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A STUDY ON EFFECTIVENESS OF SWEET ORANGE AGAINST BACTERIAL WOUND ISOLATES

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ABSTRACT: Sweet orange is currently being consumed as a health promoting and disease preventing food. Citrus fruits are good source of natural bioactive compounds such as vitamin C, carotenoids, flavonoids, limnoids, essential oils, acridone alkaloids, minerals and vitamin B. It also has antioxidant properties. The aim of this study is to evaluate the antimicrobial activity of *Citrus sinensis* (sweet orange) juice extract against different microbial isolates.The screening of antibacterial activity was done by agar well diffusion technique at 15µl concentration. The extracts were tested for antibacterial activity against various wound pathogens such as Gram negative isolates(*Pseudomonas aeruginosa, Klebsiella pneumonia, Proteus mirabilis, Acinetobacter baumannii*)and Gram positive isolates(*Staphylococcus aureus*).The citrus juice extract showed various levels of inhibitory zone on different wound isolates, ranging from 20-29mm and the highest zone of inhibitory activity was against *Pseudomonas aeruginosa* with the zone of 29mm.Therefore citrus juice extract can be used as an effective antibacterial agent against multidrug resistant bacteria, and moreover it is readily available, safe, cheap and has no side effects .
Key words: Agar well diffusion, antimicrobial activity, juice extract, sweet orange, wound isolates and zone of inhibition.

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INTRODUCTION

Fruits are known to be an integral part of diet and are consumed as fresh juices, salads or fruit based drinks. Besides their delicious taste and flavour, the fruits are also known to reduce the risk of several chronic diseases including cancer. The protective nature of fruits are due to the presence of phyto constituents such as poly phenolic compounds. Among various fruits consumed, citrus fruits are widely used in almost all countries.The genus citrus belongs to the family Rutaceae. [1]

Citrus fruits are acidic fruits, rich in nutritional content that works wonders for the body. It acts as a fabulous and rich source of vitamin C and provides wide variety of essential nutrients that are required for the body. Fresh fruits and their hand-squeezed or industrially processed juices contain mostly flavanones and flavones. [2,3]

Citrus fruits are the main source of important phytochemical nutrients and for a long period have been valued for their wholesome nutritious and antioxidant properties. It is scientifically proven that oranges being rich in vitamins and minerals have many health benefits.

Moreover, it is well appreciated that other biologically active, non-nutrient compounds found in citrus fruits such as phytochemical antioxidants, soluble and insoluble dietary fibers are known to be helpful in reducing the risk for cancers, many chronic diseases like arthritis, obesity and coronary heart diseases.[4]

Sweet orange, *Citrus sinensis*, is one of the delicious fruits consumed by the human beings and it is considered as industrially important among the major fruit crops. Globally, the cultivation of *Citrus* is approximately nine billion hectares, leading to 122.3 million tonnes of fresh fruit harvest. [5] Sweet oranges contains low calories, no saturated fats or cholesterol, but is rich in dietary fibers and pectin which are very effective in people with obesity. Pectin as a bulk laxative protects the mucous membrane from exposure to toxic substances, as well as binds to carcinogenic chemicals in the colon. Pectin also helps in the reduction of blood cholesterol levels by decreasing its re-absorption in the colon by binding to bile acids in the colon (Walton *et al.* 1945). [6]

Sweet orange is currently being consumed as a health promoting and disease preventing food. *Citrus* is a good source of natural bioactive compounds such as vitamin C, carotenoids, flavonoids, limnoids, essential oils, acridone alkaloids, minerals and vitamin B. [7] Infectious diseases are the reason for death world wide. Antibiotic resistance has become a global concern. [8] Researchers are increasingly turning their attention to folk medicine. Continuous research leading to development of better drugs against microbial infections. [9]

Several medicinal plants are being screened for their potential microbial activity due to increased failure rate on chemotherapy and antibiotic resistance exhibited by pathogenic agents. [10] Plant extracts and phytochemicals with antimicrobial activity have been used as therapeutics since ancient times. [11]

Many medicinal plants have been found effective in the treatment of bacterial diseases. Due to increasing antibiotic resistance against microorganisms and also the adverse effects of various synthetic antibiotics, medicinal plants are now more effective in the treatment of bacterial infections. In most of the developing countries, traditional medicines and medicinal plants have been used as the therapeutic agents for the maintenance of good health that has been widely observed. Medicinal plants are considered as clinically effective and safer alternatives to synthetic antibiotics. [12]

According to World Health Organization (WHO), medicinal plants would be the best source to obtain a variety of drugs. Extensive research done in the area of isolation and characterization of the active principles of these plants are required so that better, safer and cost effective drugs for treating bacterial infections can be developed. [13]

Therefore, this present study was conducted to evaluate the antibacterial activity of sweet orange against bacterial isolates from wound of the affected patients.

MATERIALS AND METHODS

Microbial isolates

5 different clinical microbial isolates (Gram negative and Gram positive bacteria) were collected and identified by using conventional biochemical tests. [14] and were cultivated in pure culture, in the Microbiology laboratory in SSSMC & RI. The microbial isolates includes *Pseudomonas aeruginosa, Klebsiella pneumoniae, E. coli, Proteus mirabilis, Acinetobacter baumannii, Staphylococcus aureus*.

Fruit collection

Fresh fruit *Citrus sinensis* (sweet orange) were obtained from the local market in and around Thiruporur. The fresh fruits were washed in running tap water in laboratory, surface sterilized with 70% alcohol, rinsed with sterile distilled water and cut open with a sterile knife and the juice pressed out into a sterile universal container separately and then filtered (using Millipore 0.45 filter paper) into another sterile container to remove the seeds and other tissues which is used freshly as crude extract without refrigeration. [15]

Antimicrobial activities

The screening of antimicrobial activities of juice extract on the tested bacteria investigation was determined on nutrient agar media, by the agar diffusion techniques using agar well diffusion method. Wells of 6 mm diameter and 5 mm depth were made on the solid agar using a sterile glass borer. [16,17] Approximately 15µl of juice extract was inoculated onto well were made in the spread plate culture of eachmicrobial isolates. The plate was incubated overnight at 37°C. After 12-24 hrs of incubation, juice extract was noted for zone of inhibition for all isolates. The diameters of the zone of inhibitions were measured by measuring scale in millimeter (mm). Sterilized distilled water or solvent (ethanol) was introduced into a well for negative control. Parallel with fruit extracts, the antibacterial activity was also analyzed with 8 commercially available standard antibiotics (Cefotaxime (30µg), Ceftazidime (30µg), Imipenem (10µg), Amoxycillin/clavulinate (20/10µg), Amikacin (30µg), Gentamicin (10 µg), Ciproploxacin (5µg), Cotrimoxazole (25µg).

RESULTS

In the present study, antibacterial activity of juice extracts obtained from sweet orange (*Citrus sinensis*) against Gram negative bacteria (*Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Proteus mirabilis, Acinetobacter baumannii*) and Gram positive bacteria (*Staphylococcus aureus*) were tested by the agar well diffusion method. The inhibitory effect (zones of inhibition) of these extract against bacterial wound isolates was measured in millimeter (mm) are shown in Figures (1, 2, 3,4& 5).

A total of 114 bacterial isolates were collected from septic wound patients. Out of these, from 25 isolates of *Pseudomonas aeruginosa* 19 (76%) were MDR strains followed by 23 number of *Klebsiella pneumoniae* from which 16 (70%) MDR strains, from 21*proteus mirabilis* 11(52%) were MDR strains, from 18 *Acinetobacter baumannii* 10(56%) were MDR strains, 27 isolates of *Staphylococcus aureus* 18(67%) were MDR strains.

Antibacterial activity of sweet orange against five different isolates of multi drug resistant strains, showed the zone of inhibition for *Pseudomonas aeruginosa* (29mm), *Klebsiella pneumonia* (27mm), *Proteus mirabilis* (24mm), *Acinetobacter baumannii* (22mm) *Staphylococcus aureus* (20mm) were recorded as shown in the Table 1.

Juice extract of sweet orange showed highest zone of inhibition against *Pseudomonas aeruginosa* of *about* 29mm. Hence this study proved the juice extract were effective in inhibiting all MDR bacterial isolates in various ranges. The result of the present investigation indicate that the sweet orange extracts of citrus fruits has antimicrobial and antioxidant activity.

In the upcoming world of bacterial multidrug resistance, sweet orange *Citrus sinensis*being a natural product has proved to be effective against the major microbial organisms encountered in clinical settings especially the indestructible nosocomial organisms like *Pseudomonas aeruginosa and Acinetobacter baumannii*. The use of such novel natural products by pharmaceuticals or consumption of fruit by people has got least side effects, greater benefit and also enhances or renews the quality of people life.

Table 1: Antibacterial activity of sweet orange juice

S.No	Organisms isolated	Total :No of isolates	MDR	Percentage	Zone of inhibition
1	*Pseudomonas aeruginosa*	25	19	76%	29mm
2	*Klebsiella pneumoniae*	23	16	70%	27mm
3	*Proteus mirabilis*	21	11	52%	24mm
4	*Acinetobacter baumannii*	18	10	56%	22mm
5	*Staphylococcus aureus*	27	18	67%	20mm

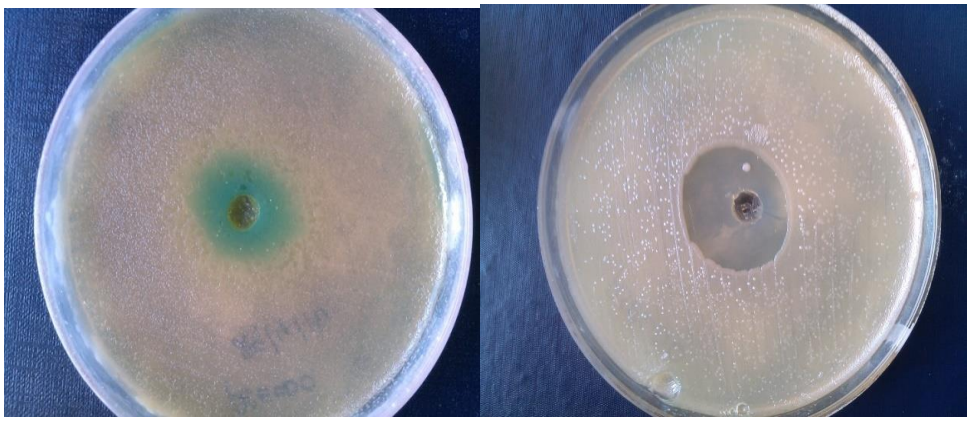

Figure 1& 2: Zone of inhibition of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*

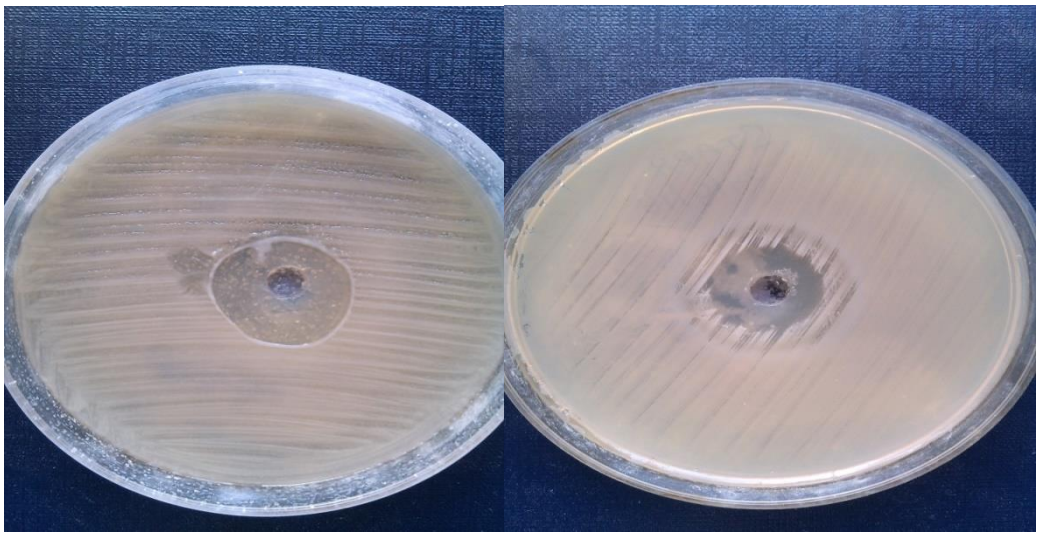

Figure 3& 4: Zone of inhibition of *Proteus mirabilis* and *Acinetobacter baumannii*

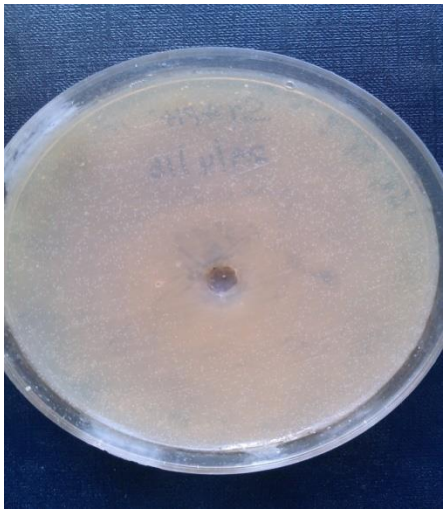

Figure 5::Zone of inhibition of *Staphylocoocus aureus*

DISCUSSION

In the present study, we have evaluated the antibacterial activity of juice extract of sweet orange against 5 different bacterial isolates from wound infection. Among which 4 isolates were found to be Gram negative and the remaining were found to be Gram positive bacteria. Out of 114 isolates, 74 isolates of MDR strains showed maximum inhibitory zone against *Pseudomonas aeruginosa* (29mm) followed by *Klebsiella pneumoniae* (27mm), *Proteus mirabilis* (24mm), *Acinetobacter baumannii* (22mm) *Staphylococcus aureus* (20mm).

In many countries these ethno botanical knowledge bases were further explored and interesting bioactive properties of *Citrus* have been recognized. Abeysinghe *et al* documented that antioxidant activity of sweet orange is attributed to many important phytochemicals present in *Citrus* fruits. [18]

The results revealed the presence of medicinally important constituents in the sweet orange juices. Many evidences gathered in earlier studies which confirmed and identified phytochemicals to be bioactive. Therefore these citrus fruit juices could be used as a good source for an effective treatment. [19] In our results, juice extracts of *C. sinensis* were significantly effective against both Gram-negative and Gram-positive organisms with inhibition zone 20-29mm.

Many studies have reported the antioxidant and antibacterial effect of juice and edible parts of oranges of different origin and from different varieties [20] The efficacy of citrus juice to inhibit bacterial growth in this study were concurrent with Mathur *et al.* [21] who showed that the aqueous extracts from citrus fruit pulp contained significant antimicrobial activity. Al-Ani *et al*. [22] revealed that the best antimicrobial activity was observed in juices of *C. aurantium*, they were active against the three types of bacteria used in their study which includes *S. aureus P. aeruginosa* and *P. vulgaris* with 16-20mm inhibitory zones and this result was almost nearer to the values (20-29 mm at 15µl concentration) which we have obtained by our study.

Thus the antibacterial activities and other medicinal attributes such as anti cancerous properties due to phytochemicals such as tannins and flavonoids clearly highpoint the importance of characterizing sweet orange. Therefore the antibacterial activity of sweet orange detected in the present study is in line with the findings of H. M. P. D. Herath et al., in their studies sweet orange cultivar also exhibited significantly higher activities against *E. coli*, *S. aures* and MRSA (9.5 mm, 10.2 mm and 6.3 mm respectively). [7] In the same way, Madhuri et al proved that the methanol extracts from peels of sweet orange were active against *Klebsiella pneumoniae* and *B. cereus* [23]

Unlike other studies, this study revealed that juice extracts of sweet orange showed greater antibacterial activity against *Acinetobacter baumannii* (22mm).Results of the current study recommend the advantages and effect of the antibacterial activity of sweet orange extracts against wound isolates compared to the standard antibiotics.

CONCLUSION

The intention of this project is to detect the antimicrobial activity of juice extract from sweet orange fruit. Due to increasing antibiotic resistance among bacteria, the results of the present study gives substantial evidence that natural sweet orange has the capacity of possessing phytochemical antioxidant property and juice extracts can be used as effective antibacterial agent against multidrug resistant bacteria such as (*Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis, Acinetobacter baumannii* and *Staphylococcus aureus*) and also sweet orange is readily available, safe, cheap and no side effects, so they are more preferred than the synthetic drugs for the wound infections.

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