EVALUATION OF ANTBACTERIAL, ANTIFUNGAL AND ANTIOXIDANT ACTIVITY OF ESSENTAIL OIL OF *CITRUS RETICULATA* FRUIT (TANGERINE FRUIT PEEL)

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Summary

The essential oil of tangerine fruit peel (*Citrus reticulata*) was extracted by steam distillation and assessed for antibacterial, antifungal and antioxidant activity. The antimicrobial activity of essential oil was tested by disc diffusion method, against different bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhymurium, Enterobacter aerogenes,*) and fungi (*Aspergillus niger*, *Aspergillus flavis*, *Aspergillus fumigatus*, *Aspergillus ficuum*, *Fusarium saloni*). Results of the study showed that it exhibited maximum zone of inhibition against *Escherichia coli (25mm) and Salmonella typhymurium* (25mm) followed by *Aspergillus niger* (19.6mm), where as the minimum zone of inhibition was shown by *Fusarium solani* (9mm). The inhibition zones, measured after 48 hours and 96 hours, showed that it is active against all tested bacteria and fungi. The results of antioxidant activity of essential oil of tangerine peel showed that it was able to reduce the stable radical DPPH to yellow-colored DPPH-H reaching 76.73% of DPPH scavenging effect at its 100% concentration comparative to ascorbic acid reference standard being a strong antioxidant reagent. The results of this study showed that essential oil of tangerine peel can be an effective medicine against different pathogenic microbes.

Key words: *Citrus reticulata,* Tangerine, Essential oils, Antimicrobial activity, Antioxidant activity

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Introduction

Essential oils, also known as volatile oils are lipophilic compounds containing volatile aroma compounds. They are generally isolated from plant materials by various methods [1]. They are used in perfumes, cosmetics, flavoring food and drinks and scenting [2]. The use of essential oils as functional ingredients in foods, drinks, toiletries and cosmetics is gaining momentum, both for the growing interest of consumers in ingredients from natural sources and also because of increasing concern about potentially harmful synthetic additives [3]. Medicinal uses proposed by sellers of essential oils vary from skin treatments to remedies for cancer. These are often based on historical uses of these oils.

The antiseptic qualities of aromatic and medicinal plants and their extracts have been recognized since antiquity [4, 5]. These claims are now subject to regulations in most countries and there is a need to ascertain some of these traditional uses scientifically [6].

Tangerine oil was obtained from the peel of *Citrus reticulata*, family *Rutaceae*. The plant family *Rutaceae* also known as 'Citrus Fruit Family' is a family of trees, shrubs and herbs of various sizes and uses [7]. They are the most widespread arboreal plants in the world and represent one of the most important crops. They are cultivated in over 130 countries between 40°N and 40°S extending over 4 million hectares [8, 9]. Twenty seven species of *Rutaceae* exist in Pakistan [10]. Tangerines are evergreen shrub with spine on trunk and branches [11].

One of the important products of *citrus* fruits is the essential oil, which is obtained from citrus peels [12]. In 1998, Caccioni [13] formulated a cosmetic lotion from *citrus limetta* essential oil for the treatment of corns, warts and other skin lesions. Sacchetti *et al.* [14] studies showed that most of the essential oils showed a moderate antimicrobial activity.

Tangerine peel oil is pleasant in taste and rich in aroma and is mainly used in food and beverages as flavoring agent [15]. It is traditionally used as an antiseptic, antispasmodic, stomachi, sedative, diuretic and to improve circulation [16, 17]. Various medicinal preparations have also been made for the treatment of diseases from tangerine [18, 19].

Increase in the emergence of new bacterial strains that are multi-resistant coupled with the non-availability and the high cost of new generation antibiotics have resulted in increase morbidity and mortality [20, 21, 22]. So, investigations into the antimicrobial activities, mode of action and potential uses of plant essential oils have regained momentum. Essential oils and their components are gaining interest because of their relatively safe status, their wide acceptance by consumers and their exploitation for potential multi-purpose functional use [23, 24]. Hence there is a need to look for potent antimicrobials from other sources. The aim of this study is to investigate the antimicrobial and antioxidant properties of tangerine peel oil from local species that may be responsible for its antiseptic property against a number of microorganisms of public health significance.

Materials and Methods

Collection of plant materials:
The fruit was collected from citrus garden near Pattoki 80Km away from Lahore on Multan road. The fruit was peeled off and cut into small pieces. The cut peels (300gm) were subjected to steam distillation linkesson apparatus for 4 hours with live steam. The steam distillate was extracted twice with ether (2×100ml). The organic extract was removed under vacuum and recovered pale yellow oil was stored for further studies.

Antibacterial and Antifungal activity of Tangerine Peel Oil:
The agar disc diffusion method was employed for the determination of antibacterial and antifungal activity of tangerine peel oil following the procedure of Baydar *et al* .[(25)] against different food borne pathogens including bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhymurium, Enterobacter aerogenes,*) and fungi (*Aspergillus niger*, *Aspergillus flavis*, *Aspergillus fumigatus*, *Aspergillus ficuum*, *Fusarium saloni*). Standard culture media (CM139, CM145, CM271, CM69, CM201 and CM7) from Oxoid were employed through out the present investigation for the purpose of culture maintenance at their respective temperatures that is 25^{o}C for fungi and 37^{o}C for bacteria.

Sterile and dried 4mm paper discs (Difco) were impregnated with filtered sterilized (0.45mm Millipore filter) newly extracted tangerine peel oil and placed on freshly seeded microbial lawns (4 discs in each plate) with a control. All experiments were conducted in triplicate. The petriplates were incubated at their respective temperatures and zones of inhibition thus developed against tested microorganisms were measured in millimeters after a period of 48 and 96 hours. The results of antimicrobial activity of tangerine peel oil against different microorganisms were expressed as resistant, intermediate and sensitive.

Antioxidant activity of Tangerine peels oil:
Antibacterial activity was evaluated by measuring the scavenging activity of the examined tangerine peel oil on the 2,2-diphenyl-1-picrylhydrazil (DPPH) radical. The DPPH assay was performed as described by Epsin *et al.*[(26)]. The samples (100 µl each) of different concentrations of 20%, 40%, 60%, 80% and 100% were mixed with 3 ml of DPPH solution. The absorbance of the resulting solutions and the blank (with only DPPH and no sample) were recorded after an incubation time of 30 minutes at room temperature against ascorbic acid as a positive control. For each sample, 3 replicates were recorded.

The disappearance of DPPH was measured spectrophotometrically at 517 nm. The percentage of radical scavenging activity was calculated using the following equation;

$$\text{DPPH scavenging effect (\%)} = (A_0 - A_1)/A_0 \times 100$$

Where A_0 is the absorbance of the control at 30 minutes and A_1 is the absorbance of the sample at 30 minutes.

Results & Discussion

The results of antibacterial and antifungal activity of Tangerine peel oil, investigated against different food borne pathogens by disc diffusion method, are presented in table I and table 2 respectively. It was found in the present study that tangerine peel oil exhibited maximum zone of inhibition against *Escherichia coli* (25mm) and *Salmonella typhymurium* (25mm) followed by *Aspergillus niger* (19.6mm), where as the minimum zone of inhibition was shown by *Fusarium solani* (9mm) after 48 hours of incubation at 37Ċ. However, *Aspergillus falvis*, *Aspergillus fumigatus*, *Aspergillus ficuum*, *Staphylococcus aureus* and *Enterobacter aerogenes* gave 12, 14, 16.6, 11 and 17.8 mm of zone of inhibition respectively after 48 hours of incubation. As the essential oil is rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids and flavonoids, that are found to have effective as antimicrobial properties [27]. The monoterpenes affect the structural and functional properties of lipid fraction of the plasma membranes of bacteria and yeasts, causing leakage of intercellular material [28] and exit of critical molecules and ions leading to death of microbes [29]. Terpenoids affect respiratory enzymes inhibiting microbial oxygen uptake and oxidative phosphorylation [30].

Table 1: Anti-bacterial Activity of Essential oil of *Citrus Tangerine* against Pathogens of Public Health Significance

Test Organisms	Colony Morphology	Incubation Temperature (°C)	Culture Media (Oxoid)	Inhibition Zone (mm) After 48h	Inhibition Zone (mm) After 96h	Efficacy
Staphylococcus aureus	Gram + cocci	37°C	CM145	11	11 0.00*	I
Bacillus subtilis	Gram + rods	37°C	CM271	12	12 0.00*	I
Escherichia coli	Gram - rods	37°C	CM69	25	25 0.00*	S
Salmonella typhimurium	Gram - rods	37°C	CM201	25	25 0.00*	S
Enterobacter aerogenes	Gram - rods	37°C	CM7	17.8	15.6 12.36*	S

R= resistant, S= sensitive and I= intermediate.
CM145: Staphylococcus medium110, CM271: Blood agar base, CM69: Eosin methylene blue, CM201: Bismithsulphite agar, CM7: Macconkey's agar
pH of the assay medium ranges from 6.4 to 7.3 depending upon the tested organisms.

*= Percentage decrease in zone of inhibition after an incubation period of 96h.

Table 2: Anti-Fungal Activity of Essential oil of *Citrus Tangerine* against Pathogens of Public Health Significance

Test Organisms	Colony Morphology	Incubation Temperature (°C)	Culture Media (Oxoid)	Inhibition Zone (mm) After 48h	Inhibition Zone (mm) After 96h	Efficacy
Aspergillus niger	White, later green/Black	25°C	CM139	19.6	15.8 19.39*	S
Aspergillus flavis	White, later green/Black	25°C	CM139	12	12 0.00*	I
Aspergillus fumigatus	White, later green/Black	25°C	CM139	14	11.8 15.71*	S
Aspergillus ficuum	White, later green/Black	25°C	CM139	16.6	15.8 4.82*	S
Fusarium solani	White cottony	25°C	CM139	9	8.8 2.22*	R

R= resistant, S= sensitive and I= intermediate.
CM139: Potato dextrose agar
pH of the assay medium ranges from 6.4 to 7.3 depending upon the tested organisms.

* = Percentage decrease in zone of inhibition after an incubation period of 96h.

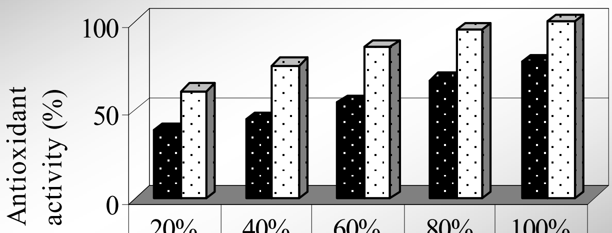
Our results showed that essential oil of tangerine exhibited more strong antibacterial activity against gram-negative organisms compared with the gram-positive ones. A same kind of observation was made by Ayoola *et al.*[31] indicating that the tangerine fruit oil has a broad spectrum antibacterial activity being more active against gram-negative organisms compared to gram-positive organisms. Johann *et al.* [32] studies also indicated that the peels of the Citrus species present substantial antimicrobial properties.

The present investigation for the assessment of antimicrobial activity of tangerine peel oil against different microbes of public health significance indicated a percent decrease in clear zone of inhibition after 96 hours ranging between 0~19% for fungi while for bacteria it ranged between 0~12%. This decrease in clear zone of inhibition after 96 hours by sterilized tangerine peel oil preparation may be either due to inactivation or low concentrations of diffusible water soluble active constituents. However, in a similar study, Wan *et al.* [33] reported that the sweet lime peel oil was found to be very effective against *Staphylococcus aureus* and P*roteus vulgaris*. Thus low or absence of activity in sterile tangerine peel oil might be due to a number of factors such as time collection of plant material, its storage, climate, which might, in turn, affect the amount of the active principal constituents (tannins, terpenoids, alkaloids, falvonoids etc) in the plant material [34, 35]. Saddiqui *et al.* [36] also observed a decrease in inhibitory zone from 15 to 26mm after a period of 96 hours of incubation, thus, showed a percentage decrease from 9.52 to 34.8% against the tested strains.

Natural compounds show stronger antioxidant activity which is likely to quench free radicals [37]. The antioxidant activity may act in various ways by scavenging the radicals, decomposing peroxides and chelating metal ions. The ability of essential oil to act as a donor for hydrogen atoms or electrons in the transformation of DPPH radical into its reduced form DPPH-H was measured spectrophotometrically. DPPH is one of the compounds that possess a proton free radical with a characteristic absorption, which decreases significantly on exposure to proton radical scavengers.

The results of DPPH scavenging activity of tangerine peel oil compared with ascorbic acid as a reference standard are shown in figure 1 indicating that tangerine peel oil has slightly lower antioxidant activity comparative to reference standard, ascorbic acid, being a strong antioxidant reagent. The essential peel oil of tangerine was able to reduce the stable radical DPPH to yellow-colored DPPH-H reaching 76.75% of DPPH scavenging effect at its 100% concentration where as the reference standard, ascorbic acid, gave a 99.67% DPPH scavenging effect at its 100% concentration. Mahmud *et al.* [38] studies also indicated that essential oil of *Zingibar officinale* (ginger) showed 76.4% of DPPH scavenging effect at its 100% concentration compared with ascorbic acid as a reference standard. Sacchetti *et al.* [14] studies also showed that essential oils of *Cananga odorata, Cymbopogon citratus, Rosmarinus officinalis* and *Curcuma longa* notably reduced the concentration of DPPH free radical indicating their strong antioxidant activities.

Fig. 1: Percentage antioxidant activity of essential oil of Tangerine in comparison with Ascorbic acid as standard reference evaluated by DPPH assay



	20%	40%	60%	80%	100%
■ Tangerine peel oil	38.76	44.7	54.5	66.98	76.75
□ Ascorbic acid Std.	60.79	75.4	85.4	95.54	99.67

Concentration

Conclusion

The results of our study showed that tangerine peel oil have the probability to be applied as a natural constituent of food preservations and medicines as they exhibit a strong antioxidant, antibacterial and antifungal activity against food borne pathogens. Scientists from divergent fields are investigating the essential oils of different plants with an eye to their anti-microbial usefulness. Laboratories of the world have found literally thousands of physiochemical that have inhibitory effects on all types of microorganisms in vitro.

More of these compounds should be subjected to animal and human studies to determine their effectiveness in whole-organism systems, including in particular toxicity studies as well as an examination of their effects on beneficial normal micro-biota. Attention to these issues could usher in a badly needed new era of chemotherapeutic treatment of infection by using plant-derived principles.

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