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COMPARATIVE STUDY OF ANTIBACTERIAL ACTIVITY OF PEEL EXTRACTS OF C*ITRUS AURANTIUM L.* (BITTER ORANGE) AND *CITRUS MEDICA L.* (LEMON) AGAINST CLINICAL ISOLATES FROM WOUND INFECTION

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ABSTRACT

The main objective of this study is antibacterial activity of Lemon (*Citrus medica* L.) and Bitter Orange (*Citrus aurantium* L.) Peel extract against clinical isolates from wound infection. As microorganism are becoming resistant to present day antibiotics, our study focuses on antimicrobial activity and future prophylactic potential of the lemon and orange peels. The antibacterial activity of Peel extract of *Citrus medica* L. and *Citrus aurantium* L. were evaluated on bacterial strains like *E. coli, Ps. aeruginosa, Kl. pneumoniae, Pr. vulgaris, Serratia marcescens , Propionibacterium acne, Enterococcus spp., Streptococcus spp., S. aureus* and *S. epidermidis.* All bacteria were isolated from wound infected patients and were multiple drug resistant to many antibiotics. Antibacterial activity of five different solvent extracts (Methanol, acetone, ethanol, petroleum ether and n-Hexane) were prepared by using Soxhlet extractor. In-vitro antibacterial activity was performed by agar well diffusion method. *Citrus medica* peel extracts showed a very good antimicrobial activity when compared to *Citrus aurantium*. Both plants peel extract showed considerable antibacterial activity against all tested bacteria. The peel extract of *Citrus aurantium* and *Citrus medica* can be considered to be as equally potent as the antibiotics used now-a-days.

KEYWORDS : Clinical isolates, antibacterial activity, wound infection, Lemon, Orange, Soxhlet extractor

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INTRODUCTION

Pharmacological industries have produced a number of new antibiotics in the last three decades; resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents [1]. The clinical efficiency of many antibiotics in existence is being treated by the emergence of multi drug-resistant pathogen [2]. To overcome this problem of resistance of bacteria, researchers concentrate their study to find out new drug from medicinal plants. Throughout the history of mankind, many infectious diseases have been known to be treated with herbal remedies .The natural herbal products either as pure compounds or as standardized plant extracts provided unlimited opportunities for new drug leads because of the uncompared availability of diversities of chemical. This results to a never ending and urgent need to discover new antimicrobial compounds with different chemical structure and new mechanisms of action for re-emerging and new infectious diseases [3]. Citrus is one of the most important commercial fruit crops grown in all continents of the world. Oranges and Lemon are an important medicinal plant of the family Rutaceae. It is cultivated mainly for its alkaloids, which are having anticancer activities and the antibacterial potential in crude extracts of different parts (viz*.,* leaves, stem, root and flower) of Lemon against clinically significant bacterial strains has been reported [4]. Citrus fruits are mainly used by juice processing industries while the peels are generally wasted. Since the juice yield of citrus is less half of the fruit weight, very large amounts of byproduct wastes, such as peels are formed every year [5]. Peel waste are highly perishable and seasonal, is a problem to the processing industries and pollution monitoring agencies. So there will be take attention in bringing useful products from citrus waste materials. The citrus peels are rich in nutrients and contain many phytochemicals, these can be efficiently used as drugs or as food supplements. The peel of *Citrus* fruits is a rich source of flavonoid glycosides, coumarins, β and γ- sitosterol, glycosides and volatile oils [6]. Many polymethoxylated flavones have several important bioactivities, which are very rare in other plants [7]. In addition the fiber of citrus fruit also contains bioactive compounds, such as polyphenols, the most important being vitamin C (or ascorbic acid), and they certainly prevent and cure vitamin C deficiency-the cause of scurvy [8]. Many studies have reported antioxidant and antibacterial effect of juice and edible parts of lemon and oranges of different varieties [9]. As far as the peel is concerned, extracts from this part of the fruit were found to have a good total radical anti-oxidative potential [10]. This study was aimed to minimize waste of fruit juice processing industries. The major waste part of lemon and oranges were peel, which were not used for any purpose. This research is for evaluating the component used as an antibacterial activity from waste peels of lemon and oranges.

MATERIALS & METHODS

1) Plant materials

The plants used in this study were *Citrus aurantium L.* (Bitter Orange) and *Citrus medica L.* (Lemon). The peels were collected from the local fruit juice shops. After collection, the peels were shade dried at room temperature (30 - 35ºC). 20 gm of peels of oranges and lemon were coarsely powdered using a mortar and pestle and were further reduced to powder using an electric blender. The powder was transferred into closed containers for further use.

2) Preparation of extracts

a) Soxhlet extraction

The dried and powdered peel materials (20 gm) were extracted with 200 ml of each solvent separately by using soxhlet extractor for 2 to 5 h at a temperature not exceeding the boiling point of the Solvent [Lin *et al*., (1999)]. The solvents used for the study were Methanol, Petroleum ether, Acetone, Ethanol and n-Hexane. The extracts were filtered and then concentrated to

dryness. The extract were transferred to glass vials and kept at 4º C before use. The extracts were dissolved in 25% aqueous dimethyl sulfoxide (DMSO) to produce a stock solution of 100 mg/ml.

3) In vitro testing of extracts for antimicrobial activity

a) Bacterial Isolates

The bacteria were isolated from wound infected sample of patients. The antibacterial activity of peel extract of orange and lemon were tested against *E. coli, S. aureus, Ps. aeruginosa, Klebsiella spp., Proteus vulgaris, Proteus mirabilis, S. epidermidis, Serratia spp., Streptococcus spp., Propionibacterium acne, Enterococcus spp.* These isolates were stored in Nutrient agar slant at 4^0C for further use.

***b)* Antimicrobial assay**

Prepare petri plates of sterile molten Mueller Hinton Agar (Hi-Media, Mumbai) at around 40ºC and solidify the petri plates. After solidification 6 mm wells were prepared. In these wells solvent extracts of the peel were added. The plate was incubated overnight at 37ºC. After incubation the zones of inhibition were measured and recorded. These studies were performed in triplicate.

RESULTS AND DISCUSSION

Citrus fruits belong to the family Rutaceae. These are rich source of vitamin-C (Ascorbic acid). The peel of the citrus fruits contains various active constituents and essential oils. In the present study the antibacterial activities of the peel of citrus fruits has been investigated. In the last few years, an increased attention has been focused on the industrial wastes, especially those containing residual phenols from the used plant raw material. Orange peel is one of the important dietary sources of antioxidant phenolics [11]. Citrus peel extracts showed a significant antibacterial activity against all the test organisms. *Citrus medica* peel extracts showed a very good antibacterial activity when compared to *Citrus aurantium*. The Ethanolic extract of *Citrus medica* showed a highest zone of inhibition against *Serratia spp.*(28 mm) followed by *Pr. mirabilis* (21 mm) and 18 mm zone was observed in *Klebsiella spp., Enterococcus spp.* and *S. aureus* [Table No. 1 & Fig. 1]. The methanolic extract of *Citrus medica* also showed good inhibitory activity against tested bacteria. The highest zone of inhibition showed against *Serratia spp.*(22 mm) subsequently *S. aureus, Ps. aeruginosa, Streptococcus spp, Enterococcus spp and Propionibacterium acne* showed 20 mm of zone of inhibition. The other solvent such as acetone, petroleum ether and n-Hexane showed least inhibition against tested bacteria. Maruti J. Dhanavade et. al., (2011) reported that *Citrus lemon* L. was effective against *Pseudomonas aeruginosa* and compounds like coumarin and tetrazene were present *lemon* peel extract [13]. The study conducted by Kabra AO showed that the ethanolic extract showed inhibitory activity against all tested bacteria [14]. Comparing their results with our study with the same test organism and solvent shows that *Citrus medica* L. extract showed relative similar results.

On the other hand, the antibacterial activity of *Citrus aurantium L.* (Bitter Orange) showed excellent inhibitory action against all tested bacteria. The Ethanolic and methanolic extract of *Citrus aurantium L.* showed maximum zone of inhibition against *Streptococcus spp.* (24 mm) followed by *Pr. mirabilis* (22mm) and *Ps. aeruginosa* (21mm) [Table No. 1 & Fig.2]. The study conducted by Ramachandra Y. L. also showed that the peel extract of *Citrus aurantium L.* was effective against *Ps. aeruginosa* [15]. Another study done by K. Ashok kumar showed that the acetone has shown highest yield as well antibacterial activity in *Citrus sinensis*[12]. But in our study ethanolic extract showed highest yields as well as antibacterial activity. The Acetone, Petroleum ether and n-Hexane extract also showed least antibacterial activity against all tested bacteria. Prasad Venu Gopal reported that the ethanolic peel extract of *Citrus aurantium L* showed activity against *S. aureus (13mm), Klebsiella spp* (13mm) and *E. coli* (12mm) while comparing their results with our study with same test organisms and solvent shows that our peel extract has a high degree of antibacterial

activity. [16]. However, this difference may be because of the difference in the phytochemical composition in various part of the plant or may be also due to the extraction method used and/or environmental factors or difference in the genotypes of the citrus plant used. The citrus peel extract exhibited higher antibacterial activity as that of the standard antibiotics used in the study. Most of the tested bacteria were resistant to many antibiotics used in this study. All bacteria tested in this study were multiple drug resistant bacteria. The difference in the antibacterial activity with the same source when extracted with different solvent has proven that not all phytochemicals that are responsible for antibacterial activity are soluble in a single solvent. Hence solvents of different polarity should be employed as discussed in this study (polar: water, acetone, ethanol; non-polar: ethyl acetate, petroleum ether). Sequential or successive solvent extraction is as good option for better solubility of many of the phytochemicals but it is always necessary to know the phytochemicals extracted by each individual solvent so as to avoid the inclusion of unnecessary solvents for extraction process as well as to understand the role of each solvent in the extraction of a individual or class of phytochemicals [12].

Table No. 1

Antibacterial activity of Citrus medica L. (Lemon) and Citrus aurantium L. (Bitter Orange) Peel extract against clinical isolates from wound infection.

Sr. No.	Bacterial isolates from wound infection	Zone of Inhibition on mm									
		Citrus medica L. (Lemon)					*Citrus aurantium L.* (Bitter Orange)				
		E	M	P	A	H	E	M	P	A	H
1.	*E. coli*	12	14	11	12	14	14	18	14	12	10
2.	*S. aureus*	18	20	14	14	15	19	19	10	12	12
3.	*S. epidermidis*	16	16	12	12	11	18	18	12	12	14
4.	*Ps. aeruginosa*	14	20	NZ	NZ	14	21	21	10	14	12
5.	*Pr. vulgaris*	14	16	NZ	NZ	14	16	16	12	13	NZ
6.	*Pr. mirabilis*	21	18	11	14	12	22	18	14	NZ	NZ
7.	*Streptococcus spp*	16	20	16	12	12	24	24	12	10	NZ
8.	*Klebsiella spp.*	18	18	NZ	14	14	14	16	NZ	8	NZ
9.	*Enterococcus spp*	18	20	14	16	12	14	19	14	12	10
10.	*Serratia spp.*	28	22	12	18	16	20	20	16	14	11
11	*Propionibacterium acne*	16	20	NZ	12	14	18	18	14	12	14

E – Ethanolic extract; M- Methanolic extract; P – Petroleum ether; A- Acetone extract; H- n-Hexane extract; NZ – No Zone

Figure 1

***Antibacterial activity of Citrus medica L.* (Lemon) against clinical isolates**

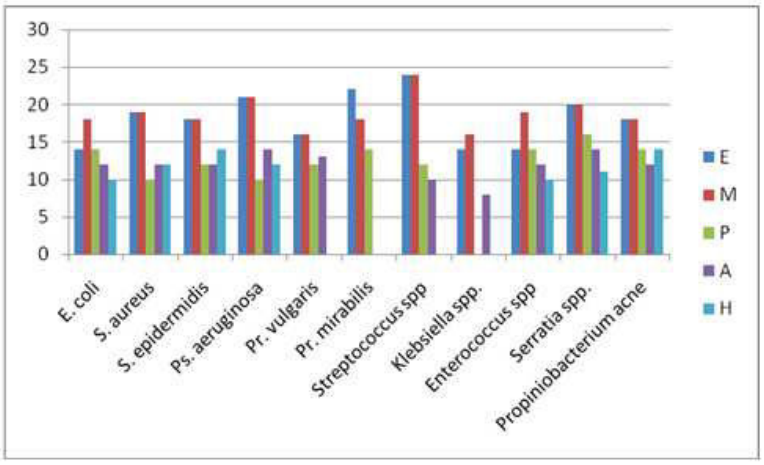
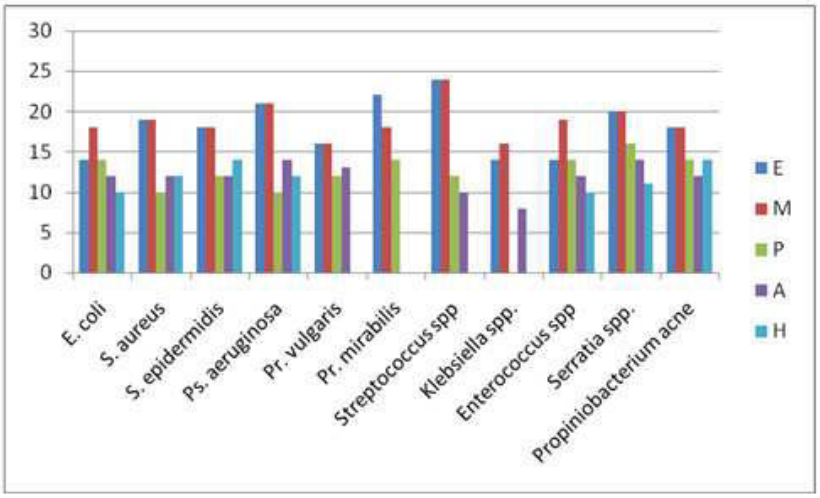


Figure 2
Antibacterial activity of *Citrus aurantium L.* (Bitter Orange) against clinical isolates



CONCLUSION

Recycling of fruit waste is one of the most important ways of utilizing it in a number of novel products which essentially required for human, animal and plant nutrition as well as in the pharmaceutical industry. This work has evaluated the antibacterial activity in *Citrus medica* and *Citrus aurantium* peels extracts obtained from different solvents against the test organisms isolated from wound infection. However, further evaluation performed with the isolation of pure compounds for the definite conclusion of the bioactive compounds contributing to the antimicrobial activity.

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