Gram negative bacteria (Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Staphylococcus typhi, Proteus spp. and Moraxella catarrhalis). Citrus juices showed the highest activity against most of the studied bacteria isolates. According to the study, moderate activity was produced by Citrus peel and the lowest activity was produced by Citrus leaves extracts [20]. Minimum inhibitory concentration of aqueous and ethanol extract of orange peel showed dilutions of various concentrations of aqueous and ethanol of peel extracts can inhibit and/or kill the isolates. Lower MIC (3.125 mg/ml) was shown by ethanol extract than aqueous extract with 6.25 mg/ml. The MBC of the extracts ranged from 12.50 – 50.00 mg/ml

The activity index of the Citrus sinensis peel extracts against standard antibiotic is presented in Table 4. The result showed that leaves ethanol extract has the highest activity index of 0.70 while the lowest activity is shown by leaves aqueous extract (0.57). The average activity index of the extracts is found to be 0.64 which indicated that the extract can compete to the standard antibiotic used. Statistical analysis of the result revealed that the table value (p value at p < 0.05) is greater than the calculated value for analysis of variance between the extracts; therefore, there is no significant different in the activity of the two extracts against the isolates used, hence null hypothesis is accepted.
Conclusion

Phytochemical screening of the seeds extracts indicated the presence of presence of alkaloid, tannin, saponin, flavonoid and phenols, terpenoid, glycoside. The antibacterial activity of the peel extracts against Salmonella typhi and Staphylococcus aureus showed that the peel leaves extracts demonstrated an antimicrobial effect against the isolates. The Minimum inhibitory Concentration (MIC) of aqueous and ethanol extract of orange peel showed dilutions of various concentrations of aqueous and ethanol of peel extracts can inhibit and/or kill the isolates. The average activity index of the extract is found to be 0.64 which indicated that the extract can compete to the standard antibiotic used. Findings from this work support the use of extracts from Orange peel for medicinal purpose.

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