

## Study Antimicrobial Activity of Lemon (*Citrus lemon L.*) Peel Extract

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**Abstract:** The main objective of the study is extraction, identification of antimicrobial compounds and demonstration of antimicrobial activity of *lemon (Citrus lemon L.)* peel against bacteria. As microorganism are becoming resistant to present day antibiotics, our study focuses on antimicrobial activity and future prophylactic potential of the lemon peel. Biologically active compounds present in the medicinal plants have always been of great interest to scientists. The peel of citrus fruits is a rich source of flavanones and many polymethoxylated flavones, which are very rare in other plants. These compounds, not only play an important physiological and ecological role, but are also of commercial interest because of their multitude of applications in the food and pharmaceutical industries. The citrus peel oils show strong antimicrobial activity. The antimicrobial activity has been checked in terms of MIC by using different solvents against microorganisms like *Pseudomonas aeruginosa* NCIM 2036 for which MIC was 1:20 in presence of methanol, for *Salmonella typhimurium* NCIM 5021 the observed MIC was 1:20 in presence of acetone. In case of *Micrococcus aureus* NCIM 5021 the observed MIC was 1:20 when ethanol was used as solvent. The compounds like coumarin and tetrazene were identified by GC/MS of *lemon* peel extract.

**Key words:** Antimicrobial, coumarin, lemon, prophylaxis, tetrazene

### INTRODUCTION

Even though 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 (Gislene *et al.*, 2000). For a long period of time, plants have been a valuable source of natural products for maintaining human health. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments (Seenivasan *et al.*, 2006). Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances e.g. the phenolic compounds which are part of the essential oils, as well as tannin (Tyagi and Malik, 2010). Essential oils are more effective in controlling biofilm cultures due to their better diffusibility and mode of contact (Al-Shuneigat *et al.*, 2005). Hence the essential oils and other extracts of plants have evoked interest as sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases (Tepe *et al.*, 2004; Dorman and Deans, 2000).

Lemon is 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 (Kawaii *et al.*, 2000). Citrus flavonoids have a large spectrum of biological activity including antibacterial, antifungal, antidiabetic, anticancer and antiviral activities (Burt, 2004; Ortuno *et al.*, 2006). Flavonoids can function as direct antioxidants and free radical scavengers, and have the capacity to modulate enzymatic activities and inhibit cell proliferation (Duthie and Crozier, 2000). In plants, they appear to play a defensive role against invading pathogens, including bacteria, fungi and viruses (Sohn *et al.*, 2004). Flavonoids are generally present in glycosylated forms in plants, and the sugar moiety is an important factor determining their bioavailability. Preparation from peel, flowers and leaves of bitter orange (*Citrus aurantium L.*) are popularly used in order to minimize central nervous system disorders (Pultrini *et al.*, 2006). The peel of *Citrus* fruits is a rich source of flavonoid glycosides, coumarins,  $\beta$  and  $\gamma$ - sitosterol, glycosides and volatile oils (Shahnah *et al.*, 2007). Many polymethoxylated flavones have several important bioactivities, which are very

rare in other plants (Ahmad *et al.*, 2006). 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 (Aronson, 2001). Antimicrobial activity of the peel extract is directly concerned with the components that they contain. The studies showed that essential oils, protopine and corydaline alkaloids, lactons, polyacetylene, acyclic sesquiterpenes, hypericin and pseudohypericin compounds are effective toward various bacteria. Nevertheless, other active terpenes, as well as alcohols, aldehydes, and esters, can contribute to the overall antimicrobial effects of the essential oils (Keles *et al.*, 2001). The lemon peel extracts in different solvents such as ethanol, methanol and acetone were subjected to antibacterial assay. The extract in solvent ethanol shows higher antimicrobial activity against tested microorganisms in comparison with the extracts of lemon peel in other solvents like methanol and acetone. The aim of this study was to evaluate the potential of plant extracts and phytochemicals on standard microorganism strains by using routine antibacterial assay techniques.

## MATERIALS AND METHODS

The study was conducted between June 2010 and January 2011. The study was carried out at the Department of Microbiology, Shivaji University, Kolhapur, India.

**Preparation of extract:** The peel of lemon was homogenized in different solvents individually and mixed well. The solvents used were ethanol, acetone, and methanol. The extracts were collected separately for further study.

**Cultures used for antimicrobial activity:** The microorganisms used were as follows, *Pseudomonas aeruginosa* NCIM 2036, *Salmonella typhimurium* NCIM 5021, and *Micrococcus aureus* NCIM 5021.

**Culture medium:** Nutrient agar medium and a mineral based medium were used in all further studies. The compositions are as shown in Table 1 and 2, respectively.

**Antimicrobial effect:** Sterile molten nutrient agar at around 40°C was taken and seeded with different microbial cultures and plates were prepared. After

Table 1: Composition of nutrient broth

Components	(%)
Peptone	1.0
Yeast extract	1.0
Sodium chloride	0.5
Agar	2.5

Table 2: Composition of mineral based medium

Components	(%)
Sodium nitrate	0.20
Dipotassium hydrogen phosphate	0.10
Potassium chloride	0.05
Glucose	1.00
Yeast extract	0.02

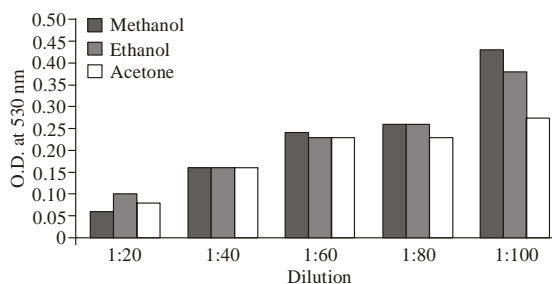


Fig. 1: Minimum inhibitory concentrations for *Pseudomonas aeruginosa*

solidification 4 mm wells were prepared. In these wells solvent extracts of the peel were added. The plate was incubated overnight at 37°C. After incubation the zones of inhibition were measured and recorded. Respective solvent controls were also run simultaneously. The above procedure was repeated using mineral based medium with added yeast extract at 0.02%.

Determination of minimum inhibitory concentration of crude extracts: Different concentration of crude extract as 1:20, 1:40, 1:60, 1:80 and 1:100 were added respectively into mineral based medium containing glucose (1%), yeast extract (0.1%). The organisms were inoculated respectively and incubated at 37°C, overnight on shaker.

Detection of phytochemicals by GCMS: Peel supernatant obtained in different solvents was analyzed by GCMS.

## RESULTS

The minimum inhibitory concentration assay conducted in nutrient broth using solvent extract are reported in Fig. 1, 2 and 3 for *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Micrococcus aureus*, respectively.

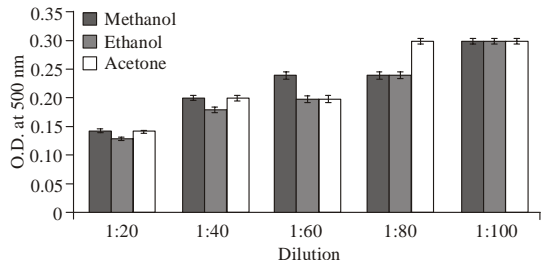


Fig. 2: Minimum inhibitory concentrations for *Salmonella typhimurium*

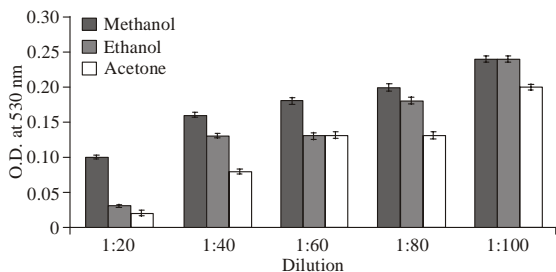


Fig. 3: Minimum inhibitory concentrations for *Micrococcus aureus*

**GCMS analysis of the extracts of the lemon peels:** The lemon peel extracts prepared in ethanol, methanol and acetone when analyzed using GCMS shows the presence of following compounds as shown in Fig. 4, 5 and 6 respectively. Figure 4, shows the presence of coumarin and Fig. 5 shows the presence of Tetrazene. Both the substances are good antimicrobials with broad spectrum activity.

## DISCUSSION

The study shows that the peel of lemon is not only an astringent but also is a good antimicrobial agent. This is an important finding as certain skin flora like *Pseudomonas* and *Micrococcus* can grow in presence of sebum, especially when it is secreted in excess (in certain person), and cause purulent skin infections. Some time it can serve as a predisposing factor for other types of skin infections like acne. Simple use of lemon juice can prevent such types of infections and could help in keeping a good and healthy skin. Of course it is needless to point out that good personal hygiene, exercise and a good diet is equally essential too.

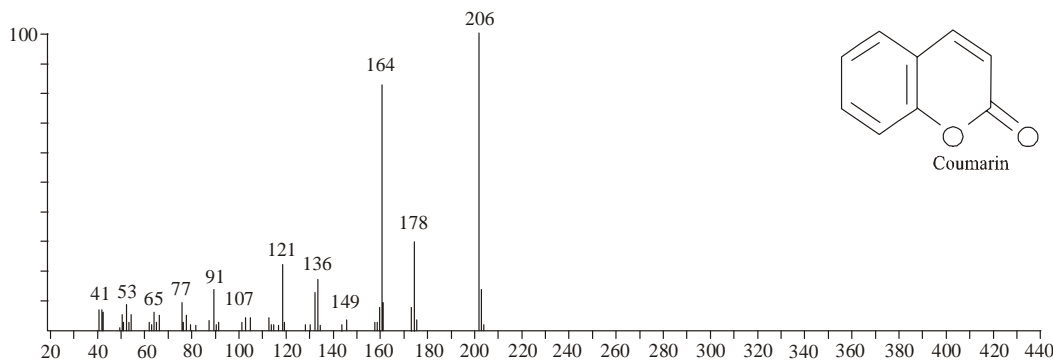


Fig. 4: GCMS analysis of lemon peel extracts shows presence of coumarin

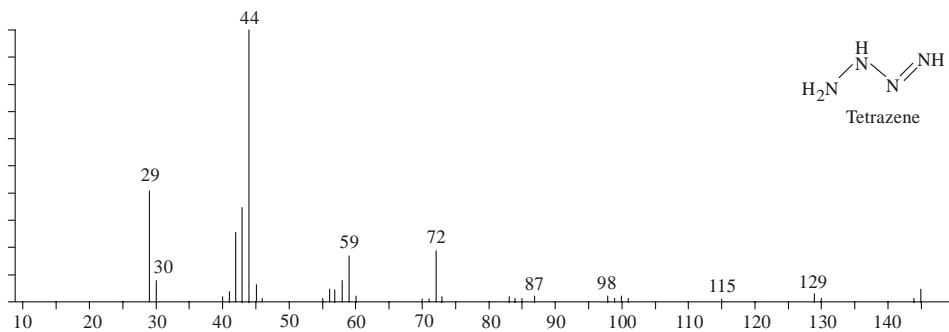


Fig. 5: GCMS analysis of lemon peel extracts shows presence of Tetrazene

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