Antibacterial Potential Of Orange (*Citrus Aurantium*) Peels Against Selected Gram-Positive and Gram-Negative Bacteria Isolated From Futa Botanical Garden Soil

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Abstract. Emerging antibacterial resistance is a great challenge to modern medicine and is an increasing problem in different regions, limiting therapeutic options. Therefore, this study aimed to use the Citrus aurantium extract and assess its effect on bacterial agents. The antibacterial potentials of orange (*Citrus aurantium*) peel were evaluated on Gram-positive and Gram-negative bacteria. Three solvents, Ethanol, Methanol and Hot water, were used for extraction. The resultant extracts were used on three Gram-positive and five Gram-negative bacteria isolated from FUTA Botanical Garden. These phytochemicals include alkaloids, glycosides, phenols, tannins, saponins, terpenoids and steroids. The agar well diffusion method assessed the antibacterial activity, reflected by the diameter of growth inhibition zones. Results showed that among the solvents used, the extracts exhibited better performance in the order of Ethanol > Methanol > Hot water, which reflects the suitability of solvents for *Citrus aurantium* peel extraction. It was found that Gram-positive bacteria were more sensitive to the extracts at 200 mg/ml. Among them, *Staphylococcus aureus* showed the highest sensitivity against extracts from (15.00±1.00 mm to 21.00±1.00 mm). Among the Gram-negative bacteria, *Klebsiella* spp was susceptible to the 200 mg/ml extracts ranging from (13.00±1.00 mm to 15.00±1.00 mm). This may be attributed to a high concentration of flavonoids and total phenolic content in the ethanolic and methanolic extracts of Citrus aurantium peel.

Keywords: Antibacterial; Resistance; Gram-positive; Gram-negative; Ethanol; Methanol; Hot water; Sensitivity; Citrus aurantium; Phytochemicals.

INTRODUCTION

Long before humanity discovered the existence of microbes, the idea that plants have some healing potential, i.e., that they contain what we will currently characterise as an antimicrobial principle, was well accepted [1]. Using higher plants and their extracts to treat infectious diseases is an age-old practice in traditional African medicine [2]. Standard medicine practice has been known for centuries in many parts of the world. However, these practices vary from country to country [2]. Nature has been a source of medicinal agents for thousands of years. Herbs are the most ancient known approach to healing [3]. World Health Organization in 1978 defined traditional medicine as the total of knowledge or practices, whether explicable or inexplicable, used to diagnose and prevent a physical, mental or social disease which may rely exclusively on experience or observation handed down from generation to generation, verbally or in writing.

Since antiquity, man has used plants to treat common infectious diseases and some of these traditional medicines are still included in the chronic treatment of various disorders [1]. Traditional medicine practitioners use Numerous plants and herbs all over Nigeria as phytomedicine. Plant extracts are given singly or as concoctions for multiple ailments. The medicine could be either powders, liquids, salves or inclusion, according to [3]. More than 70% of people living in Nigeria depend on these various concoctions

and herbal decoctions to treat some diseases [4]. Resistant bacteria represent a challenge in treating various well-known infections and necessitate finding new substances with antimicrobial properties to be used against these microorganisms. Humanity has been gifted with resources for existence much earlier than the arrival of life on Earth.

According to WHO, a medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes or that are precursors to the synthesis of valuable drugs. The World Health Organization estimates that about 80% of the population still depends upon herbal medicines to treat various diseases due to easy availability, economic reasons and lesser side effects. Herbal remedies for ages have constructed grounds of medical pharmacology and have formed a basis for traditional systems of medicine. The popularity gained by herbal medication is due to better patient acceptance. Availability of medicinal plants is not a problem, especially in developing countries like India, which has rich agroclimatic, cultural and ethnic biodiversity. India is the largest producer of medicinal herbs and is appropriately called the botanical garden of the world. Plants with therapeutic effects are essential to traditional medicine in virtually all cultures. Medicinal plants are incredibly beneficial in curing diseases and act as crucial sources of materials for various human ailments. Some researchers have shown that using plants with medical effects is better than common antibiotics.

Herbal compounds have been used numerous times as fabulous sources of a wide range of essential oils and nutrients the body requires. Fresh fruits, edible plants, and industrially processed juices contain mostly flavanones and flavones [5]. *Citrus aurantiun* contains bioactive compounds known as phytochemicals, which can be used as an alternative antibacterial to reduce the spread of increasingly drug-resistant organisms. *Citrus aurantium* has a long history of usage and is believed to alleviate heart diseases and act as an anti-depressant and tonic among people living in the north of Iran [6]. Information about possible bioactivities of this part of the plant is somewhat limited; therefore, an experiment was conducted to determine phytochemical analysis, including phenolic and flavonoid compounds and their biological activities such as antioxidant properties, anti-inflammatory and anticancer activities. Researchers are interested in finding and producing new drugs with medicinal effects from plants to overcome bacteria's antibiotic resistance. Many infectious diseases are treated with herbal remedies. The natural herbal products used as standardised plant extracts provide unbounded opportunities for new drug leads due to their rich chemical properties. This calls for discovering new antibacterial compounds with different structures and new mechanisms of action and treatment for new emerging and reemerging infectious diseases.

Literature Review

Citrus aurantium L., commonly known as bitter orange, is a widely and readily available plant belonging to the family Rutaceae. The leaves, fruit, bark, flower and root are traditionally used to treat a broad panel of diseases. It is also known as sour orange, Seville orange, or bigarade orange. It can also be utilised as a flavouring and acidifying agent for food [7]. It is 3rd most crucial fruit crop in India. Its ethnomedicinal application has been well-known for a long time.

Citrus aurantium is native to southeastern Asia. Natives of the South Sea Islands, especially Fiji, Samoa, and Guam, believe the tree was brought to their shores in prehistoric times. Arabs are thought to have carried it to Arabia in the 9th Century. It was reported to grow in Sicily in 1002 AD and was cultivated around Seville, Spain, at the end of the 12th Century. For 500 years, it was the only orange in Europe and the first orange to reach the New World. It was naturalised in Mexico by 1568 and Brazil by 1587, and not long after, it was running wild in Cape Verde Islands, Bermuda, Jamaica, Puerto Rico and Barbados. Sir Walter Raleigh took sour orange seeds to England. They were planted in Surrey, and the trees began bearing regular crops in 1595 but were killed by cold in 1739. Spaniards introduced the sour orange into St. Augustine, Florida. It was quickly accepted by the early settlers and local Indians in 1763. Sour oranges were being exported from St. Augustine to England. It spread northeastward to Japan, westward through India to the Middle East, and then to Europe, where it rapidly became established in the Mediterranean 1000 years ago. It became widespread in Spain, hence its vernacular name, Seville orange. It was one of the first citrus taken to South America in the 16th Century, where it soon escaped from cultivation and naturalised in many areas [8]. It is now cultivated in tropical and subtropical coun-

tries but rarely in South-East Asia. *Citrus aurantium* has been grown in France since the early 1400s, initially mainly as an ornamental. Later, particular perfumery cultivars grown for their fragrant flowers were developed in the French Riviera region and became known as "Bouquetiers".

The climate is the most critical parameter for selecting the location of an orange field. The climate determines the success of orange farms and the quality of citrus fruits, while soil and water determine the productivity of orange trees. Cold is the most crucial enemy of an orange tree. Citrus crop thrives well in frost-free sub-tropical to semitropical climates. However, most of the species tolerate light frost. Being evergreen, it has no specific requirement of winter chilling, but a cessation of growth during winter helps in flower bud induction, resulting in spring flowering. Best growth occurs within the temperature of 29-35 °C. An annual rainfall of 700 mm is sufficient if it is well-distributed.

Unevenly distributed rainfall can be supplemented by irrigation, and the best-quality fruits are obtained in semi-arid, sub-tropical regions. It has been suggested the use of water resources by the citrus industry in the Middle East is a contributing factor to the desiccation of the region. Another significant element in the full development of the fruit is the temperature variation between summer and winter and between day and night. In cooler climates, oranges can be grown indoors. As oranges are sensitive to frost, there are different methods to prevent frost damage to crops and trees when subfreezing temperatures are expected. A typical process is to spray the trees with water to cover them with a thin layer of ice that will stay just at the freezing point, insulating them even if air temperatures drop far lower. This is because water continues to lose heat if the environment is colder than it is, and so the water turning to ice in the environment cannot damage the trees. This practice, however, offers protection only for a very short time. Another procedure is burning fuel oil in smudge pots put between the trees. These devices burn with a great deal of particulate emissions, so condensation of water vapour on the particulate soot prevents condensation on plants and raises the air temperature very slightly. Smudge pots were developed for the first time after a disastrous freeze in Southern California in January 1913 destroyed a whole crop.

Citrus aurantium thrives well in deep, loose, well-aerated soils devoid of any hard pan of calcium carbonate in the rooting zone. The ideal soil pH is 5.5 to 7.5, but with proper management, it can grow successfully even in highly acidic soils up to 4.5 and those containing free lime with pH 8. The crop is susceptible to water logging in the root zone and is vulnerable to salt injury; hence, it does not thrive in saline and alkaline soils. Loamy soils with comparatively heavier sub-soils or heavy soils with good drainage are ideal for the crop.

Table 1 – Nutritional value of orange (*Citrus aurantium*) per 100 g

Nutritional properties	Nutrient value
Energy	197 kj (47 kcal)
Carbohydrates	11.89 g
Sugars	9.35 g
Dietary fibre	2.5 g
Fat	0.12 g
Protein	0.94 g
Vitamin A	11 μg
Thiamine (B1)	0.087 mg
Riboflavin (B2)	0.04 mg
Niacin (B3)	0.282 mg
Pantothenic acid (B5)	0.25 mg
Vitamin B6	0.06 mg
Folate (B9)	30 μg
Choline	8.4 mg
Vitamin C	53.2 mg
Vitamin E	0.18 mg
Calcium	40 mg
Iron	0.1 mg
Magnesium	10 mg
Manganese	0.025 mg
Phosphorus	14 mg
Potassium	181 mg
Zinc	0.07 mg

Economic and Health Benefits. Citrus aurantium has several local common names in different countries where it is used for food, fragrance, and medical applications. Bitter oranges are used to produce bitter orange juice concentrate as raw material. It is commonly used as a sauce in salads and soups because of its bitter taste [9]. Fruit, peel, leaves, flowers, seeds, and essential oil (EO) of *Citrus aurantium* are used in perfumes, cosmetics, and the food and confectionery industry [10]. Bitter orange oil, obtained from the pressure of fresh peels, is widely used as a fla-

vouring agent in the food industry and for beverages, particularly liqueurs and soft drinks. *Citrus aurantium* is rich in flavonoids and polyphenolic compounds with numerous pharmacological properties, such as inhibiting the oxidation of low-molecular-weight proteins and platelet accumulation, thus contributing to immune cell stability. It also treats mental disorders, inflammation, viral infections, and allergies [11]. *Citrus aurantium* essential oil, known as neroli oil, is widely used in aromatherapy. It has been suggested that it stimulates the central nervous system, lowers blood pressure, and has sedative, analgesic, anti-inflammatory, antispasmodic, carminative, digestive, and diuretic effects [12].

Medical Benefits. Citrus fruit intake is associated with a reduced risk of stomach cancer. *Citrus aurantium* has the highest concentration of citrate of any citrus fruit, and daily consumption of lemonade has been shown to decrease the rate of kidney stone formation. Before the development of fermentation-based processes, bitter orange was the primary commercial source of citric acid. The major active biological constituents in citrus herbs are flavonoids, especially hesperidin and naringin, and alkaloids, mainly synephrine, which have beneficial medical effects on human health. It is traditionally known to help treat a broad panel of diseases like stomach ache, vomiting, blood pressure, cough, cold, bronchitis, earache, dysentery, diarrhoea, abdominal pain and fever. *Citrus aurantium* is also used in herbal medicine as a stimulant and appetite suppressant. It has also been used in traditional Chinese medicine to treat nausea, indigestion, and constipation, cancer, cardiovascular effects, sedative. However, the claim that it replaces the banned ephedra stimulant without its side effects has made bitter orange well-known and popular. Because of this, C. aurantium is a popular weight-loss ingredient used in various diet pills and fats. Bitter orange has been substituted into "ephedra-free" herbal weight-loss products by dietary supplement manufacturers.

Phytochemical Components of Orange (Citrus aurantium) with Antibacterial Activity. Phytochemicals are chemicals derived from plant sources. Plants contain hundreds of phytochemicals, such as flavonoids and phenolic acids. Research indicates phytochemicals such as polyphenols have high antioxidant activity [13]. The phytochemical category includes compounds recognised as essential nutrients, which are naturally contained in plants and are required for normal physiological functions, so they must be obtained from humans' diets. Plants containing beneficial phytochemicals may supplement the human body's needs by acting as natural antioxidants.

1. Alkaloids: The name "alkaloids" was introduced in 1819 by the German chemist Carl F. W. Meissner. Alkaloids are a structurally d verse group of over 12,000 cyclic nitrogen-containing compounds found in over 20 % of plant species. Alkaloids contain a ring with nitrogen. Many alkaloids have dramatic effects on the central nervous system. Caffeine is an alkaloid that provides a mild lift, but the alkaloids in Datura cause severe intoxication and even death. They are widely used in medicine to develop drugs [14].

2. Terpenes: Terpenes are a large and varied class of organic compounds produced primarily by wide varieties of plants, particularly conifers. Vitamin A is an example of a terpene. When terpenes are modified chemically by oxidation or rearrangement of the carbon skeleton, the resulting compounds are generally called terpenoids. Each terpene consists of two paired isoprenes. The names monoterpenes, sesquiterpenes, diterpenes and triterpenes are based on the number of isoprene units.

3. Phenolics: Major bioactive compounds known for health benefits are phytochemicals, especially phenolics in fruits and vegetables. Citrus species of various origins have been evaluated for their phytochemical composition and contribution to health promotion. In organic chemistry, phenols, sometimes called phenolics, are a class of chemical compounds consisting of a hydroxyl group (-OH) bonded directly to an aromatic hydrocarbon group. The simplest of the classes is phenol, also called carbolic acid $C6H5OH$. They have higher acidity due to the aromatic ring's tight coupling with oxygen and a relatively loose bond between oxygen and hydrogen. Studies have reported that plant phenolics are present not only in edible parts of plants but also in non-edible parts of the plants, with multiple biological effects. Phyto phenols effectively prevent and treat free radical-mediated diseases such as cancer, diabetes, and neurodegenerative diseases [15]. In addition, many of the antioxidants in plants exhibit a wide range of biological effects, including antibacterial, antiviral, anti-inflammatory, antiallergic, antithrombotic and vasodilatory

actions [16]. One of the most popular world fruit crops, citrus (Citrus L. from Rutaceae), contains a host of active phytochemicals that can protect health. In addition to this, it provides an ample supply of vitamin C, folic acid, potassium and pectin.

4. Flavonoids: Flavonoids are polyphenolic compounds having a phenyl benzopyrone structure, representing two benzene rings (C6) joined by a linear three-carbon chain (C3), with a carbonyl group at the C position. Although flavonoids are generally regarded as non-nutritive agents, their potential role in the prevention of major chronic diseases has attracted the focus of many researchers. The citrus flavonoids include a class of glycosides, namely, hesperidin and naringin and another class of O-methylated aglycones of flavones, such as nobiletin and tangerine, which are two relatively common polymethoxylated flavones (PMFs). In citrus fruits, peels are reported to possess the highest amounts of PMFs compared to other edible parts of the fruit. Citrus flavonoids have been found to have health-related properties, which include anticancer, antiviral, and anti-inflammatory properties, reduced capillary fragility, and restricted human platelet aggregation [17]. Flavonoids are hydroxylated phenolic substances but occur as a C6-C3 unit linked to an aromatic ring. Since plants are known to be synthesised by plants in response to microbial infection [18], it should not be surprising that they have been found in vitro to be effective antimicrobial substances against various microorganisms. Flavones are phenolic structures containing one carbonyl group (as opposed to the two carbonyls in quinones). The addition of a 3-hydroxyl group yields a flavonol.

Antibacterial review. Antibacterial is an agent that interferes with the growth and reproduction of bacteria. These agents kill or prevent bacteria by fighting against bacteria. Heat, chemicals such as chlorine, and all antibiotic drugs have antibacterial properties. Antibiotics and antibacterials both attack bacteria. Antibacterials are now most commonly described as agents used to disinfect surfaces and eliminate potentially harmful bacteria. Antibacterials are divided into two groups according to their speed of action and residue production. Bacteriostats, disinfectants, sanitisers and sterilisers are different groups of antibacterials. Antibacterials are classified based on the Inhibition of cell wall synthesis, inhibition of protein synthesis, and Inhibition of bacterial nucleic acid synthesis.

Antibacterials, a subclass of antibiotics, can be classified into five groups: type of action, source, spectrum of activity, chemical structure, and function [19].

Classification based on the type of action. Generally, antibacterials can be classified based on their type of action: bacteriostatic and bactericidal. Antibacterial, which kills bacteria by targeting the cell wall or cell membrane of the bacteria, are termed bactericidal; those that slow or inhibit the growth of bacteria are referred to as bacteriostatic [20].

Table 2 – List of Some Bacteriostatic and Bactericidal Antibacterial

A. Bacteriostatic antibacterial	*Functions*
Sulphonamides	They act to inhibit folate synthesis at the initial stages
Amphenicols, e.g. chloramphenicol	They work as protein synthesis inhibitors.
Spectinomycin	It binds to the 30S ribosomal subunit, thereby interrupting protein synthesis.
Trimethoprim	It disturbs the tetrahydrofolate synthesis pathway.
Erythromycin, clarithromycin and azithromycin are macrolides. Linezolid is a member of the oxazolidinone class.	It is a protein synthesis inhibitor. It binds reversibly to the 30S bacterial ribosomal subunit, which blocks amino-acyl-tRNA binding to the acceptor site on the mRNA complex.
Doxycycline, tetracycline, and minocycline belong to tetracyclic synthesis.	They work as inhibitors of protein synthesis.
B. Bactericidal antibacterials	*Functions*
Gentamicin, tobramycin, and amikacin are aminoglycosides	They inhibit protein synthesis.
Quinolones and flouroquinolones, such levofloxacin,	This blocks bacterial DNA replications.

A. Bacteriostatic antibacterial	*Functions*
ciprofloxacin, and oxifloxacin	
Vancomycin is a glycopeptide.	These inhibit cell wall synthesis.
Polymyxin B and colistin are	These antibacterial disrupt the cell membrane

Classification based on the source of antibacterial agents. Antibacterials are the subclass of antibiotics, which can be naturally obtained from fungal sources and semi-synthetic members, chemically altered natural and/or synthetic products. Cephalosporins, rifamycins, benzylpenicillin, and gentamicin are well-known natural antibiotics/antibacterials. Natural antibiotics/antibacterials often exhibit higher toxicity than synthetic antibacterials. Ampicillin and amikacin are semi-synthetic antibiotics developed to show low toxicity and increase effectiveness. Synthetic antibiotics are also designed to have even greater efficacy and less toxicity and, thus, have an advantage over natural antibiotics in that the bacteria are not exposed to the compounds until they are released. Moxifloxacin and norfloxacin are promising synthetic antibiotics.

Classification based on the spectrum of activity. This is another way of classifying antibiotics or antibacterial agents based on their target specification. In this category, the antibacterial may be either narrow or broad spectrum [21]. Limited-spectrum antibacterials are considered to be those that can work on a limited range of microorganisms; that is, they act against Gram-positive-only or Gram-negative-only bacteria. Unlike narrow-spectrum antibacterials, broad-spectrum antibacterials affect many pathogenic bacteria, including Gram-positive and Gram-negative ones. Usually, the narrow-spectrum antibacterial is considered the ideal antibacterial and is preferred over the broad-spectrum antibacterial. The reason is that narrow-spectrum antibiotics do not kill as many of the typical microorganisms in the body as broad-spectrum antibiotics and thus have less ability to cause superinfection. Also, narrow-spectrum antibiotics cause less resistance to bacteria as they deal with only specific bacteria.

Classification Based on Chemical Structure. Different skeleton-containing antibiotics display other therapeutic behaviours; therefore, it is necessary to classify antibacterials based on their chemical structure. This classification is also essential as similar structural units have similar patterns of toxicity, effectiveness, and other related properties. Usually, on a structural basis, antibacterial has been classified into group A (β-lactams) and group B (aminoglycosides). However, more elaborately, the antibacterials can be classified into β-lactams, β-lactam/β-lactamase inhibitor combinations, aminoglycosides, macrolides, quinolones, and fluoroquinolones.

Function-based Classification of Antibacterial Drugs. Function means how a drug works or what its mode of action is. This is one of the most critical factors related to each antibacterial. The significant processes or functions responsible for bacterial growth are cell wall synthesis, cell membrane function, protein synthesis, nucleic acid synthesis, and so on. All such processes are targets for antibiotics; therefore, antibacterial, which interfere with or disturb these processes in different ways, can be subdivided into four groups: such as cell wall synthesis inhibitors, inhibitors of membrane function, inhibitors of protein synthesis, and inhibitors of nucleic acid synthesis.

Antibacterial Resistance among Gram-Positive and Gram-Negative Bacteria. Antibacterial resistance is bacteria's ability to resist an antibiotic's effects. Antibacterial resistance occurs when bacteria change in some way that reduces or eliminates the effectiveness of drugs, chemicals, or other agents designed to cure or prevent infections. The bacteria survive and continue to multiply, causing more harm. The widespread use of antibiotics over the past decades has led to the emergence of antibiotic-resistant strains of many bacteria. Both Gram-positive and Gram-negative have acquired resistance to Antibacterial drugs. Among various pathogenic Gram-positive bacteria, *Staphylococcus aureus, Streptococcus pneumoniae*, and *Enterococci* stand out as responsible for global resistance challenges, significant public health burden, and cost to healthcare [22]. Multidrug resistance among gram-positive bacteria, especially methicillin-resistant Staphylococcus aureus (MRSA), has been a worldwide healthcare concern [23]. Extended-spectrum beta-lactamase ESBL is an enzyme that has developed resistance to bat lactam antibiotics such as cephalosporin. ESBL enzymes are produced mainly by two bacteria, i.e., *Escherichia coli* and *Klebsiella pneumonia*. They predominantly occur in the family of Enterobacteriaceae. They are responsible for the

outbreak of nosocomial infections in intensive care units and burns, oncology, and neonatal units.

MATERIALS AND METHODS

Materials: oranges (*Citrus aurantium*), distilled water, sterile syringes (5 ml) and needles, sterile Petri dishes, conical flask, measuring cylinder, aluminium foil, sterile cotton wool, pipette, inoculating loop, test tubes, bijou bottles, fermenter, microscope, microscope slides, Bunsen burner, paper tape, test tubes, test tube rack, bucket, bowl, marker, nose mask, hand gloves, funnel, cavity slide and Durham tubes, Whatman No 1 filter paper, membrane filter.

Reagents: Ethanol, Methanol, hydrogen peroxide, serum, crystal violet, lugol's iodine, safranin, blood plasma, 70% ethanol, methylene blue, sugars (glucose, mannitol, lactose, sucrose, maltose, immersion oil, standard saline solution, Dimethyl sulfoxide (DMSO).

Media: Nutrient agar (NA), MacConkey agar, Salmonella Shigella agar, Muller Hinton agar, peptone water.

Equipment: refrigerator, incubator, autoclave, spectrophotometer, microscope, electric weighing balance, rotary evaporator.

Procurement of Plant Material. Oranges were purchased from Shasha Market Akure, Ondo State and conveyed to the Microbiology Laboratory, Federal University of Technology Akure, Ondo State.

Determination of Percentage Yield. The percentage yield of the crude extract was determined for each solvent. The percentage yield of the Ethanol, Methanol and Hot water extracts was calculated as:

$$\text{Percentage yield} = \frac{\text{Weight of extract afterextraction}}{\text{Weight of extract beforeextraction}} \times 100$$

Preparation of the Mcfarland's Standard. The preparation involves the mixture of both Tetraoxosulphate (6) acid (sulphuric H_2S0_4) (1%) and Barium Chloride (1.17%). 9.95 ml of H_2S0_4 was added to 0.05 ml of Bacl to form a precipitate suspension. This served as the 0.5 McFarland's turbidity standard for the test organisms.

Preparation of the cell suspension (inocular). The Test organisms were sub-cultured on a nutrient agar plate and incubated at 37 °C for 15-24 hours. The growth from each plate was transferred into a test tube containing 5 ml of 0.9% sterile saline, and the volume was adjusted to attain a turbidity that matches that of 0.5 McFarland's standard. This means the cell suspension appropriately contains 1.5 x 10^8 colony-forming units per ml (cfu/ml).

Serial dilutions of the extract. An electronic weighing balance was used to weigh 1g of the extract. This was transferred into a sterilised universal bottle containing 5 ml of 30% Dimethyl sulfoxide (DMSO). It was stirred using a glass rod to help it dissolve. This gives a concentration of 200 mg/ml. Serial dilution was made by mixing 1 ml of the concentration from this universal bottle into another one containing 1 ml of 30% dimethyl sulfoxide (DMSO) and mixing it to obtain a 100 mg/ml solution. This procedure prepared concentrations of 50, 25 and 12.5 mg/ml.

Serial dilutions of the standard antibiotics. For Septrin. The tablet composition was 480 mg. Therefore, one tablet was dissolved in 0.96ml of sterile water to achieve the concentration of 500 mg/ml. From the 500 mg/ml solution, 1 ml was taken and put into another 10 ml of sterile water to achieve a 50 mg/ml concentration. 1 ml was taken from the 50 mg/ml and put into another 10 ml sterile water to achieve 5mg/ml. From the 5 mg/ml solution, 1 ml was taken into another 10 ml of clean water to achieve 0.5mg/ml.

For Amoxicillin. The tablet composition was 500 mg. Therefore, one tablet was dissolved in 1 ml of sterile water to achieve a 500mg/ml concentration. From the 500 mg/ml solution, 1 ml was taken and put into another 10 ml of sterile water to achieve a 50 mg/ml concentration. 1 ml was taken from the 50mg/ml and put into another 10 ml sterile water to achieve 5mg/ml. From the 5mg/ml solution, 1 ml was taken into another 10 ml sterile water to achieve 0.5 mg/ml.

Antibiotic Sensitivity Test using Disc Diffusion Method. An antibacterial susceptibility test was performed using the Kirby-Bauer disc diffusion method on Muller-Hinton agar plates [24]. The Gram-positive bacteria were screened against Rocephin 25 µg, Ciprofloxacin 10 µg, Gentamycin 30 µg, Streptomycin 30 µg, Septrin 30 µg,

Erythromycin 10 µg, Pefloxacin 30 µg, Ampiclox 30 µg, Zinnacef 30 µg, Amoxicillin 30 µg. The Gram-negative bacteria were screened against Septrin 30 µg, Chloramphenicol 30 µg, Sparfloxacin 10 µg, Ciprofloxacin 30 µg, Amoxicillin 30 µg, Augmentin 10 µg, Gentamycin 30 µg, Pefloxacin 30 µg, Tarivid 10 µg and Streptomycin 30 µg. The cell suspension was used to swab the entire surface of the Muller Hinton agar plate as follows: a sterile swab stick was dipped into the bacterial suspension and was dispensed in the MH agar surface. The swab stick was used to swab the nutrient agar surface all over while rotating it anticlockwise. Multidiscs containing the antibiotics above were aseptically placed on the Muller Hinton agar plate inoculated with the test organism. The discs were allowed for 1 hour to diffuse through the plate and were then incubated at 37 °C for 24 hours. After incubation, the diameter of the zone of inhibition around each antibiotic disc was recorded in millimetres to determine the sensitivity or resistant categories according to the standard chart of Hi-media. This process was carried out in duplicate.

Screening for Antibacterial Activity of Orange (*Citrus aurantium*) peel. The antibacterial screening was carried out using the agar well diffusion method. Double-strength Muller-Hinton agar was prepared according to the manufacturer's specifications. The plates were seeded with the standardised test inoculum. The Muller-Hinton agar was punched with a 7 mm sterile cork borer to make six wells: three wells for the same concentrations of the extracts (200 mg/ml), two wells for the standard antibiotics Septrin and Amoxicillin (0.5 mg/ml) used as positive control and the 6th well for the negative control Dimethyl sulfoxide (DMSO). 0.1 ml of the Ethanol, Methanol and Hot water extracts, positive control and negative control, were introduced to the wells and labelled appropriately. The culture plates were allowed to stand on the working bench for 30 minutes for pre-diffusion and were then incubated at 37 °C for 24 hours. After 24 hours, antibacterial activity was determined by measurement of diameter zones of inhibition (mm) (against the test organisms) around each of the extracts and the positive control. The antibacterial screening was performed using different extract concentrations at 12.5, 25, 50, 100 and 200 mg/ml without positive control. This process was carried out in duplicate.

Statistical analysis. The data obtained were analysed using SPSS (Statistical Package for Social Sciences) version 20. Descriptive statistics (Mean value and SE) and a comparison in the mean zone of inhibition between the extracts at different concentrations were performed using one-way analysis of variance (ANOVA) with Duncan *post hoc*. The confidence and significance levels were 95% and 5%, respectively.

RESULTS AND DISCUSSION

Identification of Bacterial. Bacteria isolated from Futa botanical garden soil include *Staphylococcus aureus, Bacillus subtilis, Clostridium perfringens, Acinetobacter iwoffi, Alcaligenes* spp, *Pseudomonas fluorescens, Klebsiella* spp, *Achromobacter* spp. 62.5% of the isolated bacteria were Gram-negative, while 37.5% were Gram-positive. The Morphological characteristics, biochemical characteristics and microscopic identification of isolated organisms are represented in Tables 3, and 4.

Extraction Yield. The extraction yield was 3.99%, 3.17%, and 2.62% for methanol, hot water, and ethanol, respectively. The methanol solvent showed a higher yield compared with the other extraction solvents. At the same time, the ethanol solvent showed the lowest yield. It is represented in a Bar chart (Figure 1). The colour of the peel extracts was yellow.

Phytochemical Screening of Orange (*Citrus aurantium*) Peels. The qualitative phytochemical screening results showed most of the tested constituents to be present in all the peel extracts (Ethanol, Methanol and Hot water) except for Anthraquinone, which was absent across all three solvents and Saponin, which was missing in both Ethanol and Hot water extracts, both present in Methanol extract (Table 5). The quantitative phytochemical screening results showed that methanol extract has a significant yield of the constituents tested compared to other extracts, particularly for the alkaloids, which was 12.03%. The results are represented in Table 6.

Antibiotic sensitivity screening. The isolated bacteria were screened against industrial antibiotic agents, both positive and negative agents. The Gram-positive bacteria were more sensitive to the antibiotic agents, while most Gram-negative isolates were resistant. The results are represented in Tables 7, 8.

Antibacterial activity of Orange (Citrus aurantium) peel. The results of the antibacterial activity of Citrus aurantium peel extracts are shown in Tables 9-16.

The peel extracts at 200 mg/ml were tested against all isolated organisms, and conventional antibiotics (Amoxicillin and Septrin) were also used as positive controls (Plates 1 and 2). The extracts at different concentrations were also tested against the isolated bacteria. The ethanol extract showed a significant diameter zone of inhibition against Gram-positive and Gram-negative bacteria. The effect exerted on the organisms decreased as the concentration decreased. The Methanol extract inhibited the bacteria isolate but was not as significant as the ethanol extract. All the organisms were resistant to the Hot water extract.

Table 3 – Colonial, Cellular and Morphological Characteristics of Isolates

Number of soil bacterial	Colour of colony	Description of colony Isolate
K1	Yellowish	Translucent, flat, smooth, circular
K2	Greyish white	Translucent, smooth, circular, encapsulated
K3	Colourless	Irregular, smooth, transparent, flat, slightly curved
K4	White (opaque)	Translucent, irregular, raised, rough edges
K5	Greenish	Translucent, large, smooth edges
K6	Greyish White	Large, mucoid, encapsulated
K7	Greenish	Translucent, flat, Large
K8	Colourless	Circular, glossy, flat, smooth

Table 4 – Biochemical Characteristics and Microscopic Identification of Isolated Bacteria

Number of isolates possible	Cell share	CR	CA	CO	UR	CI	MO	IN	LA	GL	SU	MA	MN	Organism
K1	Cocci	+	+	+	+	+	-	-	AG	AG	AG	AG	AG	*Staphyloccus aureus*
K2	Cocci	-	+	-	-	+	-	-	OO	OO	OO	OO	OO	*Acinetobacter iwaffi*
K3	Thin Rod	-	+	-	-	+	+	-	OO	OO	OO	OO	OO	*Alcaligenes spp*
K4	Thick Rod	+	+	-	+	+	+	-	AG	AG	AG	AG	AG	*Bacillus subtilis*
K5	Straight Rod	-	+	-	+	+	+	-	OO	OO	OO	OO	OO	*Pseudomonas fluorescens*
K6	Straight Rod	+	-	-	-	+	-	-	AG	AG	AG	AG	AG	*Klebsiella* spp
K7	Elongated Rod	+	-	-	-	+	-	-	AG	AG	AG	AG	AG	*Clostridium perfringens*
K8	Straight Rod	-	+	-	-	-	-	-	OO	AG	OO	OO	OO	*Achromobacter* spp

Notes: GR – Gram reaction; CA – Catalase; CO – Coagulase; UR – Urease; CI – Citrate; MO – Mobility; IN – Indole; LA – Lactose; GL – Glucose; SU – Sucrose; MA – Maltose; AG – Acid gas; OO – No reaction; + is Positive; - is Negative.

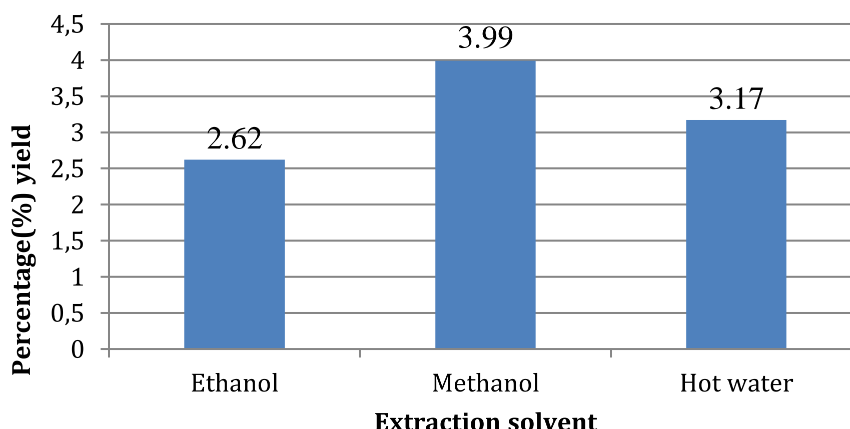

Figure 1 – Graph (Bar chart) of % Yield of Extract

Table 5 – Qualitative Phytochemical Screening of Ethanol, Methanol and Hot Water Extract of Orange (*Citrus aurantium*) peel

Phytochemical constituents	Peel extracts		
	Ethanol	Methanol	Hot water
Tannins	+	+	+
Saponins	-	+	-
Flavonoids	+	+	+
Terpenoids	+	+	+
Alkaloids	+	+	+
Phenol	+	+	+
Anthraquinone	-	-	-
Cardiac glycosides	+	+	+
Legal's test	+	+	+
Keller kiliani's test	+	+	+
Salkwoski's TEST	+	+	+
Lieberman's test	+	+	+

Notes: + present; - absent.

Table 6 – Quantitative Phytochemical Screening of Ethanol, Methanol and Hot Water Extract of Orange (*Citrus aurantium*) peel

Phytochemical constituents, mg/g	Peel extracts (Mean±SE)		
	Ethanol	Methanol	Hot water
Tannins	6.08 ± 0.20^{b}	7.98 ± 0.46^{c}	4.57 ± 0.10^{a}
Saponins	0.00 ± 0.00^{a}	8.30 ± 0.13^{b}	0.00 ± 0.00^{a}
Flavonoids	3.32 ± 0.00^{a}	6.00 ± 0.41^{b}	2.57 ± 0.01^{a}
Terpenoids	7.66 ± 0.02^{b}	4.92 ± 0.32^{a}	5.53 ± 0.03^{a}
Alkaloids (%)	9.75 ± 0.14^{b}	12.03 ± 0.21^{c}	7.60 ± 0.03^{a}
Phenolics	4.55 ± 0.16^{b}	4.83 ± 0.00^{b}	3.93 ± 0.09^{a}
Glycosides	7.31 ± 0.78^{b}	5.15 ± 0.08^{a}	5.35 ± 0.37^{a}
Steroids	5.72 ± 0.04^{a}	9.13 ± 0.09^{c}	6.32 ± 0.10^{b}

Notes: The test applied – One-way ANOVA with Duncan *post hoc*, $P < 0.05$ (statistically significant); *Post hoc*: Values with the same letter superscripted do not vary significantly.

Table 7 – Diameter Zones of Inhibition of Antibiotic Agents on Gram-Positive Bacteria (mm) (Mean±SE)

Isolates	PEF (30 µg)	CN (30 µg)	APX (30 µg)	Z (30 µg)	AM (30 µg)	R (25 µg)	CPX (10 µg)	S (30 µg)	SXT (30 µg)	E (10 µg)
Staphyloccus aureus	18.00 ± 2.00^{c}	2.00 ± 0.00^{a}	6.00 ± 0.00^{ab}	14.00 ± 2.00^{c}	6.00 ± 0.00^{ab}	12.00 ± 0.00^{bc}	18.00 ± 2.00^{c}	16.00 ± 4.00^{c}	18.00 ± 2.00^{c}	4.00 ± 0.00^{a}
Bacillus subtilis	12.00 ± 0.00^{a}	10.00 ± 2.00^{a}	6.00 ± 0.00^{a}	10.00 ± 2.00^{a}	6.00 ± 2.00^{a}	10.00 ± 2.00^{a}	12.00 ± 2.00^{a}	12.00 ± 4.00^{a}	8.00 ± 0.00^{a}	6.00 ± 0.00^{a}
Clostridium perfringens	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	2.00 ± 0.00^{a}	2.00 ± 0.00^{a}	14.00 ± 0.00^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Notes: The test applied – One way ANOVA with Duncan *post hoc*, $P < 0.05$ (statistically significant); *Post hoc*: Values with the same letter superscripted do not vary significantly; PEF – Pefloxacin; CN – Gentamycin; APX – Ampiclox; Z – Zinnacef; AM – Amoxicillin; R – Rocephin; CPX – Ciprofloxacin; S – Streptomycin; SXT – Septrin; E – Erythromycin.

Table 8 – Diameter Zones of Inhibition of Antibiotic Agents on Gram-Negative Bacteria (mm) (Mean±SE)

Isolates	SXT (30 μg)	CH (30 μg)	SP (10 μg)	CPX (30 μg)	AM (30 μg)	AU (10 μg)	CN (30 μg)	PEF (30 μg)	OFX (10 μg)	S (10 μg)
Acinetobacter iwaffi	4.00±0.00^{a}	2.00±0.00^{a}	2.00±0.00^{a}	14.00±2.00^{c}	6.00±2.00^{ab}	4.00±0.00^{a}	10.00±2.00^{bc}	14.00±2.00^{c}	14.00±2.00^{c}	12.00±0.00^{ac}
Alcaligenes spp	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	18.00±2.00^{d}	0.00±0.00^{a}	4.00±0.00^{b}	0.00±0.00^{a}	16.00±0.00^{d}	16.00±2.00^{d}	12.00±0.00^{c}
Pseudomonas fluorescens	16.00±0.00^{d}	12.00±2.00^{c}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	12.00±0.00^{c}	6.00±2.00^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
Klebsiella spp	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Achromobacter spp	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Notes: The test applied – One way ANOVA with Duncan *post hoc*, P˂0.05 (statistically significant); *Post hoc*: Values with the same letter superscripted do not vary significantly; SXT – Septrin; CH – Chloramphenicol; SP – Sparfloxacin; CPX – Ciprofloxacin; AM – Amoxicillin; AU – Augmentin; CN – Gentamycin; PEF – Pefloxacin; OFX – Tarivid; S – Streptomycin.

Table 9 – Antibacterial Activity of Extracts at 200 mg/ml with positive and negative control on Gram Positive bacteria

Isolates	Extract concentration and diameter zone of inhibition (mm) (Mean±SE)			Control antibiotics		Negative control
	Ethanol (200 mg/ml)	Methanol (200 mg/ml)	Hot water (200 mg/ml)	AMX (0.5 mg/ml)	SXT (0.5 mg/ml)	DMSO (30%)
Staphylococcus Aureus	21.00±1.00^{c}	15.00±1.00^{b}	0.00±0.00^{a}	14.00±0.00^{b}	15.00±1.00^{b}	0.00±0.00^{a}
Bacillus subtilis	15.00±1.00^{d}	12.00±0.00^{c}	0.00±0.00^{a}	5.00±1.00^{b}	0.00±0.00^{a}	0.00±0.00^{a}
Clostridium perfringes	15.00±1.00^{c}	12.00±2.00^{b}	0.00±0.00^{a}	9.00±1.00^{b}	0.00±0.00^{a}	0.00±0.00^{a}

Notes: Test applied – One way ANOVA with Duncan *post hoc*, P˂0.05 (statistically significant); *Post hoc*: Values with the same letter superscripted do not vary significantly

Table 10 – Antibacterial Activity of Extracts at 200 mg/ml with positive and negative control on Gram Negative bacteria (mm)

Isolates	Extract Concentration and Diameter Zone of Inhibition (mm) (Mean±SE)			Control Antibiotics		Negative Control
	Ethanol (200 mg/ml)	Methanol (200 mg/ml)	Hot Water (200 mg/ml)	AMX (0.5 mg/ml)	SXT (0.5 mg/ml)	DMSO (30 %)
Acinetobacter iwoffi	12.00±0.00^{c}	7.00±1.00^{b}	1.00±0.00^{a}	11.00±1.00^{c}	7.00±1.00^{b}	0.00±0.00^{a}
Alcaligenes spp	13.00±1.00^{b}	10.00±2.00^{b}	1.00±1.00^{a}	9.00±1.00^{b}	21.00±1.00^{c}	0.00±0.00^{a}
Pseudomonas fluorescens	6.00±0.00^{b}	7.00±1.00^{b}	0.00±0.00^{a}	0.00±0.00^{a}	32.00±2.00^{c}	0.00±0.00^{a}
Klebsiella spp	15.00±1.00^{c}	13.00±1.00^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
Achromobacter spp	13.00±1.00^{d}	10.00±0.00^{c}	0.00±0.00^{a}	13.00±1.00^{d}	5.00±1.00^{b}	0.00±0.00^{a}

Notes: Test applied – One way ANOVA with Duncan *post hoc*, P˂0.05 (statistically significant); *Post hoc*: Values with the same letter superscripted do not vary significantly.

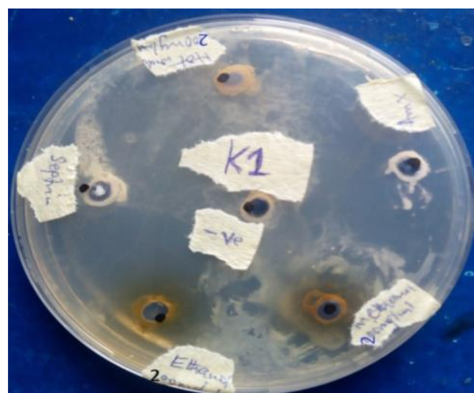

Plate 1 – Agar plate showing the effect of Orange (*Citrus aurantium) peel* extract on *Staphylococcus aureus*

Plate 2 – Agar plate showing the effect of Orange (*Citrus aurantium) peel* extract on *Klebsiella spp*.

Table 11 – Antibacterial Activity of Ethanol Extract at different concentrations on Gram-Positive Bacteria (mm)

Isolates	Ethanol Extract				
	Extract Concentration (mg/ml) and Diameter Zone of Inhibition (mm) (Mean±SE)				
	200 (mg/ml)	100 (mg/ml)	50 (mg/ml)	25 (mg/ml)	12.5 (mg/ml)
Staphylococcus Aureus	19.00±1.00^{c}	11.00±1.00^{b}	9.00±1.00^{ab}	8.00±0.00^{a}	8.00±0.00^{a}
Bacillus subtilis	15.00±1.00^{c}	13.00±1.00^{c}	6.00±2.00^{b}	5.00±1.00^{ab}	1.00±1.00^{a}
Clostridium perfringes	15.00±1.00^{b}	13.00±1.00^{b}	8.00±2.00^{a}	7.00±1.00^{a}	4.00±0.00^{a}

Notes: Test applied – One way ANOVA with Duncan *post hoc*, P<0.05 (statistically significant); *Post hoc*: Values with the same letter superscripted do not vary significantly.

Table 12 – Antibacterial Activity of Ethanol Extract at Different Concentrations on Gram-Negative Bacteria

Isolates	Ethanol Extract (Mean±SE)				
	Extract Concentration (mg/ml) and Diameter Zone of Inhibition (mm)				
	200 (mg/ml)	100 (mg/ml)	50 (mg/ml)	25 (mg/ml)	12.5 (mg/ml)
Acinetobacter iwoffi	12.00±0.00^{c}	11.00±1.00^{c}	9.00±1.00^{bc}	6.00±2.00^{b}	0.00±0.00^{a}
Alcaligenes spp	13.00±1.00^{c}	11.00±1.00^{bc}	9.00±1.00^{b}	3.00±1.00^{a}	0.00±0.00^{a}
Pseudomonas fluorescens	7.00±1.00^{c}	5.00±1.00^{bc}	3.00±1.00^{b}	0.00±0.00^{a}	0.00±0.00^{a}
Klebsiella spp	15.00±0.00^{c}	13.00±1.00^{c}	9.00±1.00^{b}	5.00±1.00^{a}	3.00±1.00^{a}
Achromobacter spp	13.00±1.00^{c}	11.00±1.00^{cd}	9.00±1.00^{bc}	7.00±1.00^{b}	3.00±1.00^{a}

Notes: Test applied – One way ANOVA with Duncan *post hoc*, P<0.05 (statistically significant); *Post hoc*: Values with the same letter superscripted do not vary significantly.

Table 13 – Antibacterial Activity of Methanol Extract at different concentrations on Gram-Positive Bacteria (mm)

Isolates	Methanol Extract, mg/ml				
	Extract Concentration and Diameter Zone of Inhibition (Mean±SE)				
	200 (mg/ml)	100 (mg/ml)	50 (mg/ml)	25 (mg/ml)	12.5 (mg/ml)
Staphylococcus Aureus	14.50±1.50^{b}	14.00±2.00^{b}	6.00±0.00^{a}	5.00±1.00^{a}	5.00±1.00^{a}
Bacillus subtilis	12.00±2.00^{a}	9.00±1.00^{a}	8.00±2.00^{a}	8.00±0.00^{a}	6.00±2.00^{a}
Clostridium perfringes	12.00±0.00^{c}	9.00±1.00^{b}	8.00±0.00^{b}	7.00±1.00^{b}	3.00±1.00^{a}

Notes: Test applied – One way ANOVA with Duncan *post hoc*, P<0.05 (statistically significant); *Post hoc*: Values with the same letter superscripted do not vary significantly.

Table 14 – Antibacterial Activity of Methanol Extract at different Concentrations on Gram Negative Bacteria

Isolates	Methanol Extract (Mean±SE)				
	Extract Concentration (mg/ml) and Diameter Zone of Inhibition (mm)				
	200 (mg/ml)	100 (mg/ml)	50 (mg/ml)	25 (mg/ml)	12.5 (mg/ml)
Acinetobacter iwoffi	7.00±1.00[b]	1.00±1.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Alcaligenes spp	10.00±2.00[c]	8.00±0.00[bc]	6.00±0.00[ab]	5.00±1.00[ab]	4.00±0.00[a]
Pseudomonas fluorescens	10.00±2.00[c]	7.00±1.00[bc]	4.00±0.00[ab]	4.00±0.00[ab]	1.00±1.00[a]
Klebsiella spp	15.00±1.00[c]	10.00±2.00[b]	5.00±1.00[a]	5.00±1.00[a]	1.00±1.00[a]
Achromobacter spp	10.00±2.00[c]	10.00±2.00[c]	7.00±1.00[ab]	5.00±1.00[ab]	3.00±1.00[a]

Notes: Test applied – One way ANOVA with Duncan *post hoc*, P<0.05 (statistically significant); *Post hoc*: Values with the same letter superscripted do not vary significantly.

Table 15 – Antibacterial Activity of Hot Water Extract at different concentrations on Gram Positive bacteria

Isolates	Hot Water Extract (Mean±SE)				
	Extract Concentration (mg/ml) and Diameter Zone of Inhibition (mm)				
	200 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml
Staphylococcus Aureus	3.00±1.00[b]	1.00±1.00[ab]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Bacillus subtilis	1.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Clostridium perfringes	3.00±0.00[c]	2.00±0.00[bc]	0.00±0.00[a]	0.00±0.00[a]	1.00±1.00[ab]

Notes: Test applied – One way ANOVA with Duncan *post hoc*, P<0.05 (statistically significant); *Post hoc*: Values with the same letter superscripted do not vary significantly.

Table 16 – Antibacterial Activity of Hot Water Extract at different concentrations on Gram Negative bacteria

Isolates	Hot Water Extract, (Mean±SE)				
	Extract Concentration (mg/ml) and Diameter Zone of Inhibition (mm)				
	200 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml
Acinetobacter iwoffi	0.00±0.00[a]	1.00±1.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Alcaligenes spp	2.00±-0.00	1.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Pseudomonas fluorescens	3.00±1.00[c]	1.00±1.00[ab]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Klebsiella spp	2.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Achromobacter spp	2.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Notes: Test applied – One way ANOVA with Duncan *post hoc*, P<0.05 (statistically significant); *Post hoc*: Values with the same letter superscripted do not vary significantly.

The present study is an effort to test the potential of *Citrus aurantium* peel as a natural antibacterial agent. The antibacterial efficacy of peel extracts of *Citrus aurantium* against eight bacteria by Agar well diffusion was evaluated. The powdered peel of *Citrus aurantium* was individually extracted with different solvents (Ethanol, Methanol and Hot water). The preliminary phytochemical screening of the extracts of *Citrus aurantium* showed the presence of alkaloids, glycosides, phenols, tannins, saponins, terpenoids and steroids (Tables 5, 6). Due to the presence of different phytochemical compounds in the *Citrus aurantium* peel ex-

tracts, it has efficient Antibacterial activity against different microorganisms, including Gram-positive and Gram-negative bacteria. Antibacterial properties of medicinal plants are being increasingly reported from different parts of the world. The World Health Organisation estimated that plant extracts or their active constituents are used as folk medicine in traditional therapies by 80% of the world population. In the present work, the extracts from bitter orange (*Citrus aurantium*) showed strong activity against most of the tested bacteria. Among the extracts tested, the ethanol extracts exerted the highest activity on bacterial agents tested compared to the methanol and hot water extracts. The ethanol extract at 200 mg/ml, for example, 21.00±1.00 mm, was recorded as a diameter zone of inhibition against *Staphylococcus aureus.* This was followed by a 15.00±1.00 mm diameter zone of inhibition each against *Bacillus subtilis*, *Clostridium perfringes* and *Klebsiella* spp, 13.00±1.00 mm diameter zone of each against *Alcaligenes* spp and *Achromobacter* spp, 12.00 mm diameter zone of inhibition against *Acinetobacter iwoffi* and 6mm diameter zone of inhibition against *Pseudomonas fluorescence* (Table 9 and 10). Whereas at the same concentration (200 mg/ml) the methanol extracts exerted the highest activity against *Staphylococcus aureus* with a diameter zone of inhibition of 15.00±1.00 mm, followed by 13.00±1.00 mm zone of inhibition against *Klebsiella* spp, 12 mm diameter zone of inhibition each against *Bacillus subtilis* and *Clostridium perfringes,* 10mm diameter zone of inhibition each against *Achromobacter* spp and *Alcaligenes* spp and 7.00±1.00mm zone of inhibition each against *Acinetobacter iwoffi* and *Pseudomonas fluorescens* (Tables 9, 10). The hot water extracts recorded the least activity (3mm diameter zone of inhibition) against *Staphylococcus aureus*, *Pseudomonas fluorescens* and *Clostridium* spp at 200 mg/ml. The differences in the observed activities of the various extracts may be due to the varying solubility of the active constituents in the three solvents used. It has been documented that different solvents have diverse solubility capacities for different phytoconstituents [25]. The difference in activities among the solvents recorded in this study may be associated with the presence of oils, wax, resins, fatty acids or pigments, which had been reported to be capable of blocking the active ingredients in the plant extract, thus preventing the plant extract from accessing the bacterial cell wall. This study showed the efficacy of the phytochemicals from the *Citrus aurantium* peel to inhibit Gram-positive bacteria to a greater extent than Gram-negative bacteria, in agreement with the study [26]. A marked inhibitory effect of ethanolic and methanolic extract of *Citrus aurantium* against a panel of bacteria was observed [27]. Activities of the various extracts were comparable to those of standard antibacterial agents Amoxicillin and Septrin ($p < 0.05$). The antibiotic sensitivity test on the isolated bacteria showed that most bacteria were resistant to the antibiotics while some were sensitive. This may result from different properties in the cell wall of bacteria, such as peptidoglycan in the walls of Gram-positive bacteria, which is a thick layer responsible for the resistance of the bacteria to osmotic pressure. Also, lipopolysaccharide in the cell wall of Gram-negative bacteria is a major component contributing greatly to the structural integrity of the bacteria and protecting the membrane from certain kinds of chemical attack [28]. The widespread use of conventional drugs inside and outside medicine plays a vital role in the emergence and re-emergence of resistant bacteria [29]. The extensive and incorrect use of antimicrobial agents has consistently led to the development of antibiotic resistance, which has become a major problem globally [29]. Authors [30] observed in recent discoveries that bacteria antibiotic resistance patterns are sometimes due to the presence of large plasmids and the ability of the plasmids to undergo the conjugation process. It is, therefore, imperative to note that orthodox medicine cannot fill the space of microbial resistance alone. As a result of this, there is a need for medicinal plants to aid antibiotics in the treatment of various diseases caused by these microorganisms. Demonstrating the antibacterial activity of Citrus aurantium peel extracts against the test bacteria indicates the possibility of sourcing alternative antibiotic substances in these plants to develop newer antibacterial agents.

CONCLUSIONS

The peels of citrus fruits are considered to be waste products. In this study, the peel extract of Citrus aurantium exerted antibacterial activity

against Gram-positive and Gram-negative bacteria associated with different infections, including pneumonia (*Klebsiella spp*) and urinary tract infections (*Staphylococcus aureus*). The study, therefore, provides the scientific basis for its traditional application as ethnomedicine. The antibacterial efficacy of the peel extracts can be ascribed to the presence of secondary metabolites. The peels of this citrus fruit can then be used to treat infectious diseases caused by Gram-positive and Gram-negative bacteria. The demonstration of activity against both Gram-positive and Gram-negative bacteria indicates a broad spectrum of activity. It thus can be used to source antibiotic substances for drug development that can be used to control these bacterial infections. Further investigations of its activity against a broader range of bacteria and fungi, identification and purification of its chemical constituents, and toxicological investigations of the plant extracts should be carried out to develop novel drugs for human consumption.

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