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Research Article

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In Vitro Antibacterial Activity of *Citrus limon* Peel Extracts against Several Bacterial Strains

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ABSTRACT

We aimed in this study to evaluate the antibacterial properties of Citrus limon peels extract against several bacterial strains. Thus, ethanolic, methanolic and acetone extracts of Citrus limon were prepared. Then we tested these extracts on five strains of bacteria (Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pneumonia, Klebsiella pneumonia and Escherichia coli), using pore method on Muller Hinton Agar. As we observed, the testes bacteria showed variant susceptibility and the most effective extract was acetone extract with the highest susceptibility against Staphylococcus epidermidis, then ethanolic extract against Streptococcus pneumonia. However, the least affected bacteria were E. coli and Klebsiella. We can conclude that lemon peel extracts are more affective against gram positive bacteria, so they can be used as food preservatives and in dermal disinfectants to inhibit their growth.

Keywords: *Citrus limon*; Antibacterial activity; Peel extracts

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INTRODUCTION

Lemon (*Citrus limon*) belongs to the family Rutaceae. It is an important dietary and medicinal plant which is cultivated in wide range lands of the world. The Citrus species have been used in Arabic Traditional Medicine as sedatives, analgesics, antiarrhythmics, a stomachic, anti-rheumatic, and for skin care [1,2].

Citrus peels are rich of flavonoid glycosides, coumarins, sitosterol, and essential oils which can be extracted and added to several cosmetic and pharmaceutical products. Other active terpenes, as well as alcohols, aldehydes and

esters contribute to the overall antimicrobial effects of the essential oils [3,4].

The antibacterial potential in crude extracts of different parts (leaves, stem, root and flower and peels) of *Citrus limon* against clinically significant bacterial strains has been reported [5,6]. Citrus flavonoids have a large spectrum of biological activity including antibacterial, antifungal, antidiabetic, anticancer and antiviral activities [7].

MATERIAL AND METHODS

Extraction Procedure

Fresh lemon fruits used in the study were obtained from the local supermarket in Latakia city. The fruits were surface disinfected with 70% alcohol and rinsed with sterile distilled water. Using a peeler, fine pieces of lemon peels were obtained and kept in dark and dry place for drying for 7 days. After that, 5 gr of lemon peels were put into separate conical flasks containing acetone, ethanol and methanol for extraction for 24 h on a rotary shaker. After filtering with a Whatman No. 1 filter paper, the filtrates were concentrated to dryness under reduced pressure at a maximum of 40°C using a rotary evaporator.

Bacterial Isolation

Several bacterial strains were isolated and identified using API strips at the laboratory section of Tishreen hospital, Latakia, Syria. Antibacterial activity was evaluated against three gram-positive bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus pneumoniae*) and two gram-negative bacteria (*Klebsiella pneumonia* and *Escherichia coli*).

Preparation of Mueller-Hinton Medium

We suspended 38 grams in 1000 ml distilled water, heated until boiling to dissolve the medium completely. Then, we sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Suspensions of microorganisms containing 10^6 cells/ ml were inoculated onto plate surfaces with a sterile cotton swab. Test plates (diameter 10 cm) were prepared with 20 ml of Mueller-Hinton agar (himedia laboratories Pvt. Ltd), and holes of 6 mm in diameter were punched in the agar plates using cork borer. Each hole was filled with 50 µl of different lemon peel extract. The diameters of the growth inhibition zones around the holes were measured after incubation for 48 h at 37°C [8].

RESULTS AND DISCUSSION

The *in vitro* antibacterial activity of lemon peel extract against the previously mentioned microorganisms was analyzed according to the presence or absence of inhibition zones, as depicted in Tables 1-3.

Table 1. Effect of acetone peel extract (concentration of 25%) on different bacterial strains

Bacterial strain	*Streptococcus pneumoniae*	*Klebsiella pneumoniae*	*E. coli*	*Staphylococcus epidermidis*	*Staphylococcus aureus*
Diameter of inhibition zone (mm)	15	14	13	24	16

Table 2. Effect of ethanolic peel extract (concentration of 25%) on different bacterial strains

Bacterial strain	*Streptococcus pneumoniae*	*Klebsiella pneumoniae*	*E. coli*	*Staphylococcus epidermidis*	*Staphylococcus aureus*
Diameter of inhibition zone (mm)	20	13	15	15	16

Table 3. Effect of methanolic peel extract (concentration of 25%) on different bacterial strains

Bacterial strain	*Streptococcus pneumoniae*	*Klebsiella pneumoniae*	*E. coli*	*Staphylococcus epidermidis*	*Staphylococcus aureus*
Diameter of inhibition zone (mm)	17	14	13	15	14

As we can observe from the previous tables that the highest efficacy of lemon peel extracts belongs to the acetone extract with the biggest diameter of inhibition zone (24 mm) against *Staphylococcus epidermidis*. This result is in concordance to Otang findings where the acetone lemon peel extract was considered the most effective extract. However, the most susceptible bacterium was *Salmonella typhimurium* in that previous study [9] Ethanolic extract also showed high antibacterial activity against *Streptococcus pneumoniae* with a (20 mm) diameter of inhibition zone.

In contrast, all the studied extracts had lower antibacterial activity against the two gram negative bacteria *E. coli* and *Klebsiella pneumonia.* Our conclusion is similar to Gupta results where lemon peel was effective against both groups of bacteria but its activity was high in Gram positive bacteria as compared to Gram-negative bacteria [10].

The difference in antibacterial activity of different extracts can be explained by extracting different class of phytochemicalS with different solvents [11].

CONCLUSION

Recycling of plant waste is one of the most important means of utilizing it to extract new products and supply the pharmaceutical industry with essential raw material, As a result of our study, the extract of lemon peel has a distinctive effect against the growth of many bacteria in vitro, especially *Staphylococcus epidermidis* and *Streptococcus pneumonia*. We may conclude that lemon peel extract are more effective at Gram positive than gram negative bacteria and therefore can be used in some pharmaceuticals such as skin lotions and cosmetics.

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