Antibacterial Activity of \textit{Citrus sinensis} and \textit{Solanum lycopersicum} on Wound Isolated from Hospitals in Kaduna Metropolis Kaduna Nigeria

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Abstract: The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has lead to the screening of several medicinal plants for their potential antimicrobial activity. The medicinal properties shown by different medicinal plants are due to the phytochemicals present in the plant. These phytochemicals are the most vital sources for the treatment of destructive diseases. Different phytochemicals have an extensive range of activities, which helps to enhance the immune system and give resistance against long term disease to protect the body from harmful pathogens. To determine the antibacterial activity of \textit{Citrus sinensis} and \textit{Solanum lycopersicum} on wound isolated from Hospitals in Kaduna Metropolis Kaduna Nigeria was the main purpose of this study. Ethanolic and aqueous extracts of powdered \textit{C. sinensis} peel and fresh fruit of \textit{S. lycopersicum} were used for the qualitative measurement of various phytochemicals present in these plants. The phytochemical screening of the extracts yielded positive results for carbohydrates, glycosides, cardiac glycosides, saponins, triterpene, tannins, flavonoids and alkaloids. Wound swab samples of patients from five (5) selected hospitals within Kaduna metropolis yielded \textit{Bacillus cereus}, \textit{Myroides Species} and \textit{Staphylococcus lentus}. The \textit{Citrus sinensis} ethanolic and aqueous extracts as well as the \textit{Solanum lycopersicum} ethanolic and aqueous extracts demonstrated a broad spectrum antibacterial activity against the three pathogenic bacteria of wound origin. The result of the tests indicate that orange peel ethanolic extract, tomato aqueous extract and tomato ethanolic extract showed the highest inhibition against \textit{Bacillus cereus} 22mm, 20mm and 15mm respectively while highest inhibition of orange peel aqueous extract 19.5mm was recorded for \textit{Myroides spp}. Lowest inhibition of tomato aqueous extract and tomato ethanolic extract were recorded for \textit{Staphylococcus lentus} 15.2mm and 13.17mm respectively. Orange peel ethanolic extract and orange peel aqueous extract did not inhibit the growth of \textit{Bacillus cereus} and \textit{Staphylococcus lentus} respectively. Extracts which exhibited high activities against one or several pathogenic wound isolates were further assayed for minimum inhibitory concentration (MIC). The present study concludes that orange peel ethanolic, tomato aqueous and tomato ethanolic extracts showed highest antibacterial activity against the organism \textit{Bacillus cereus} while orange peel aqueous showed highest antibacterial activity against \textit{Myroide spp}. and there was a significant difference in the level of inhibition among the organisms isolated.

Keywords: Ethanolic Extract, Aqueous Extract, Phytochemical, \textit{Bacillus cereus}, \textit{Myroides Species}, \textit{Staphylococcus lentus}, Antibacterial Activity, Minimum Inhibitory Concentration (MIC)

1. Introduction

The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity [1]. The secondary metabolites (phytochemicals) present in plants have been linked with the healing properties of plant [2, 3]. Herbs that play a role in the wound healing process encourages blood clotting, fights infections and accelerate the wound healing
process in general [4, 5].

2. Theoretical Background

2.1. Citrus Sinensis

Citrus sinensis (sweet orange) is one of the most important commercial fruit crops grown in all countries of the world. Citrus is a term commonly used for genus of flowering plants in the family Rutaceae originating in tropical and subtropical south-east regions of the world [6]. The group also encompasses other citrus fruits such as Citrus reticulata, Citrus viti, Citrus aurantifolia, Citrus medica and Citrus limonum [7]. Citrus fruits are commonly consumed because they contain a high amount of vitamins, minerals and antioxidant compounds, such as flavonoids. Flavonoids are a family of phenolic compounds that have many biological properties, including hepatoprotective, antithrombotic, antibacterial, antiviral and anticancer activity [8]. Citrus fruits are also low in fat and in overall dietary energy – a major consideration given the increasing rate of obesity in both adults and children. It also has a relatively low glycaemic index which helps in maintaining a more stable blood glucose level and generally healthier carbohydrate metabolism [9]. A decoction of the dried leaves and flowers was given in Italy as an antispasmodic, cardiac sedative, carminative. Decoction of husked orange seeds was used as antimicrobial [13], antioxidant [14], carminative, anti-yeast, antimutagenic agent [15]. Citrus peel waste are highly perishable and seasonal, is a problem to the processing industries and pollution monitoring agencies. There is always an increased attention in bringing useful products from waste materials and citrus wastes are no exception. Suitable methods have to be adopted to utilize them for conversion into value-added products [8].

2.2. Solanum Lycopersicum

Solanum lycopersicum (tomato) is one of the most important vegetables worldwide because of its high consumption, year round availability and large content of health related components [16]. Solanum lycopersicum belongs to the plant family solanaceae and genus solanum and it is spread throughout the world [17]. Solanum lycopersicum is used as juice, soup, puree, ketchup or paste [18]. In terms of human health, Solanum lycopersicum is believed to benefit the heart, among other organs. They contain the carotene lycopenone, one of the most powerful natural antioxidants. Lycopene has also been shown to improve the skin’s ability to protect against harmful UV rays [19]. In addition to its flavor properties, Solanum lycopersicum are reported to possess numerous beneficial nutritional and bioactive components that may also benefit human health. These include the nutrients vitamin A, vitamin C, iron, and potassium [20, 21]. Solanum lycopersicum are also an excellent source of free radical-scapenging bone-healthy vitamin K., vitamin B6 and folate. In addition, Solanum lycopersicum are a good source of heart-healthy magnesium, niacin, and vitamin E, vitamin B1, and phosphorus; muscle-building protein, and bone-healthy copper [22]. Nonnutritive digestible and indigestible dietary fiber; the antioxidant compounds; β-carotene and lutein [20] and the cholesterol lowering and immune system enhancing glycoalkaldoids tomatine and dehydrotomatine [23]. Consumption of tomatoes, tomato products, and isolated bioactive tomato ingredients is reported to be associated with lowered risk of cancer [24], heart disease [25], diabetes [26] and hypertension [27]. These considerations suggest that edible tomato contains antimicrobials which may have multiple benefits [28].


The Genus Myroides comprises yellow-pigmented, non-motile, Gram-negative, non- fermentative bacilli that releases a fruity odor during growth appears as lightly yellow-pigmented because of the presence of flexirubin pigment [29-32]. Myroides are aerobes, having a strictly respiratory type of metabolism with oxygen as the terminal electron acceptor. Good growth occurs on nutrient agar and MacConkey agar. No hemolysis occurs on blood agar. Growth occurs at 18–22 and 37°C, but not at 5 or 42°C. NaCl is not required, but growth occurs in the presence of at least 5% NaCl. Myroides species are widely distributed in nature [33- 36]. Though not part of the human microbiota, Myroides species are a rare source of human infection [37]. The organism has been isolated from urine, blood, wounds, and respiratory secretions [38, 39].

Antimicrobial susceptibility testing was done on Mueller- Hinton Agar by Kirby-Bauer disc diffusion method and interpreted as per Clinical Laboratory Standards Institute standards [40], using the zone diameters for Pseudomonas aeruginosa (as there are no established standards for Myroides spp.)[41].

2.4. Bacillus Cereus

Bacillus cereus is a Gram-positive, rod-shaped, aerobic, motile, beta hemolytic bacterium commonly found in soil and food. Some strains are harmful to humans and cause foodborne illness, while other strains can be beneficial as probiotics for animals [42, 43].
2.5. *Staphylococcus Lentus*

*Staphylococcus lentus* is a member of *Staphylococcus sciuri* group which are widespread in nature, and they can be isolated from a variety of farm animals, pets, and wild animals, as well as from various food products of animal origin [44, 45, 46]. Although they are principally associated with animals, members of the *S. sciuri* group may colonize humans, and it has been estimated that they may constitute 0.79 to 4.3% of the total number of coagulase-negative *Staphylococci* isolated from clinical samples [47, 48]. However, they have been associated with serious infections such as endocarditis [49], peritonitis [50], septic shock [51], urinary tract infection [52], endophthalmitis [53], pelvic inflammatory disease [52], and most frequently, wound infections [54].

3. Materials and Experiments

3.1. Plant Collection and Identification

Fresh *Citrus sinensis* and *Solanum lycopersicum* were obtained from Station and Mogadishu Markets Kaduna, Nigeria. The botanical identity of both *Citrus sinensis* and *Solanum lycopersicum* were further confirmed, authenticated and voucher specimen No: 990 and 2159 for *Citrus sinensis* and *Solanum lycopersicum* respectively were deposited at the Herbarium section of the Botany unit of the Department of Biological Sciences, Ahmadu Bello University Zaria, Kaduna, Nigeria.

3.2. Phytochemical Extraction Method

The fruits were taken to Laboratory and were washed in running tap water. Fruit surfaces were sterilized separately with 70% alcohol and then rinsed with sterile distilled water [55].

For extraction, surface sterilized *Citrus sinensis* peel was dried in an oven at 50°C for 48 hrs followed by grinding into a fine powder using a sterile blender. The powdered material was stored in air tight jars [56]. While fresh fruits of *Solanum lycopersicum* were blended in a sterile blender [57]. Two extractants i.e., water and 95% ethanol were used for the phytochemical extraction of both *Citrus sinensis* and *Solanum lycopersicum*.  

3.2.1. Extraction Protocols

According to [58], 1000g of the fine powder of the *Citrus sinensis* peels was weighed and percolated with 10000cm³ of 95% ethanol and water respectively and incubated in a shaker incubator at 250 rpm at 37°C for 24 hours. The percolates were then filtered using sterilized Whatman no.1 filter [55] and solvents (ethanol and water) evaporated at room temperature to obtain the ethanolic and aqueous extracts of the peels [59]. Same protocol was repeated for the fresh fruits of *Solanum lycopersicum*. The extracts were stored in a sterile bottle at room temperature.

3.2.2. Phytochemical Screening of the Plant Extract

The extracts were analysed for the presence of alkaloids, carbohydrates, flavonoids, saponins, tannins, steroids, glycosides, triterpenoids, phytosterols and cardiac glycosides.

3.3. Preparation of Extract Concentration

The stock solutions of the plant extracts were prepared in screw capped bijou bottles containing dimethyl sulphoxide (D. M. S. O). One gram of each extract was weighed on a mettler balance, and dissolved in 10cm³ of D.M.S.O to arrive at 10,000,000 µg/cm³ concentration of stock solution. Seven varied extract concentrations of both ethanolic and aqueous extracts of *Citrus sinensis* and *Solanum lycopersicum* were prepared at concentrations of 10; 5; 2.5; 1.3; 0.6; 0.3; 0.2 from the stock solution (10,000,000 µg/ml) using 2-fold serial dilution [55]. Extracts were obtained in semi-solid form.

3.4. Sample Collection, Transportation and Isolation

Wound swab samples of patients presented with, stitches, burn, boil, accident, abscess, cuts and injury were collected with the help of experienced Senior Nursing staff using sterile swab stick [60]. The samples were transported to the laboratory. The samples were processed using standard microbiological techniques [61]. The isolates were maintained on a freshly prepared nutrient agar slant and kept at 4°C until required for use.

3.5. Preparation of McFarland Turbidity Standard

Barium sulphate suspension at 1.0% w/v was prepared as follows: One percent (1% v/v) solution of sulphuric acid was prepared by adding 1cm³ of concentrated H₂SO₄ in 99cm³ of water. One percent (1% w/v) solution of barium chloride was also prepared by dissolving 0.5g of barium chloride in 50cm³ distilled water. Barium chloride solution (0.6cm³) was added to 99.4cm³ of sulphuric acid solution to yield 1.0% w/v barium suspension. The turbid solution formed was transferred into a test tube as the standard for comparison [62].

3.6. Standardisation of Bacterial Inoculum

For inoculum preparation density of wound isolated bacterial cultures was adjusted equal to that of 0.5 McFarland standard (1.5 x 10⁶ CFU/ml) by adding sterile distilled water. McFarland standard was used as a reference to adjust the turbidity of microbial suspension so that number of microorganisms will be within a given range. To aid comparison the test and standard were compared against a white background with a contrasting black line [63].

3.7. Determination of Antibacterial Activity

Antibacterial activity was tested on Mueller Hinton Agar (MHA). The antibacterial activity of four (4) crude extracts (aqueous and ethanolic) of two (2) fruits against three (3) wound isolates was evaluated by using agar well diffusion method [64, 65]. Plate count agar (PCA) plates were inoculated with 100µl of standardized inoculum (1.5 x10⁸ CFU/ml) of each bacterium (in triplicates) and spread with...
sterile swabs. Wells of 8 mm size were made with sterile borer into agar plates containing the bacterial inoculum and the lower portion was sealed with a little molten agar medium. 100µl volume of the fruit extract was poured into a well of inoculated plates. Sterilized distilled water was used instead of fruit extract. The plates thus prepared were left at room temperature for ten minutes allowing the diffusion of the extracts into the agar [55]. After incubation for 24 hrs at 37°C, the plates were observed. If antibacterial activity was present on the plates, it was indicated by an inhibition zone around the well containing the fruit extract. The diameter of inhibition zone (DIZ) was measured and expressed in millimetres. The mean values of the diameter of inhibition zones were calculated.

3.8. Determination of the Minimum Inhibitory Concentration (MIC)

Extrac.ts which exhibited high activities against one or several pathogenic wound isolates were further assayed for their minimum inhibitory concentration (MIC). This was carried out by the two fold serial dilution of the tested extracts in nutrient broth (2 ml volumes), then inoculated with 100µl inoculum size with the test organisms. The alcoholic and aqueous crude extracts were prepared at concentrations of 10; 5; 2.5; 1.3; 0.6; 0.3; 0.2% (w/v). The MIC was determined by the broth dilution method [55].

Mueller Hinton broth samples (10 ml) were inoculated with different concentrations of the crude extracts and with 100µl of active inoculum of bacterial wound isolates (1.5 x 10^8 CFU/ml) in tubes and incubated for 24 hrs at 37°C. The MIC was determined as the lowest concentration of the extract which inhibited the organism [66].

3.9. Statistical Analysis

The data obtained were subjected to descriptive statistical analysis such as mean, standard deviation and analysis of variance [67].

4. Results

Table 1. Physical Characteristics of Citrus sinensis Peel and Solanum lycopersicum Fruit Extracts.

<table>
<thead>
<tr>
<th>Plant Part</th>
<th>Solvent</th>
<th>Initial Weight (g)</th>
<th>Final Weight (g)</th>
<th>Colour</th>
<th>Odour</th>
<th>Texture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orange Peel</td>
<td>Ethanol</td>
<td>200</td>
<td>90.23</td>
<td>Orange</td>
<td>Fruity</td>
<td>Oily</td>
</tr>
<tr>
<td>Orange Peel</td>
<td>Aqueous</td>
<td>200</td>
<td>102.45</td>
<td>Orange</td>
<td>Fruity</td>
<td>Oily</td>
</tr>
<tr>
<td>Tomato Fruit</td>
<td>Ethanol</td>
<td>1000</td>
<td>171.42</td>
<td>Red</td>
<td>Fruity</td>
<td>Smooth</td>
</tr>
<tr>
<td>Tomato Fruit</td>
<td>Aqueous</td>
<td>1000</td>
<td>90.18</td>
<td>Red</td>
<td>Fruity</td>
<td>Smooth</td>
</tr>
</tbody>
</table>

Table 2. Qualitative Analysis of Phytochemicals in Citrus sinensis Peel Extracts and Solanum lycopersicum Fruit Extracts.

<table>
<thead>
<tr>
<th>Constituent</th>
<th>OPE</th>
<th>OPA</th>
<th>TOE</th>
<th>TOA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac Glycosides</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Triterpene</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

KEY: OPE= Orange Peel Ethanic, OPA=Orange Peel Aqueous, TOE= Tomato Ethanolic, TOA= Tomato aqueous, + = Present, - = Absent

Table 3. Colonial and Cell Morphology of Bacterial Cultures Isolated from Wound Samples.

<table>
<thead>
<tr>
<th>Colonial Characteristics</th>
<th>Grams Nature and Morphology</th>
<th>Capsule</th>
<th>Spore</th>
<th>Motility</th>
<th>Flagellum</th>
<th>Probable Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smooth, circular and golden yellow colonies</td>
<td>Gram positive oval cells in bunches</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Staphylococcus spp</td>
</tr>
<tr>
<td>Smooth yellow colonies with fruity odour</td>
<td>Gram negative rods</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>Myroides spp</td>
</tr>
<tr>
<td>Dark grey and opaque colonies with a rough matted surface and zones of beta-hemolysis</td>
<td>Gram positive rods</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Bacillus spp</td>
</tr>
</tbody>
</table>

KEY: ++Present, - = Absent

Table 4. Biochemical and Sugar Fermentation Test of Bacterial Cultures Isolated from Wound Samples.

<table>
<thead>
<tr>
<th>Cat</th>
<th>Coag</th>
<th>In</th>
<th>MR</th>
<th>VP</th>
<th>Cit</th>
<th>Urease</th>
<th>H₂S</th>
<th>Glu</th>
<th>Suc</th>
<th>Lac</th>
<th>Probable Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Staphylococcus Spp</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Myroides Spp</td>
</tr>
</tbody>
</table>

KEY: Oxi=oxidase; Coag=coagulase; In =indole; MR=methyl red; VP=voges proskauer; Cit=citrate; H₂S=hydrogen sulphide; Glu=glucose; Suc=sucrose; Lac=lactose; ++ positive; = negative.
pathogens have been reported [70-77]. Properties, together with alkaloids and tannins in synergistic manner, are responsible for growth inhibition of the microorganisms. Results of the current study indicate that plants are important sources of antimicrobial agents and phytochemical characteristics of Citrus sinensis and Solanum lycopersicum were evaluated. Both ethanolic and aqueous extracts of Citrus sinensis and Solanum lycopersicum had effects on the bacterial wound isolates. The extracts of Citrus sinensis and Solanum lycopersicum had minimum inhibitory concentration on the wound bacterial isolates.

Tannins have been reported to reversibly form complexes with proline rich proteins resulting in the inhibition of cell protein synthesis as well as production of typical tanning effect which is important in treating inflamed or ulcerated tissues, burns, wounds, pneumonia and dysentery [68]. Saponins and flavonoids in plant materials exert antibacterial properties, together with alkaloids and tannins in synergistic manner, are responsible for growth inhibition of the pathogens [69].

Bacillus cereus, Myroides spp and Staphylococcus lentus were isolated from wound in the present study. Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli and Staphylococcus aureus were isolated from patients with wound infections in the work carried out by [55]. Similar pathogenic organisms have been reported [70-77].

In the present study, orange peel ethanolic extract, tomato aqueous extract and tomato ethanolic extract showed the highest inhibition against Bacillus cereus 22mm, 20mm and 15mm respectively while highest inhibition of orange peel aqueous extract 19.5mm was recorded for Myroides spp. Lowest inhibition of tomato aqueous extract and tomato ethanolic extract were recorded for Staphylococcus lentus 15.2mm and 13.17mm respectively. Orange peel ethanolic extract and orange peel aqueous extract did not inhibit the growth of Bacillus cereus and Staphylococcus lentus respectively, this result is in agreement with the work of [59, 78-81]. That the extracts inhibited the growth of the isolates is an indication that they contain substance(s) that are active against bacterial species [82, 83, 84]. That Orange peel ethanolic extract and orange peel aqueous extract did not inhibit the growth of Bacillus cereus and Staphylococcus lentus respectively maybe due to the fact that the bacteria possess mechanisms for detoxifying or removing the active principles. The observed antibacterial activities of the extracts may be due to alkaloids, carbohydrates, flavonoids, saponins, tannins, steroids, glycosides, triterpenoids, phytosterols and cardiac glycocoydes identified in the ethanolic and aqueous extracts of Citrus sinensis and Solanum lycopersicum.

Ethanol extracts of all the fruits used exhibited higher antibacterial effect than aqueous extracts [8, 55]. The water solvent was ineffective in extracting the components of the fruit peels [8] which contradict the present study where orange peel aqueous extract was ranked second highest with 21.85mm zone of inhibition. The present study indicate that Citrus sinensis peel extracts have appropriate antimicrobial effects on Gram positive and Gram negative bacteria which is in line with the work of [85]. Preparing extracts in this study with organic solvents was shown to provide a better antibacterial activity in accordance with the results obtained by [8, 86, 87].

In a research where citrus juice, peel and leaves were used, results showed that citrus juices had the highest antibacterial activity against most of the studied bacterial isolates, moderate activity produced by citrus peel while citrus leaves showed the lowest activity produced [88].

A research was conducted using both ripe and unripe C. sinensis peels extract [84]. The unripe C. sinensis peels extract showed strong inhibition on the isolates. Its highest inhibition was on Pseudomonas aeruginosa (12.75mm), followed by S. aureus (11.00mm), but no effect on E. coli. The ripe peel of C. sinensis had no effect whatsoever on any of the wound pathogens while the positive control antibiotics, gentamicin inhibited all the isolates moderately with the most successful being S. aureus and the least successful being E. coli. This result however has proved that Gram negative organisms are generally more resistant to antimicrobial

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### Table 5. Antibacterial Activity of Citrus sinensis Peel and Solanum lycopersicum Extracts against Some Clinical Bacterial Wound Isolates.

<table>
<thead>
<tr>
<th>Bacteria Isolate</th>
<th>Average zone of inhibition (mm)</th>
<th>MIC Value (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus cereus</td>
<td>OPA: 22±0.0, TOA: 20±0.87, TOE: 15±0.0</td>
<td></td>
</tr>
<tr>
<td>Myroides species</td>
<td>OPA: 19.5±0.58, TOA: 16.07±0.12, TOE: 10.67±0.29</td>
<td></td>
</tr>
<tr>
<td>S. lentus</td>
<td>OPA: NI, TOA: 15.2±0.2, TOE: 13.17±0.16</td>
<td></td>
</tr>
</tbody>
</table>

*mean ± std (mm)*

### Table 6. Minimum Inhibitory Concentration (MIC) of Citrus sinensis Peel and Solanum lycopersicum Extracts against Some Clinical Bacterial Isolates.

<table>
<thead>
<tr>
<th>Fruit Extract</th>
<th>Test Organism</th>
<th>MIC Value (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPA</td>
<td>Myroides spp</td>
<td>0.25</td>
</tr>
<tr>
<td>OPE</td>
<td>Bacillus cereus</td>
<td>0.13</td>
</tr>
<tr>
<td>TOA</td>
<td>Bacillus cereus</td>
<td>0.13</td>
</tr>
<tr>
<td>TOE</td>
<td>Bacillus cereus</td>
<td>0.13</td>
</tr>
</tbody>
</table>

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5. Discussion

Microorganisms develop resistance even to the most powerful antimicrobial agents. Results of the current study on the “Antibacterial Activity of Citrus sinensis and Solanum lycopersicum on Wound Isolates” indicate that plants are important sources of antimicrobial agents and phytochemical characteristics of Citrus sinensis and Solanum lycopersicum were evaluated. Both ethanolic and aqueous extracts of Citrus sinensis and Solanum lycopersicum had effects on the bacterial wound isolates. The extracts of Citrus sinensis and Solanum lycopersicum had minimum inhibitory concentration on the wound bacterial isolates.

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Ethanol extracts of all the fruits used exhibited higher antibacterial effect than aqueous extracts [8, 55]. The water solvent was ineffective in extracting the components of the fruit peels [8] which contradict the present study where orange peel aqueous extract was ranked second highest with 21.85mm zone of inhibition. The present study indicate that Citrus sinensis peel extracts have appropriate antimicrobial effects on Gram positive and Gram negative bacteria which is in line with the work of [85]. Preparing extracts in this study with organic solvents was shown to provide a better antibacterial activity in accordance with the results obtained by [8, 86, 87].

In a research where citrus juice, peel and leaves were used, results showed that citrus juices had the highest antibacterial activity against most of the studied bacterial isolates, moderate activity produced by citrus peel while citrus leaves showed the lowest activity produced [88].

A research was conducted using both ripe and unripe C. sinensis peels extract [84]. The unripe C. sinensis peels extract showed strong inhibition on the isolates. Its highest inhibition was on Pseudomonas aeruginosa (12.75mm), followed by S. aureus (11.00mm), but no effect on E. coli. The ripe peel of C. sinensis had no effect whatsoever on any of the wound pathogens while the positive control antibiotics, gentamicin inhibited all the isolates moderately with the most successful being S. aureus and the least successful being E. coli. This result however has proved that Gram negative organisms are generally more resistant to antimicrobial
agents, probably due to their complex cell wall structure as well as possession of antibiotic resistance plasmids and production of enzymes called Extended Spectrum Beta Lactamases (ESBL) by organisms such as *E. coli* [89]. The ripe extract of *C. sinensis* peels did not show any antimicrobial activity throughout the study. This therefore may be due to the higher concentration of aliphatic aldehydes and oxygen containing monoterpenes and sesquiterpenes with little antimicrobial potentials than the peels of the unripe *C. sinensis*. The pH of the unripe *C. sinensis* was 4.8 while the pH of the ripe *C. sinensis* was 6.8. The acidic nature of the unripe peel extract could be responsible for the antibacterial activity evident by the work of [90] where they observed that of caffeic acid with pH 4.0 was enough to inhibit the growth of some of the studied microorganisms’ whole pH requirements range from 5.0 to 7.0. As observed from the preliminary phytochemical screening result, the unripe peel extracts contains alkaloids, tannins, saponins, phenol, cyanogenic glycosides and flavonoids.

*Solanum lycopersicum* extracts (ethanolic and aqueous) in the present study indicated the growth of all the isolates tested, the growth of *B. cereus* was inhibited to a greater extent compared to the *Myroides* spp and *S. lentus*. *Solanum lycopersicum* proved to be effective against microorganisms such as *Staphylococcus aureus*, *Proteus*, *Bacillus* & antifungal *Candida albicans*, *Aspergillus niger* [91, 92]. In the study by [28], it was shown that tomato extract produced antimicrobial effect on some Gram-negative microorganisms selected, the results obtained showed antibacterial action is linked with the presence of active compound of tomato extract on some of the isolated microbes *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Acinetobacter sp*. But tomato extract has higher antibacterial properties when combined with other substance like honey, the zone of inhibition was higher when the extract was diluted with honey [16, 55, 93].

In the present study, t-test result of the antibacterial activity of orange peel aqueous extract on the bacterial isolates indicates that orange peel aqueous (OPA) extract has the highest antibacterial activity against *Myroides* spp (21.83mm) as compared to *staphylococcus lentus* (12mm). The result shows that there is a significant difference in the level of inhibition among the organisms isolated (\(t_{cal}= 29.5, P<0.05, df=4\)).

The t-test result of antibacterial activity of orange peel ethanolic extract on the bacterial isolates indicates that orange peel ethanolic (OPE) extract has the highest antibacterial activity against *Bacillus cereus* (22mm) as compared to *Myroides* spp (19.5mm). The result shows that there is a significant difference in the level of inhibition among the organisms isolated (\(t_{cal}= 74.0, P<0.05, df=4\)).

The result of the analysis of variance (ANOVA) on Tomato aqueous extracts indicates that there is significant difference in the level of inhibition among the three organisms isolated (\(F_{cal}=73.311, P<0.05, df=2\)).

In the Scheffe’s Post Hoc test, the result reveals that the average zone inhibition of tomato aqueous extract against *Bacillus Cereus* significantly differs with that of *Myroides* spp (mean difference= 3.93, \(P<0.05\)) and *staphylococcus lentus* (mean difference=4.80, \(P<0.05\)). Tomato aqueous extract shows no evidence of significant difference in the level of inhibition between *Myroides* spp and *staphylococcus lentus* (mean difference=0.87, \(P>0.05\)).

The result of the analysis of variance (ANOVA) on Tomato ethanolic extract indicates that there is significant difference in the level of inhibition among the three organisms isolated (\(F_{cal}=399.22, P<0.05, df=2\)).

In the Scheffe’s Post Hoc test, the result reveals that the average zone inhibition of tomato ethanolic extract against *Bacillus Cereus* significantly differs with that of *Myroides* spp (mean difference= 4.33, \(P<0.05\)) and *staphylococcus lentus* (mean difference=1.83, \(P<0.05\)). Also, the extract shows evidence of significant difference in the level of inhibition between *Myroides* spp and *staphylococcus lentus* (mean difference=2.50, \(P>0.05\)).

### 6. Conclusion

The evaluation of phytochemical characteristics in this study reveals the presence of various phytochemicals such as carbohydrates, glycosides, cardiac glycosides, triterpene, tannins, flavonoids alkaloids anthraquinones, saponins and steroids in both *Citrus sinensis* and *Solanum lycopersicum* aqueous and ethanolic extracts. Three (3) bacterial wound isolates were characterized. The bacteria include *Bacillus cereus*, *Myroides* spp and *Staphylococcus lentus*. The *Citrus sinensis* ethanolic and aqueous extracts as well as the *Solanum lycopersicum* ethanolic and aqueous extracts demonstrated a broad spectrum antibacterial activity against the three pathogenic bacteria of wound origin. The present study concludes that Orange peel ethanolic, Tomato aqueous and Tomato ethanolic extracts showed highest inhibition against the organism *Bacillus cereus* while Orange peel aqueous showed highest inhibition against *Myroides* spp. The t-test result indicates that *Citrus sinensis* peel aqueous (OPA) extract has the highest antibacterial activity against *Myroides* spp (21.83mm) as compared to *Staphylococcus lentus* (12mm). The result shows that there is a significant difference in the level of inhibition among the organisms isolated (\(t_{cal}=29.5, P<0.05, df=4\)). The t-test result of *Citrus sinensis* ethanolic extract indicates that orange peel ethanolic (OPE) extract has the highest antibacterial activity against *Bacillus cereus* (22mm) as compared to *Myroides* spp (19.5mm). The result shows that there is a significant difference in the level of inhibition among the organisms isolated (\(t_{cal}=74.0, P<0.05, df=4\)). Tomato aqueous extract shows no evidence of significant difference in the level of inhibition between *Myroides* spp and *Staphylococcus lentus* (mean difference=0.87, \(P>0.05\)). Also, the extract shows evidence of significant difference in the level of inhibition between *Myroides* spp and *Staphylococcus lentus* (mean difference=2.50, \(P>0.05\)). Minimum inhibitory concentration of the extracts were also determined. OPA MIC value 0.25 *Myroides* spp, OPE, TOA and TOE 0.13 *Bacillus cereus*
7. Recommendation

The result obtained from the current study has proven the antibacterial activity of Citrus sinensis and Solanum lycopersicum extracts on wound isolates. It is therefore recommended that further studies can be carried out in order to fractionize and incorporate fruit extracts into synthetic drugs and ointments and also test them on wound isolates and some other isolates.

References


Abdulrashid Maz’uma: Antibacterial Activity of *Citrus sinensis* and *Solanum lycopersicum* on Wound Isolated from Hospitals in Kaduna Metropolis Kaduna Nigeria


