

Antibacterial Activity of *Citrus sinensis* and *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 led 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 *Citrus sinensis* and *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 *C. sinensis* peel and fresh fruit of *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 *Bacillus cereus*, *Myroides Species* 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 result of the tests indicate that 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. 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 *Bacillus cereus* while orange peel aqueous showed highest antibacterial activity against *Myroide spp.* and there was a significant difference in the level of inhibition among the organisms isolated.

Keywords: Ethanolic Extract, Aqueous Extract, Phytochemical, *Bacillus cereus*, *Myroides Species*, *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 sub-tropical south-east regions of the world [6]. The group also encompasses other citrus fruits such as *Citrus reticulata*, *Citrus vitis*, *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, antiemetic, digestive and remedy for flatulence. The inner bark, macerated and infused in wine, was taken as a tonic and carminative. Decoction of husked orange seeds was prescribed for urinary ailments in China and the juice of fresh orange leaves or a decoction of the dried leaves was taken as a carminative or emmenagogue or applied on sores and ulcers. An orange seed extract was given as a treatment for malaria in Ecuador but it was known to cause respiratory depression and a strong contraction of the spleen [10]. Peels represent between 50 to 65% of total weight of the fruits and remain as the primary byproduct [11]. *Citrus sinensis* peels contains volatile essential oils which are said to be effective in inhibiting microbial growth and in disinfecting wounds; among its other medicinal capabilities [12]. It has also been used as antimicrobial [13], antioxidant [14], carminative, insect repellent, antibacterial, larvicidal, antiviral, uricosuric, anti-yeast, antihepatotoxic and 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 lycopene, 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-scavenging 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 antioxidative compounds: β -carotene and lutein [20] and the cholesterol lowering and immune system enhancing glycoalkaloids 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].

2.3. *Myroides Spp.*

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 sulphur oxide (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 10⁸ 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 as a negative control which was introduced into a well 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 surrounding 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)

Extracts 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 (1.5×10^8 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.

Plant Part	Solvent	Initial Weight (g)	Final Weight (g)	Colour	Odour	Texture
Orange Peel	Ethanol	200	90.23	Orange	Fruity	Oily
Orange Peel	Aqueous	200	102.45	Orange	Fruity	Oily
Tomato Fruit	Ethanol	1000	171.42	Red	Fruity	Smooth
Tomato Fruit	Aqueous	1000	90.18	Red	Fruity	Smooth

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

Constituent	OPE	OPA	TOE	TOA
Carbohydrates	+	+	+	+
Antraquinones	-	-	-	-
Glycosides	+	+	+	+
Cardiac Glycosides	+	+	+	+
Saponins	-	-	+	-
Steroids	-	-	-	-
Triterpene	+	+	+	+
Tannins	+	-	-	-
Flavonoid	+	+	-	+
Alkaloid	+	+	+	+

KEY: OPE= Orange Peel Ethanolic, 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.

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

KEY: +=Present, - = Absent

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

Cat	Coag	In	MR	VP	Cit	Urease	H ₂ S	Glu	Suc	Lac	Probable Species
+	+	-	+	-	-	-	+	+	+	+	<i>Staphylococcus</i> Spp
+	+	-	+	-	+	+	+	+	-	-	<i>Myroides</i> Spp
+	-	-	-	+	+	-	-	+	-	-	<i>Bacillus</i> Spp

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.

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

Bacteria Isolate	Average zone of inhibition (mm)			
	OPA	OPE	TOA	TOE
<i>Bacillus cereus</i>	NI	22±0.0	20±0.87	15±0.0
<i>Myroides species</i>	21.83±0.29	19.5±0.58	16.07±0.12	10.67±0.29
<i>S. lentus</i>	12±0.50	NI	15.2±0.2	13.17±0.16

KEY: OPE= Orange Peel Ethanolic, OPA: Orange Peel Aqueous, TOE= Tomato Ethanolic, TOA= Tomato aqueous, += Present, - = Absent, NI= No Inhibition, *mean ± std (mm)

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

Fruit Extract	Test Organism	MIC Value (mg/ml)
OPA	<i>Myroides spp</i>	0.25
OPE	<i>Bacillus cereus</i>	0.13
TOA	<i>Bacillus cereus</i>	0.13
TOE	<i>Bacillus cereus</i>	0.13

KEY: OPE= Orange Peel Ethanolic, OPA: Orange Peel Aqueous, TOE= Tomato Ethanolic, TOA= Tomato aqueous

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.

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 glycosides identified in the ethanolic and aqueous extracts of *Citrus sinensis* and *Solanum lycopersicum*.

Ethanolic 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 inhibited 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

- [1] Westh, H., Zinn, C. S. and Rosdahl, V. T. (2004). An International Multi Cancer Study of Antimicrobial Consumption and Resistance in *S. aureus* Isolated from 15 hospitals in 14 Countries. *Journal of Microbial Drug Resistance*. 10: 169-176.
- [2] Stafford, G. I., Jäger, A. K. and van Staden J. (2004). Effect of Storage on the Chemical Composition and Biological Activity of Several Popular South African Medicinal Plants. *Journal of Ethnopharmacology*. 97: 107-115.
- [3] Parekh, J. and Chanda S. (2007). In Vitro Antimicrobial Activity of *Trapa nantans* L. Fruit Rind Extracted in Different Solvents. *African Journal of Biotechnology*. 6: 776-770.
- [4] O'meara, S. N., Callum, N. A., Majid, M. and Sheldon, T. A. (2001). Systematic Review of Antimicrobial Agents Used for Chronic Wounds. *British Journal of Surgery*. 88 (1): 421.
- [5] Parekh, J. and Chanda S. (2007). In Vitro Antimicrobial Activity of *nantans* L. Fruit Rind Extracted in Different Solvents. *African Journal Biotechnology*. 6: 776 -770.
- [6] Obi, R. K., Nwanebu, E. C., Ndubuisi-Nnaji U. U., Onuoha, L. N and Chiegboka, N. (2011). Ethanolic Extraction and Phytochemical Screening of Two Nigerian Herbs on Pathogens Isolated from Wound Infections. *International Journal of Comprehensive Pharmacy*. 10 (2): 1-5.
- [7] Scazzocchio, F., Cometa, M. F., Tomassini, L. and Palmery, M. (2001). Antibacterial Activity of *Hydrastis canadensis* Extract and Its Major Isolated Alkaloids. *Journal of Plant Medicine*. 67: 561-563.
- [8] Okwi, D. E. and Emenike, I. N. (2006). Evaluation of the Phytonutrients and Vitamins Contents of *Citrus* Fruits. *International Journal of Molecular Medicine and Advance Sciences*. 2 (1): 1-6.
- [9] Nisha, N. S., Anu, S. A. and Syed N. R. J. (2013). Antibacterial Activity of *Citrus sinensis* Peel against Enteric Pathogens. *International Journal of Pharmaceutical and Biological Science* 2 (5): 1-13.
- [10] Al- Snafi, A. E. (2013). Nutritional Value and Pharmacological Importance of *Citrus species* Grown in Iraq. *IOSR Journal of Pharmacy*. 6 (8): 76- 108.
- [11] Milind, P. and Dev, C. (2012). Orange: Range of Benefits. *International Journal Research Pharmacy*. 3 (7): 59-63.
- [12] Mandalari, G., Bennett, R. N., Bisignano, G., Saija, A., Dugo, G., Faulds, C. B. and Waldron K. W. (2006). Characterization of Flavonoids and Pectin from Bergamot (*Bergamia risso*) Peel: A Major Byproduct of Essential Oil Extraction. *Journal of Agricultural and Food Chemistry*. 54: 197-203.
- [13] Roger, G. D. P. (2002). Education and Health Library (editorial). *Encyclopedia of Medicinal Plants*. Safeliz SL. Spain. 1: 153-154.
- [14] Omobuwajo, O. R., Gbolade, A. A., Nia, R. and Adewoyin, F. B. (2005). The 11th Symposium of the Natural Product Research Network for Eastern and Central Africa (NAPRECA) August 12th-19th. Madagascar, Antananarivo. 72.
- [15] Kumar A. K. (2011). Antimicrobial Activity and Phytochemical Analysis of Citrus Fruit Peels Utilization of Fruit Waste. *International Journal of Engineering Science and Technology*. 3 (6): 5414- 5421.
- [16] Kanaze, F. I., Termentzi, A., Gabrieli C., Niopas, I., Georgarakis, M. and Kokkalou, E. (2008). The phytochemical Analysis and Antioxidant Activity Assessment of Peel (*Citrus sinensis*) Cultivated in Greece-Crete indicates a New Source Hesperidin. *Journal of Biomedical Chromatography*. 23: 239-249.
- [17] Krishna, M. J., Bhaumik, A. and Kumar, S. P. (2013). Phytochemical Analysis and Antimicrobial Studies of Various Extracts of Tomato (*Solanum lycopersicum* L.). *Scholars Academic Journal of Biosciences* (SAJB). 1 (2): 34-38.
- [18] Sarah, C. D., Sandra, K. and Iris, E. P. (2003). Taxonomy of Tomatoes in the Galápagos Islands: Native and Introduced Species of *Solanum Lycopersicon* (Solanaceae). *Journal of Systematics and Biodiversity*. 1 (1): 29-53.
- [19] Dilis, B. and Trichopoulou, A. (2010). *The Journal of Nutrition*. Bethesda. 140 (7): 1274- 1279.
- [20] Dorais, M., Ehret, D. L. and Papadopoulos, A. P. (2008) Tomato (*Solanum lycopersicum*) Health Components: from the Seed to the Consumer. *Journal of Phytochemical Reviews*. 1-20.
- [21] Rajkumar, S. and Jebanesan, A. (2004). Ovicidal Activity of *Solanum trilobatum* Linn (Solanaceae); Leaf Extract against *Culex quinquefasciatus* Say and *Culex triaeniorhynchus* Gile (Diptera: Culicidae). *International Journal of Tropical Science*. 24: 340-342.
- [22] Frusciante, L., Carli, P., Ercolano, M. R., Pernice, R., Di Matteo, A., Fogliano, V. and Pellegrini, N. (2007). Antioxidant Nutritional Quality of Tomato. *Journal of Molecular Nutrition and Food Research*. 51 (5): 609-617.
- [23] Jacob, K., Garcia, F. J. and Ros, G. (2010). *Archivos Latinoamericanos De. Nutricion* 60 (2): 192.
- [24] Morrow, W. J. W., Yang, Y-W and Sheikh, N. A. (2004). Immunobiology of the Tomatine Adjuvant Vaccine. 22 (19): 2380 -2384. DOI: 10.1016/j.vaccine.2004.03.022.
- [25] Friedman, M. Henika, P. R., Levin, C. E., Mandrell, R. E. (2007). Recipes for Antimicrobial Wine Marinades against *Bacillus cereus*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella enterica*. *Journal of Food Science*. 72: M207-M213.
- [26] Willcox, J. K., Catignani, G. L. And Lazarus, S. (2003). Tomatoes and Cardiovascular Health. *Journal of Critical Reviews on Food Science and Nutrition*. 43 (1): 1-18.
- [27] Bose, K. S. C. and Agrawal, B. K. (2007). Effect of Short Term Supplementation of Tomatoes on Antioxidant Enzymes and Lipid Peroxidation in Type-II Diabetes. *Indian Journal of Clinical Biochemistry*. 22 (1): 95-98.

- [27] Engelhard, Y. N., Gazer, B. and Paran, E. (2006). Natural Antioxidants from Tomato Extract Reduce Blood Pressure in Patients with Grade-I Hypertension: A Double-Blind, Placebo-controlled Pilot Study. *American Heart Journal*. 151 (1): 100.
- [28] Al- Oqaili, R. M. S., MohammedIstabreq, B. B. and Salman, M. A. (2014). In Vitro Antibacterial Activity of *Solanum lycopersicum* Extract against Some Pathogenic Bacteria. *Journal of Food Science and Quality Management*. 27: 12-17.
- [29] Holmes, B., Snell, J. J. S., Lapage, S. P., (1977). Revised Description, from Clinical Isolates, of *Flavobacterium odoratum* Stutzer and Kwaschnina 1929, and Designation of the Neotype Strain. *International Journal of Systematic Bacteriology*. 27 (4): 330–336.
- [30] Bernardet, J.-F., Segers, P., Vancanneyt, M., Berthe, F., Kersters, K. and Vandamme, P. (1996). Cutting a Gordian Knot: Emended Classification and Description of the Genus *Flavobacterium*, Emended Description of the Family Flavobacteriaceae, and Proposal of *Flavobacterium hydatidis* nom. nov. (Basonym, *Cytophaga Aquatilis* Strohl and Tait 1978). *International Journal of Systematic Bacteriology*. 46: 128–148.
- [31] Cho, S. H., Chae, S. H., Im, W. T. (2011). *Myroides marinus* sp. nov., a Member of the Family Flavobacteriaceae, Isolated from Seawater. *International Journal of Systematic Evolution in Microbiology*. 61 (4): 938-947.
- [32] Maraki, S., Sarchianaki, E., Barbagadakis, S., (2012). *Myroides odoratimimus* Soft Tissue Infection in an Immunocompetent Child Following a Pig Bite: Case Report and Literature Review. *Brazilian Journal of Infectious Diseases*. 16 (4): 390-392.
- [33] Mammeri, H., Bellais, S., Nordmann, P., (2002). Chromosome- encoded β -lactamases TUS-1 and MUS-1 from *Myroides odoratus* and *Myroides odoratimimus* (formerly *Flavobacterium odoratum*), New Members of the Lineage of Molecular Subclass B1 Metalloenzymes. *Journal of Antimicrobial Agents and Chemotherapy*. 46 (11): 3561- 3567.
- [34] Ktari, S., Mnif, B., Koubaa, M. (2012). Nosocomial Outbreak of *Myroides odoratimimus* Urinary Tract Infection in a Tunisian Hospital. *Journal of Hospital Infection*. 80 (1): 77-81.
- [35] Suganthi, R., Priya, T. S., Saranya, A. (2013). Relationship between Plasmid Occurrence and Antibiotic Resistance in *Myroides odoratimimus* SKS05-GRD Isolated from Raw Chicken Meat. *International Journal of Microbiology and Biotechnology*. 29 (6): 983 990.
- [36] Ravindran, C., Varatharajan, G. R., Raju, R. (2015). Infection and Pathogenicity of *Myroides odoratimimus* (NIOCR-12) Isolated from the Gut of Grey Mullet (*Mugil cephalus* (Linnaeus, 1758)). *Journal of Microbial Pathology*. 88: 22-28.
- [37] Tiana, R., Yazdani, E., Dhiman, N., Benavides, R. and Spak, C. W. (2015). *Myroides odoratimimus* Bacteremia in a Diabetic Patient. *Baylor University Medical Center Proceedings*. 28 (3): 342–343.
- [38] Holmes, B., Snell, J. J. & Lapage, S. P. (1979). *Flavobacterium odoratum*: A Species Resistant to a Wide Range of Antimicrobial Agents. *Journal of Clinical Pathology* 32, 73–77.
- [39] Yağci, A., Cerikçioğlu, N., Kaufmann, M. E., Malnick, H., Söyletir, G., Babacan, F. and Pit, T. L. (2000). Molecular Typing of *Myroides odoratimimus* (*Flavobacterium odoratum*) Urinary Tract Infections in a Turkish Hospital. *European Journal of Clinical and Microbiological Infectious Diseases*. 19 (9): 731–732.
- [40] Clinical and Laboratory Standard Institute (CLSI) (2013). Performance Standards for Antimicrobial Susceptibility Testing, 23rd Informational Supplement M100-S23. Wayne, PA: Clinical and Laboratory Standards Institute.
- [41] Elantamilan, D., Lyngdoh, V. W., Choudhury, B., Khyriem A. B. and Rajbongshi J. (2015). Septicaemia Caused by *Myroides* spp.: A Case Report. *Journal of Medical Microbiology Case Reports*. DOI 10.1099/jmmcr.0.000097.
- [42] Ryan, K. J. and Ray, C. G. (2004). Sherris Medical Microbiology: An Introduction to Infectious Disease. Fourth Edition. New York: McGraw Hill.
- [43] Charalampopoulos, D. and Rastall (2009). Prebiotics and Probiotics Science and Technology. Springer Science & Business Media. 627.
- [44] Stepanović, S., Dimitrijević, V., Vukovic, D., Dakic, I., Savic, B. and Švabic-Vlahović, M. (2001). *Staphylococcus sciuri* as a Part of Skin, Nasal and Oral Flora in Healthy Dogs. *Journal of Veterinary Microbiology*. 82: 177–185.
- [45] Garcia, M. C., Rodriguez, M. J., Bernardo, A., Tornadijo, M. E. and Carballo, J. (2002). Study of *Enterococci* and *Micrococci* Isolated Throughout Manufacture and Ripening of San Simon Cheese. *Journal of Food Microbiology*. 19: 23–33.
- [46] Hauschild, T., and S. Schwarz. (2003). Differentiation of *Staphylococcus sciuri* Strains Isolated from Free-Living Rodents and Insectivores. *Journal of Veterinary Medicine*. 50: 241–246.
- [47] Guirguitsova, B., Chankova, D. and Zozikov, B. (2002). *Staphylococci* as Uropathogens Frequency of Isolation in Hospitalized Patients and Sensitivity to Antimicrobial Agents. *Annales D'Urologie Journals* (Paris). 36: 341–347.
- [48] Stepanovic, S., Jezek, P., Vukovic, D., Dakic, I. and Petras, P. (2003). Isolation of Members of the *Staphylococcus sciuri* Group from Urine and their Relationship to Urinary Tract Infections. *Journal of Clinical Microbiology*. 41: 5262–5264.
- [49] Hedin, G. and Widerstrom, M. (1998). Endocarditis due to *Staphylococcus sciuri*. *European Journal of Clinical Microbiological Infectious Diseases*. 17: 673–675.
- [50] Wallet, F., Stuit, L., Boulanger, E., Roussel-Delvallez, M., Dequiedt, P. and Courcol, R. J. (2000). Peritonitis due to *Staphylococcus sciuri* in a Patient on Continuous Ambulatory Peritoneal Dialysis. *Journal of Infectious Diseases*. 32: 697–698.
- [51] Horii, T., Suzuki, Y., Kimura, T., Kanno, T and Maekawa, M. (2001). Intravenous Catheter Related Septic Shock Caused by *Staphylococcus sciuri* and *Escherichia vulneris*. *Journal of Infectious Diseases*. 33: 930–932.
- [52] Stepanović S., Dakic, I., Morrison, D., Hauschild, T., Ježek, P., Petra, P., Martel, A., Vukovic D., Shittu, A. and Devriese L. A. (2005). Identification and Characterization of Clinical Isolates of Members of the *Staphylococcus sciuri* Group. *Journal of Clinical Microbiology*. 43 (2): 956–958.
- [53] Benz, M. S., Scott, I. U., Jr. Flynn, H. W., Unonius, N. and Miller, D. (2004). Endophthalmitis Isolates and Antibiotic Sensitivities: A 6-year Review of Culture-Proven Cases. *American Journal of Ophthalmology*. 137: 38–42.

- [54] Shittu, A., Lin, J., Morrison, D. and Kolawole, D. (2004). Isolation and Molecular Characterization of Multiresistant *Staphylococcus sciuri* and *Staphylococcus haemolyticus* Associated with Skin and Soft-Tissue Infections. *Journal of Medical Microbiology*. 53: 51-55.
- [55] Unnisa N., Tabassum H., Ali N. M., Ponia K. (2012). Evaluation of Antibacterial Activity of Five Selected Fruits on Bacterial Wound Isolates. *International Journal of Pharmaceutical and Biological Sciences*. 3 (4): 531-546.
- [56] Ram, K. P. and Pranay, J. (2010). Comparative Studies on the Antibacterial Activity of Black pepper (*Piper nigrum*) and Turmeric (*Curcuma longa*) Extracts. *International Journal of Applied Biology and Pharmaceutical Technology*. 1 (2): 491-501.
- [57] Azwanida, N. N. (2015). A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation. *Journal of Medicinal and Aromatic Plants*. 4: 196.
- [58] Wu, T., Guan, Y. and Ye, J. (2007). Determination of Flavonoids and Ascorbic Acid in Grapefruit Peel and Juice by Capillary Electrophoresis with Electrochemical Detection. *Journal of Food Chemistry*. 100: 1573-1579.
- [59] Lawal, D. I., Bala, J. A., Aliyu, S. Y. and Huguma, M. A. (2013). Phytochemical Screening and In Vitro Antibacterial Studies of the Ethanolic Extract of *Citrus sinensis* (Linn.) Peel against Some Clinical Bacterial Isolates. *International Journal of Innovation and Applied Studies*. 2 (2): 138-145.
- [60] Agu, G. C. and Thomas, B. T. (2012). Antibacterial Activity of Ethanol and Aqueous Extracts of Five Nigerian Medicinal Plants on Some Wound Pathogens. *Journal of Nature and Science*. 10 (12): 78-84.
- [61] Girish, H. V. and Satish S. (2000). Antibacterial Activity of Important Medicinal Plants on Phytopathogenic *Xanthomonas campestris* Pathovars. *Letters in Microbiology*. 28: 145- 147.
- [62] Cheesbrough, M. (2002). *District Laboratory Practice in Tropical Countries*, Cambridge University Press, London. 2: 137-140.
- [63] Andrews, J. M. (2001). Determination of Minimum Inhibitory Concentration. *Journal of Antimicrobial Chemotherapy*. 48: 5-16.
- [64] Ahmad, I. and Beg, A. Z. (2001). Antimicrobial and Phytochemical Studies on 45 Indian Medicinal Plants against Multidrug Resistant Human Pathogens. *Journal of Ethnopharmacology*. 74: 113-123.
- [65] Srinivasan, D., Nathan, S., Suresh, T. and Perumalsamy, O. (2001). Antimicrobial Activity of Certain Indian Medicinal Plants Used in Folkloric Medicine. *Journal of Ethnopharmacology*. 74: 217-220.
- [66] Chehregani, A., Azimishad, F. and Alizade, H. J. (2007). Study on Antibacterial Effect of Some *Allium species* from Hamedan- Iran. *International Journal of Agriculture and Biology*. 9 (6): 873-876.
- [67] Mukhtar, F. B. (2013). *An Introduction to Biostatistics*. Third Edition. Ahmadu Bello University Press, Zaria- Kaduna, Nigeria. 24, 31, 110-119.
- [68] Phan, T. T., Wang, L., Sel, P., Grayer, R. J., Chans, Y. and Lee, S. T. (2001). Phenolic Compounds of *Chromolaena odorata* Protect Cultured Skin Cells from Oxidative Damage: Implication for Cutaneous Wound Healing. *Biological Bulletin*. 24: 1373- 1379.
- [69] Trease, G. E. and Evans, W. C. (1989). *A Textbook of Pharmacology Thirteenth Edition* Bailliere Tindall Ltd. London. <https://www.abebooks.com> Trease and Evans.
- [70] Carter, G. R. and Chengappa, M. M. (1991). *Essentials of Veterinary Bacteriology and Mycology*. Fourth edition, Lea and Febiger, Philadelphia, USA. 71- 263.
- [71] Talaro, K. and Talaro, A. (1993). *Foundations in Microbiology*. Wm. Brown C Publishers, Dubuque, IA., USA, pp. 87, 341, 355-356, 769.
- [72] Mahon, C. R. and Manuselis, G. *Textbook of Diagnostic Microbiology*. Fifth Edition. Saunders W. B. Co., Philadelphia, USA, pp. 237-239, 280-281, 294-295, 875.
- [73] Nester, E. W., Robert, C. E., Pearsall, W. W., Anderson, D. G. and Nester, M. T. (1998). *Microbiology: A Human Perspective*, Second Edition, WCB/McGraw- Hill CO., Boston, USA, pp. 260, 265, 651-657.
- [74] Stepp, C. A. and Woods M. (1998). *Laboratory Procedures for Medical Office Personnel*. Saunders W. B. Co., Philadelphia, USA. 351- 366.
- [75] Baron, S. (1996). *Medical Microbiology*, Fourth edition, The University of Texas Medical Branch, Galveston, TX, USA. 265-266, 270-271, 282, 351.
- [76] Subrahmanyam, M. Archan, H. and Pauer, S. G. (2001). Antibacterial Activity of Honey on Bacterial Isolated from Wounds. *Journal of Annals of Burns and Fire Disaster*. 14 (1): 124-128.
- [77] Wiley, J. M., Sherwood, L. M. and Woolverton, C. J. (2008). Prescott, Harley and Klein's *Microbiology*, Seventh edition, McGraw-Hill Higher Education, Boston, USA, pp. 581.
- [78] Gulay, K. F., Tavmen, A., Dulger, B. and Turker, G. (2009). Antimicrobial Activity of Turkish *Citrus* Peel Oils. *Pakistan Journal of Botany*. 4 (16): 3207-3212.
- [79] Jacob, A. Sumathy, J. H. (2010). Effect of Citrus Fruit Peel Extracts on Pathogens Causing Gastrointestinal Disorders. *Journal of Advanced Biotechnology*. 10 (3): 38-44.
- [80] Vivek, V. K., Nondini, S., Shashadhara, K. and Anitha, S. (2010). Anti-Typhoid Activity of Aqueous Extract of Fruit Peel *Citrus sinensis* (L), *International Journal of Pharmaceutical Research and Development*. 2 (9): 3-11.
- [81] Nwankwo, I. U., Onwuakor, C. E. and Aninweze, O. N. (2014). Antibacterial Activity of Ethanolic Extracts of *Citrus sinensis* Peels on *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* Isolated from Wound infections. *International Journal of Advances in Pharmacy, Biology and Chemistry*. 3 (4): 941-947.
- [82] Akujobi, C. E., Anyanwu, B. N., Onyeze, C. G. and Ibekwe, V. I. (2004). Antibacterial and Preliminary Phytochemical Screening of Four Medicinal Plants. *Journal of Applied Sciences*. 7 (3): 4328-4338.
- [83] Nweze, E. I., Okafor, J. I. and Njoku, O. (2004). Antimicrobial Activities of Methanolic Extracts of *Trema guineensis* (Schumm and Thorn) and *Morinda lucida benth* used in Nigerian Herbal Medicinal Practice. *Journal of Biological Research and Biotechnology*. 2 (10): 39- 46.

- [84] Osadebe, P. O. and Ukwueze, S. E. (2004). A Comparative Study of the Phytochemical and Antimicrobial Properties of the Eastern Nigerian Species of the African Mistletoe (*Loranthus micranthus*) Sourced from Different Host Trees. *Journal of Biological Research and Biotechnology*. 2 (1): 18-23.
- [85] Nannapaneni, R., Muthaiyan, A., Crandall, P. G., Johnson, M. G., O'Bryan, C. A., Chalova, V. I., Callaway, T. R., Carroll, J. A., Arthington, J. D., Nisbet, D. J. and Rieke, S. C. (2008). Antimicrobial Activity of Commercial Citrus-Based Natural Extracts against *Escherichia coli* O157:H7 Isolates and Mutant Strains. *Journal of Food Borne Pathogenic Diseases*. 5: 695-699.
- [86] Nair, R., Kalariya, T. and Sumitra, C. (2005). Antibacterial Activity of Some Selected Indian Medicinal Flora. *Turkish Journal of Biology*. 29: 41-47.
- [87] Tumane, P. M., Meshram, V. G. and Wasnik, D. D. (2014). Comparative Study of Antibacterial Activity of Peel Extracts of *Citrus aurantium* (Bitter Orange) and *Citrus medica* (Lemon) against Clinical Isolates from Wound Infection. (2014). *International Journal of Pharmacology and Biological Sciences*. 5 (1): 382-387.
- [88] Hindi, N. K. K., Chabuck, Z. A. G. and Hindi, S. K. K. (2014). Antibacterial Evaluation of Aqueous Extracts of Four *Citrus* Species in Hilla, Iraq. *International Journal of Pharmacological Screening Methods*. 4 (1): 43- 48.
- [89] Kadar, A. A. (2005). Prevalence and Antimicrobial Susceptibility of Extended Spectrum Beta Lactamases (ESBL) Producing *Escherichia coli* and *Klebsiella pneumoniae* in a General Hospital. *Journal of Clinical Microbiology*. 25 (3): 239-242.
- [90] Almajano, N. P. Carbo, R. C. Delgado, M. E. Gordon, M. H. (2007). Effect of pH on the Antimicrobial Activity and Oxidative Stability of Oil- In- Water Emulsion Containing Caffeic Acid. *Journal of Food Science*. 72 (5): 258-263.
- [91] Sung, W. S., Lee, I. S. and Lee, D. G. (2007). Damage to the Cytoplasmic Membrane and Cell Death Caused by Lycopene in *Candida albicans*. *Journal of Microbiology and Biotechnology*. 17 (1): 797-804.
- [92] Pavlović, R., Mladenović, J. and Radovanović, B. (2013). In Vitro Antimicrobial Activity of Ethanol Tomato Extracts. Conference VIVUS, 24th-25th April 2013, Biotechnical centre Naklo, Strahinj 99, Naklo Slovenia, Biotechnical Centre Naklo Higher Vocational College, Strahinj 99, Naklo, Slovenia.
- [93] Omodamiro, O. D. and Amaechi, U. (2013). The Phytochemical Content, Antioxidant, Antimicrobial and Anti-inflammatory Activities of *Lycopersicon esculentum* (Tomato). *Asian Journal of Plant Science and Research*. 3 (5): 70-81.