

# ***Antibacterial* activity of sweet orange (*Citrus sinensis*) on *Staphylococcus aureus* and *Escherichia coli* isolate from wound infections**

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**Abstract** :The antibacterial activity of sweet orange ( *citrus sinensis* ) on the microorganisms like *Staphylococcus aureus*, *Escherichia coli* are isolated from wound infections. Totally of 15 wound samples of different age range were collected. The two bacterial wound isolates were identified after subjecting them to different biochemical tests. The dried and powdered plant materials were extracted using standard qualitative procedure and agar well diffusion method was used for antibacterial assay. The peel extracts of *Citrus sinensis* were determined with values ranging from 0.6% to 10%. The peel extracts acts as an inhibition against the three isolated pathogenic bacteria present in the wound. The results of the phyto-chemical screening of the peel revealed the presence of alkaloids , terpenoids , flavonoids , tannins , saponins , reducing sugar and amino acids. The result of the sensitivity pattern of both orange extract and antibiotics on *S. aureus* and *E. coli* showed that some of the isolated organisms were susceptible to both orange extract and antibiotics. The result showed that the zone of inhibition of the orange extract were minimal on both *S. aureus* and *E. coli*. Its active ingredient vitamin C boosts the immune system inside the body increasing the production of B and T cells and other white blood cells including those that destroy micro organisms.

**Keywords** : *Staphylococcus aureus* , *E. Coli* , antibacterial activity , sweet orange , wound infections.

## **I. INTRODUCTION**

Orange is the fruit, comes under citrus species which is scientifically known as *citrus sinensis* ( sweet orange). The sweet orange is full of genome sequenced. That is it is the hybrid between pomelo and mandarin. The protective nature of the fruit is due to the presence of phyto constituents such as poly phenolic compounds. Peels are generally wasted while the citrus fruits are mainly used in juice processing industries. The pollution of environment can also be reduced by this. The orange peels are rich in nutrients, which can be used as drugs or food supplements too. Oranges are cultivated in many countries like India, UK, France, Germany, Holland, Brazil, China, USA and Spain. The peel and juice of the sweet orange is used to make medicines. Sweet orange contains large amounts of vitamin C. Vitamin C also has antimicrobial and antioxidant activity. An antimicrobial is a substance which kills or inhibits the growth of many types of microorganisms such as bacteria, fungi or protozoans. Antimicrobial drugs either kill or prevent the growth of microbes. Antimicrobials are substances which are used on nonliving objects as disinfectants. Citrus fruit products act as antimicrobial agents against bacteria and fungus. It has an important physiological role because of its commercial value in food and pharmaceutical industries of the entire world. The antioxidant property is present in the plant materials due to many active photochemicals which include vitamins, flavonoids, terpenoids, carotenoids, coumarins, lignin, saponin, plant sterols etc. Citrus fruits and their juices are an important source of bioactive compounds, the most important to human nutrition which include antioxidants such as ascorbic acid, phenolic compounds, flavonoids and pectins.

### **1.1 Bacterial species**

#### **a. *Staphylococcus aureus***

*Staphylococcus* is a gram positive bacteria which is small round shaped and it is grape like clusters. It is one of the five most infection causing organisms. It affects all mammalian species which also includes human beings. It also transmits from human to animals. It transmits through air droplets or aerosols. It has a variety of extracellular proteins and polysaccharides. These are correlated with virulence. The antibodies will neutralize staphylococcal toxins and enzymes, but vaccines are not available. Some species of staphylococci have been recognized biochemically and analyzed and particularly by DNA-DNA hybridisation.

#### **b. Morphology:**

- Gram positive cocci about 0.5 – 1.0 µm in diameter.
- They are in pairs and grow as clusters.
- Non motile
- Non spore forming
- Catalase : positive
- Oxidase : negative
- Facultatively anaerobic

**c. E. Coli :**

*Escherchia coli* also known as *E .coli*. It is found in the lower intestine of warm blooded organisms. Mostly their strains are harmless but they cause food poisonings. It also causes food contamination. They can be grown and cultured easily and inexpensively in a laboratory. It is the most important species in the field of biotechnology and microbiology. It is also used for majority of work with recombinant DNA. The generation time of *E.coli* is 20 minutes. Outside the normal habitat it causes some serious infections. When wound gets contaminated with *E.coli* may lead to wound infection. It is a common cause of infections in surgical wounds. The antibodies that are best for the *E.coli* are azithromycin, ciprofloxacin and levofloxacin.

**d. Morphology:**

- They are gram negative bacteria and 1-3 x 0.4-0.7 µm in size and 0.6 to 0.7µm in volume
- They are facultative anaerobes
- They are both fermentative and respiratory
- They are either non –motile or motile by peritrichous flagella
- They are non sporing
- Growth occurs over a wide range of temperatures from 15-45 degree celcius.
- Their cell wall is thin with only 1 or 2 layers of peptidoglycan.

**II. MATERIALS AND METHODS****2.1 Media preparation :**

The growth of an organism on a medium is called culture. The food base that support the growth of an organism is called culture medium. The culture media are devised in such a way that the organism should get all the nutritional requirements. These culture media are prepared in a laboratory by weighing ingredients. Generally the media contains both the organic and inorganic matter but for cultivating organisms specialised media are prepared. To solidify the media , agar is mixed with other ingredients.

**2.2 Nutrient agar:**

It is used for the general purpose like nutrient medium is used for the cultivation of microbes supporting growth of some non fastidious organisms. It grows variety of types of bacteria and fungi and contains many nutrients needed for the bacterial growth.

**a. Composition of nutrient agar:**

- 0.5% peptone
- 0.3% beef extract
- 1.5% agar
- 0.5% NaCl
- Distilled water
- pH – neutral (7.4) at 25 degree celcius

**b. Preparation of nutrient agar:**

Take 28g of nutrient agar powder in 1 litre of distilled water. Heat this mixture while stirring to fully dissolve all component. Autoclave the dissolved mixture at 121 degree celcius for 15 min for sterilization. After sterilization of the nutrient agar, allow it to cool but do not solidify it. Pour nutrient agar into each plate and the plate has to be kept in the sterile surface for solidification. Replace the lid of each petri dish and store the plates in a refrigerator.

**TABLE 1- COMPOSITION OF NUTRIENT AGAR**

TYPICAL FORMULA	NUTRIENT AGAR ( gm/litre)
Lab lemco powder	1.0
Yeast extract	2.0
Peptone	5.0
Sodium chloride	5.0
Agar	15.0

**2.3 Mac conkey agar:**

Mac conkey agar was the first solid differential media to be formulated which was developed at 20<sup>th</sup> century century by Alfred Theodore mac conkey. Mac conkey agar is a selective and differential media used for the isolation and differentiation of non fastidious gram negative rods , particularly members of the family enterobacteriaceae and the genus pseudomonas.

**a. Composition of Mac conkey agar :**

- Peptone -17 gm
- Poteose peptone- 3gm
- Lactose monohydrate -10gm
- Bile salts – 1.5gm
- Sodium chloride -5gm
- Neutral red – 0.03gm
- Crystal violet – 0.001gm
- Agar- 13.5 gm
- Distilled water – 1 liter
- pH- 7.1

**b. Preparation of Mac conkey agar :**

Suspend 49.53 grams of dehydrated medium in 1000ml distilled water. Boil it to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure for 15 mins. Cool to 45-50 degree celcius. Mix well before pouring into sterile petri plates. Once the agar solidifies , the agar is ready to use .

**2.4 Sample collection :**

The sweet oranges was purchased from Reliance fresh super market in Coimbatore, tamilnadu. The fruits was transported to microbiology laboratory of DR.NGP arts and science college, Coimbatore. The fruits were washed with distilled water and sterilizing using 70% alcohol and kept in a sterile zip polythene bag until ready for use.

**2.5 Disk diffusion method:**

The plates were inoculated by dipping a sterile swab into the inoculum. The excess inoculum was removed by pressing and rotating the swabs firmly against the side of the tube above the level of the liquid. Prepared the lawn culture by streaking the swab all over the surface of the medium three times, rotating the plate through an angle of 60 degree after each application. Finally, passed the swab round the edge of the agar surface. Left the inoculum to dry for a few minutes at room temperature with the lid closed .The plate was inverted and divided into four parts using the marker Plain white discs were placed on three parts on the inoculated plate using sterile forceps. The sample ( orange peel methanol extract) of different concentrations were prepared of dilutions 1:1,1:2,1:3\_Using a microscope 500µl of the extract of different dilutions were placed on the white plain discs. At the fourth part of the inoculated plate the antibiotic disc was placed using a pair of sterile forceps ( A sterile needle tip may also be used to place the antibiotic disc on the plate. Alternatively, an antibiotic disc dispenser can be used to apply the discs to the inoculated plate).The plates were then incubated at 37 degree Celsius for 24 hours and observed for results.

**III. RESULTS AND DISCUSSION****FIGURE 1**



FIGURE 2

TABLE 2-ANTIBIOTIC SUSECEPTIBILITY TEST

Antibiotic type	Concentration	Size of clear zone	<i>S. aureus</i>	<i>E.coli</i>
Sweet orange	5mg/ml	Small	-	-
	10mg/ml	Small	+	-
	15mg/ml	Medium	+	+
	25mg/ml	Large	+	+
Tetracycline	30mg/ml	Very small	-	+
Chloramphenicol	10mg/ml	Large	+	+
Penicillin	30mg/ml	Medium	-	+

The result of the findings are presented in the table. The result showed that the *S.aureus* and *E.coli* are highly sensitive to concentrations of 0.75ml/20ml of agar media in using disk diffusion method. The isolates of *E.coli* was a bit resistant at concentration of 0.5ml/28 ml of media. This is because of the permeability of *E.coli*, that is 20% of membrane of *E.coli* is made up of lipid while that of *S.aureus* is only made up of 2% lipid.

We have also observed that as the concentration of the orange extract increases increases, we have also observed that the efficiency increased and their growth and inhibition has been diminished. As observed from the above tables, large clear zones at higher concentrations. This implies that, sweet orange has both bacteriostatic and bactericidal effect.

Natural and environmentally friendly antimicrobials, antibiotics, antioxidants have become the priority search for pharmaceutical industries. citrus is the only fruit that has the major role. In the study of antibacterial activity and phytochemical analysis of *citrus sinensis* peel extract against three organisms isolated from wound infection which have also been associated with wound infection. These includes gram positive and gram negative bacteria. In this study, the peel extract of the *C.sinensis* showed good antibacterial activities against tested organisms. The ability of these extracts to inhibit the growth of the tested bacteria in varying degrees is an indication of the presence of the active principles for antimicrobial actions. The extracts of *C.sinensis* fruits contain antimicrobial substances.

#### IV. CONCLUSION:

The result of the study shows that extracts of *C.sinensis* have varying degree of antimicrobial activity against *S.aureus* and *E.coli*. The extracts of *citrus sinensis* are beneficial in developing an antibacterial agent that can be used in treatment of infections. Pharmaceutical industries shows much interest towards these new leads that are cheap and easily available raw materials in the era of threat of resistance by many pathogenic bacteria. The antimicrobial activity of these extracts may not be unconnected to the phytochemical constituents.

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