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Research Article

ANTIMICROBIAL ACTIVITY OF *CITRUS SINENSIS* AND *CITRUS AURANTIUM* PEEL EXTRACTS

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ABSTRACT
The present study was conducted to determine antimicrobial activity of peels extract of two Citrus fruits *viz*., *Citrus sinensis* and *Citrus aurantium*. The peels were separated from fruits, shade dried, powdered and extracted using methanol. Antibacterial and antifungal activity of peel extracts was determined by agar well diffusion assay and poisoned food technique respectively. Among bacteria, *Klebsiella pneumoniae* and *Bacillus cereus* were inhibited to high and least extent respectively. *C. sinensis* peel extract had marked antibacterial activity than *C. aurantium* peel extract. In case of antifungal activity, *C. aurantium* inhibited mycelial growth of *Colletotrichum capsici* to high extent when compared to *C. sinensis*. The peel extracts of selected citrus fruits can be used against infectious agents and to control anthracnose of chilli caused by *C. capsici*.
Keywords: *Citrus sinensis*, *Citrus aurantium*, Peel extract, Agar well diffusion, Poisoned food technique, *Colletotrichum capsici*

INTRODUCTION

Fruits are known to be an integral part of diet and are consumed fresh and as juices, salads or fruit based drinks. Besides their delicious taste and flavor, the fruits are known to reduce the risk of several chronic diseases including cancer. The protective nature of fruits is due to the presence of phytoconstituents such as poly phenolic compounds. Among various fruits that are consumed, citrus fruits are widely used in almost all countries. The genus Citrus belongs to the family Rutaceae and is native to tropical and subtropical areas in Southeast Asia. The citrus plants are grown worldwide and ranks top in world production and trade among the fruit trees. Citrus fruits are richer sources of bioactive compounds having beneficial effect on human health such as vitamin C, carotenoids, flavonoids, limonoids, essential oils, acridone alkaloids, minerals and vitamin B complex. Majority of citrus fruits are eaten fresh (such as sweet orange, mandarins, grapefruits etc). Many citrus fruits are used to prepare juices, pickles and other recipes as flavoring agents. The peel of citrus fruits is an important byproduct of citrus processing industries. A large amount of peel is produced and is considered as waste. The citrus peels are divided into epicarp or flavedo and mesocarp or albedo. The flavedo is colored and is the outermost surface of the peel whereas the albedo is the white, soft inner layer of the peel. The citrus peels contain high quantity of phenolic compounds including several flavonoid compounds. The citrus peel extracts and essential oils are known to exhibit various biological activities such as antimicrobial and antioxidant activities[1-6]. The objective of the present study was to determine antimicrobial activity of peel extract of two citrus fruits namely *C. aurantium* and *C. sinensis*.

MATERIALS AND METHODS

Collection of Citrus fruits

The ripe fruits of *C. aurantium* (bitter orange, kanchi) and *C. sinensis* (sweet orange, mosambi) that are free from insect infestation and other kinds of damage were collected at Maragalale, Thirthahalli Taluk of Shivamogga District, Karnataka, India.

Extraction

The fruits were washed several times using clean water, peels were separated, cut into small pieces, dried under shade and powdered in a blender. A known quantity of each peel powder (25 g) was added into separate conical flasks containing 100 ml of methanol (HiMedia, Mumbai, India) and left for 48 hours with occasional stirring. The content of flask was filtered through sterile Whatman No. 1 filter paper and evaporated to dryness. The condensed peel extracts were used for determining antimicrobial activity.

Antibacterial activity of Citrus peel extracts

Agar well diffusion assay was carried out to determine antibacterial activity of citrus peel extracts. One Gram positive bacterium *Bacillus cereus* and two Gram negative bacteria *Shigella flexneri* and *Klebsiella pneumoniae* were screened for their susceptibility to citrus peel extracts. In brief, 24 hours old Nutrient broth (HiMedia, Mumbai, India) cultures of test bacteria were aseptically swab inoculated on sterile Nutrient agar (HiMedia, Mumbai, India) plates followed by punching wells of 6mm diameter using a sterile cork borer. 100 µl of peel extracts (20 mg/ml of 25 % Dimethyl sulfoxide [DMSO; HiMedia, Mumbai, India]), standard (Streptomycin, 1 mg/ml) and DMSO (25 %, in sterile water) were transferred into labeled wells. The plates were incubated at 37^{o}C for 24 hours in upright position and the zone of inhibition formed around the wells was measured[7].

Antifungal activity of Citrus peel extracts

Poisoned food technique was performed to investigate antifungal efficacy of citrus peel extracts against *Colletotrichum capsici*. Potato dextrose agar (HiMedia, Mumbai, India) was poisoned with citrus peel extracts (1 mg extract/ml of medium). Spore suspension of *C. capsici* was prepared and inoculated at the centre of control (without extract) and poisoned plates by point inoculation method. The plates were incubated at 28°C for 5 days in upright position. Using a ruler, the diameter of fungal colonies in both control and poisoned plates were measured in mutual perpendicular

directions. Antifungal activity of peel extracts was determined using the formula:

Inhibition of mycelial growth (%) = (C– T/ C) × 100,
Where 'C' is diameter of colony in control plates and
'T' is diameter of colony in poisoned plates[8]

Statistical analysis

All experiments were done in triplicates. The results are mentioned as Mean ± Standard Deviation (S.D).

RESULTS

Extract yield was more and less in case of *C. aurantium* (12.35 %) and *C. sinensis* (08.00 %) respectively. The color of both peel extracts was yellow. The result of antibacterial activity of citrus peel extracts is shown in Table 1. The peel extracts were effective in inhibiting all test bacteria but to a varied extent. Among test bacteria, marked inhibitory effect was observed against *K. pneumoniae* while *B. cereus* was inhibited to least extent. Among peel extracts, *C. sinensis* inhibited test bacteria to higher extent than *C. aurantium*.

Table 1: Antibacterial activity of Citrus peel extracts

Treatment	Zone of inhibition in cm (Mean±S.D)		
	B. cereus	*S. flexneri*	*K. pneumoniae*
C. sinensis	1.2 ± 0.0	1.3 ± 0.0	1.4 ± 0.0
C. aurantium	0.8 ± 0.0	1.2 ± 0.0	1.3 ± 0.0
Antibiotic	3.1 ± 0.0	2.6 ± 0.2	2.9 ± 0.2
DMSO	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

The result of inhibitory potential of citrus peel extracts against *C. capsici* is shown in Table 2 and Figure 1 and 2. Both peel extracts caused marked inhibition of mycelial growth of *C. capsici* with an inhibition of >50 %. Extract of *C. aurantium* (75 %) inhibited fungus to higher extent than extract of *C. sinensis* (59.37 %).

Table 2: Colony diameter (in cm) of *C. capsici* on control and poisoned plates

Treatment	C.D (Mean ± S.D)
Control	3.2 ± 0.1
C. sinensis	1.3 ± 0.1
C. aurantium	0.8 ± 0.0

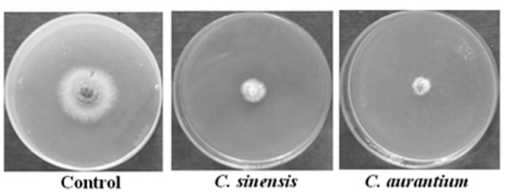

Figure 1: Growth of *C. capsici* on control and poisoned plates

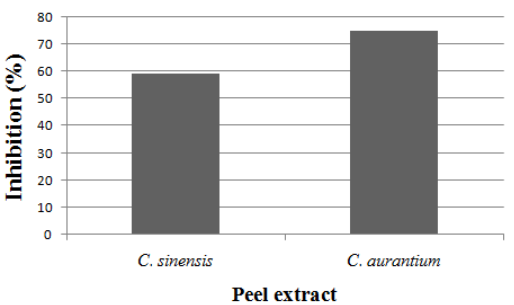

Figure 2: Inhibition of *C. capsici* (%) by Citrus peel extracts

DISCUSSION

In the present study, we evaluated antibacterial efficacy of peel extract of *C. sinensis* and *C. aurantium* against 3 bacteria by Agar well diffusion assay. *K. pneumoniae* exhibited higher susceptibility to peel extracts while *B. cereus* was least affected. *C. sinensis* peel extract had high inhibitory potential than peel extract of *C. aurantium*. It has been experimentally shown that extracts and essential oil from peels of Citrus fruits exhibit inhibitory activity against microorganisms. In a study, Kirbaşlar *et al*.[9] evaluated antibacterial activity of peel oil from Citrus fruits. It was observed that peel oil of *C. sinensis* and *C. aurantium* exhibited more or less similar inhibition of Gram positive and Gram negative bacteria. The study of Siddique *et al*.[4] showed the efficacy of essential oil from peel of *C. aurantium* to inhibit Gram positive bacteria but not Gram negative bacteria. The ethanolic extract from peel of *C. sinensis* was shown to inhibit Gram positive to higher extent than Gram negative bacteria[10]. Tumane *et al*.[11] observed marked inhibitory effect of ethanolic and methanolic extract of *C. aurantium* against a panel of bacteria. In the present study, we determined inhibitory effect of peel extract of *C. aurantium* and *C. sinensis* against *C. capsici* isolated previously from chilli anthracnose by poisoned food technique. The peel extracts inhibited mycelial growth of the fungus to >50 %. Among peel extracts, *C. aurantium* had high antifungal effect than that of *C. sinensis*. The essential oil and extracts from peels of citrus fruits are shown to exhibit antifungal activity. It has been found that the peel extract of *C. sinensis* significantly inhibited the growth of *Fusarium oxysporum* to higher extent when compared to leaf extract[12]. The essential oil from fully ripe fruit peel of *C. reticulata* was shown to exhibit inhibitory activity against *Alternaria alternata*, *Rhizoctonia solani*, *Curvularia lunata*, *Fusarium oxysporum* and *Helminthosporium oryzae*[13]. It has been found that essential oil from peel of *C. sinensis* exhibit inhibitory activity against growth of *Aspergillus flavus*[14].

CONCLUSION

The peels of citrus fruits are considered to be waste product of citrus processing industries. In the present study, the peel extracts of *C. aurantium* and *C. sinensis* exhibited inhibitory effect against bacteria and *C. capsici*. The antimicrobial efficacy of peel extracts can be ascribed to the presence of secondary metabolites. The peels of these citrus fruits can be used in treatment of infectious diseases and to control *C. capsici* causing anthracnose of chilli.

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