Antimicrobial Activity of Six Different Parts of the Plant

*Citrus medica* Linn.

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**Abstract**

Introduction: Antimicrobial activity of fruit juice and ethanolic extracts of root, leaf, bark, peel and pulp of citron (*Citrus medica* Linn., Rutaceae) was examined against seven bacteria (*Bacillus subtilis, Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Proteus vulgaris*), two fungi (*Aspergillus flavus* and *A. niger*) and a yeast *Candida albicans* of clinical origin. Methods: The level of antimicrobial effects was established using an *in vitro* disc diffusion method; minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) were determined by standard agar dilution method. Results: All extracts and fruit juice showed varied level of antibacterial activity against one or more test bacteria. Antifungal activity was shown by only root extract and fruit juice while *C. albicans* was resistant to all tested plant samples. Broad spectrum antimicrobial activity was shown by fruit juice (MIC <1% to 3.5% and MBC 1% to 7% v/v) and fruit pulp (MIC 25 mg/ml and MBC 30 to 75 mg/ml). Root extract was found highly potent with MIC as small as 0.5 mg/ml and MBC 1 mg/ml against *S. aureus*. Among all tested plant samples leaf and peel extracts have shown less antimicrobial activity. Conclusion: It is concluded that fruit juice and juiceless fruit pulp extract have shown broad antimicrobial activity while root extract was very effective against some tested microorganisms.

Key words: Antibacterial, antifungal, antimicrobial activity, citron, *Citrus medica* Linn.

**Introduction**

In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world.[1-3] However, the situation is critical in developing and developed countries due to indiscriminate use of antibiotics. The drug-resistant bacteria and fungal pathogens have further complicated the treatment of infectious diseases in immunocompromised AIDS and cancer patients.[4] The public is becoming increasingly aware of problems with the over prescription and misuse of traditional antibiotics. It is reported that on average, two or three antibiotics derived from microorganisms are launched each year.[5] Scientists realize that due to resistance of microorganisms the effective life span of any antibiotic is limited.[6] In the present scenario of emergence of multiple drug resistance to human pathogens, this has necessitated to search new antimicrobial substances from other sources including plants.

The substances that can inhibit the growth of microorganisms or kill them and have little or no toxicity to human cells are considered candidates for developing new antimicrobial drugs. Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties.[7-9] In recent years antimicrobial properties of medicinal plants have been increasingly reported worldwide.[10-17] In the present study, we have selected *Citrus medica* Linn. commonly known as citron; which was not previously screened for antimicrobial properties. However, most of the *Citrus* species were previously screened against pathogenic organisms.[18-22] Many parts of this plant are used in the Indian traditional system of medicine against various ailments. Ripe fruits are used in sore throat, cough, asthma, thirst, hiccough, earache, vomiting; potent antiscorbutic, stomachic, tonic, stimulant, expellant of poison, correct fetid breath; distilled water of the fruit is sedative; fruits and seeds are cardiac tonic and useful in palpitation; fruit decoction is analgesic. Roots, flowers, seeds, peels and leaves are also used in many ailments.[23-24] Fruit extracts have also shown good antioxidant...
activity. The fruit wrapped in cloth is used to protect clothes from moth indicates its insect-repellent activity. In ancient literature citron was mentioned as an antidote of every kind of poison.

It was expected that screening of fruit juice and extracts of root, leaf, bark, peel and pulp against wide variety of microorganisms would be helpful in obtaining broad-spectrum new antimicrobial substances. The aim of this study was to test plant extracts against a diverse range of organisms including gram-positive and gram-negative bacteria, two fungi and a yeast.

MATERIALS AND METHODS

Plant material
All samples of C. medica Linn. were collected locally. The plant was identified as C. medica Linn. by an authorized taxonomist at National Botanical Research Institute, Lucknow. Voucher specimen number 97840 has been deposited in the same institute.

Preparation of plant extracts
Fresh leaves and peels were shade dried and powdered. Bark and root samples were sun dried and powdered. Ripe fruits after removing peels were squeezed to collect juice and stored at 4-6°C until used. Juiceless pulps were dried under sun and powdered. Extracts of bark and pulp were prepared by soaking 100 gm of powdered samples in 250 ml of 70% ethanol while 100 gm of root, leaf and peel samples were soaked in 500 ml of 70% ethanol for 96 h. Each mixture was stirred every 24 h using sterile glass rod. At the end of extraction each extract was passed through Whatman filter paper no. 1 (Whatman, UK). The filtrates were concentrated on a rotary evaporator at 40°C, dried and weighed. The percentage of extract with respect to crude drug was found as 7.43% (root), 9.6% (leaf), 5.8 % (bark), 24.6% (peel) and 23.26% (pulp).

Microorganisms used
Microorganisms were obtained from the Institute of Microbial Technology (IMTECH), Chandigarh, India. Bacterial cultures were Bacillus subtilis MTCC 441, Staphylococcus aureus MTCC 737, Enterococcus faecalis MTCC 439, Escherichia coli MTCC 1687, Klebsiella pneumoniae MTCC 109, Pseudomonas aeruginosa MTCC 1688 and Proteus vulgaris MTCC 1771. The filamentous fungi used were Aspergillus flavus MTCC 277 and Aspergillus niger MTCC 1344; and a yeast Candida albicans MTCC 227. All cultures were Micribial Type Culture Collection (MTCC).

Culture media and inoculum
The fungi were maintained on Sabouraud Dextrose Agar (SDA; Hi-Media, India) and bacteria on Nutrient Agar (NA; Hi-Media, India) at 4°C temperature until used in the study. Before use, the fungal cultures were revived in Sabouraud Dextrose Broth (SDB; Hi-Media, India) and bacterial cultures on Nutrient Broth (NB; Hi-Media, India). Inoculated broths were incubated overnight at 37°C for bacteria and at 28°C for fungi. The freshly grown microbial cultures were appropriately diluted in sterile broth media to obtain the cell suspension of 10^6 cfu/ml.

Antimicrobial assay
The agar disc diffusion method was used; 0.1 ml of diluted inoculum (10^6 cfu/ml) of test organism was spreaded on SDA/NA plates. Sterilized Whatman papers discs (6 mm diameter) were soaked in fruit juice and solutions of extracts (100 or 200 mg/ml) in dimethyl sulfoxide (DMSO, Sigma) and solvent blank (DMSO). Chloramphenicol discs (25 µg/disc; CDH, Central Drug House, New Delhi) were used in the test system as positive control against test bacteria while itraconazole against test fungi and yeast. The bacterial plates were incubated for 24 h at 37°C while fungal and yeast plates were incubated for 48 h at 28°C. The antimicrobial activity was evaluated by measuring the zone of inhibition (ZOI) against test organisms. Experiments were performed in triplicate.

MIC and MBC determinations
Agar dilution method was adopted to find minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of active extracts. MIC is defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation, and MBC as the lowest concentration of antimicrobial that will prevent the growth of an organism after subculture on to antimicrobial-free media.

RESULTS
Antimicrobial activity of six different extracts of C. medica Linn. is shown in Table 1 in terms of zone of inhibition. DMSO as control did not show any inhibition against all tested microorganisms. All extracts failed to inhibit growth of C. albicans. Fruit juice inhibited all tested Gram-positive, Gram-negative bacteria and two fungi A. niger and A. flavus with zone of inhibition comparable to standard drugs. Semidried extract of juiceless fruit pulp have also shown similar results as fruit juice except against B. subtilis, A. niger and A. flavus. Only difference was in the zone size which was less in case of fruit pulp than juice.

Both fungi A. niger and A. flavus were inhibited by root extract and fruit juice. Root, leaf and bark extracts inhibited S. aureus, E. faecalis and P. vulgaris with maximum inhibition by root extract comparable to standard antibiotic. Fruit
Peels have shown least activity among all extracts and slightly inhibited growth of *S. aureus*, *K. pneumoniae* and *P. vulgaris*. The yeast *C. albicans* was not inhibited by any extract. Among bacteria *S. aureus* and *P. vulgaris* were highly susceptible to all extracts while *B. subtilis* was highly resistant and inhibited by only fruit juice.

Table 2 shows MIC and MBC of active extracts against susceptible microorganisms. Root extract had the lowest MIC 0.5 mg/ml and MBC 1 mg/ml against *S. aureus*. The maximum MIC of extracts was 50 mg/ml and MBC 75 mg/ml. The minimum MIC of juice was <1% and MBC 1% against *P. vulgaris* while maximum MIC was 3.5% and MBC 7%.

**DISCUSSION**

Various publications have documented the antimicrobial activity of plant extracts and essential oils including lemon juice against HIV, syphilis, gonorrhea, and topical microbicide. Published results on the antimicrobial activity of *Citrus* extracts suggested screening of such activity of *C. medica* Linn. variety available in Kumaun region of Uttarakhand, India which was not previously screened for antimicrobial activity.

In the present study fruit juice was found to be the most effective agent against all tested bacteria and fungi; juiceless pulp was the next effective extract. The basis of varying degree of sensitivity of test organisms may be due to the intrinsic tolerance of microorganisms and the nature and combinations of phytoconstituents present in the crude extract. One or more of the common phytoconstituents like alkaloids, tannins, phenols, glycosides, flavonoids and acids were already reported in some of these active extracts. These major phytoconstituents are known to have antimicrobial activity.

On the basis of the present investigation it can be highlighted that some of the extracts of *C. medica* Linn. showed promising antibacterial and antifungal properties and could...
be exploited in herbal preparations for both external and internal uses. Since the fruit is edible and used by the people of Kumaun in the preparation of pickles, juices and other recepies so can be used in herbal preparations for internal use also. Root is also reported in ancient Indian literature to be used as anthelmintic and in urinary calculus, so it can also be used internally.[31]

CONCLUSION

The active extracts can also be screened against more human pathogens including human immunodeficiency virus (HIV) and antibiotic resistant strains. The active principles of these extracts are required to be isolated, characterized and tested for their safety and efficacy to uncover their therapeutic potential in modern medicine against infectious diseases. Furthermore of these compounds can 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.

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