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Research Article

A COMPARATIVE STUDY OF ANTIMICROBIAL ACTIVITY OF CITRUS MAXIMA AND CITRUS AURANTIUM PLANT EXTRACTS

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ABSTRACT

The aim of the present study was to compare the anti microbial activity of aqueous and methanolic extracts of various parts of the plants like pulp, leaf, seed, fruit peel and bark of *Citrus maxima* and *Citrus aurantium* on *Escherichia coli, Staphylococcus aureus* and *Klebsiella pneumoniae.* The methanolic extracts of the leaf, seed, fruit peel and bark of *Citrus maxima* exhibited anti bacterial activity against *Escherichia coli* and *Klebsiella pneumoniae* but no zone of inhibition was noticed with *Staphylococcus aureus*. The methanolic and aqueous extracts of the *Citrus maxima* pulp showed considerable antibacterial activity against all three bacteria. Therefore parts of these citrus plants contain compounds with medicinal and pharmacological importance.

INTRODUCTION

From time immemorial plants have been used in medical treatment. The active secondary metabolites produced by the plants are effective antimicrobial agents and can be of great significance in pharmaceuticals . Many plants have been used because of their antimicrobial properties which are due to compounds synthesized in the secondary metabolism of the plant (Tyagi and Malik, 2010). Essential oils are effective in controlling biofilm cultures due to better diffusibility and mode of contact (Al-Shuneigat *et al.*, 2005). Extracts of plants have evoked interest because of their potential use as alternative remedies for treatment of infectious diseases.

Citrus is one of the most important commercial fruit crops grown in all continents of the world. Oranges and Lemon are important medicinal plants of the family Rutaceae cultivated mainly for alkaloids, which have anticancer activities. 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 by Kawaii *et al*., 2000.The peels of *Citrus* fruits are rich in flavonoids, especially many polymethoxylated flavones, which are very rare in other plants (Ahmad *et al.,* 2006). The antimicrobial activities of several *Citrus* species, namely, *C. aurantium, C. bergamia, C. limonum*, C. *maxima, C. paradisi, and C. reticulata,* have been investigated, but the related tests have typically involved microorganisms in planktonic cultures. (Adedjl *et al*., 2007; Kirbaslar *et al.,* 2009; Sokovic *et al.,* 2010; Ashok Kumar *et al.,* 2011).The plant *Citrus maxima* is commonly known as Pomelo and is indigenous to tropical parts of Asia. The antibacterial activity of the ethanolic extracts of its leaves against *Escherchia coli* and *Pseudomonas aeruginosa* was studied by Swarnamoni Das *et al.,* 2013. The antibacterial and antioxidant properties of leaf extracts of three citrus plants *Citrus microcarpa, Citrus aurantium and Citrus maxima* was studied by Rich Milton Dulay and Castro, 2016. All the three citrus leaves extract exhibited antibacterial activity against *Staphylococcus aureus* but not against *Escherchia coli.*

Preparation from peel, flowers and leaves of *Citrus aurantium* are popularly used in order to minimize Central Nervous System disorders (Pultrini *et al.,* 2006). Maruti *et al.,* 2011 reported the antimicrobial activity of Lemon peel extract against different microorganisms.

Antimicrobial assay showed that Chloroform, Ethanol and water extracts of *Citrus aurantium* leaves exhibited *in vitro* anti bacterial activity against Gram positive and Gram negative bacteria whereas significant activity was not observed in petroleum ether extract. (Prasad Venugopal, 2012).

In order to find out cheap, effective and natural products for

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treatment of diseases this study investigated and compared the anti microbial activites of different parts of *Citrus maxima* and *Citrus aurantium.*

MATERIALS AND METHODS

The study was carried out in collaboration with Leads clinical Research and Bio services Pvt Ltd, Bangalore.

Preparation of the extracts*: Citrus maxima* and *Citrus aurantium* parts were taken from an urban garden in Bangalore. The different parts of the plants were homogenized in water and methanol individually and mixed well. This was kept in a shaker for 3 days and filtered. The extracts were collected separately for further study.

Cultures used for anti microbial activity*:* The microorganisms used were *Escherichia coli, Staphylococcus aureus* and *Klebsiella pneumoniae.*

Measurement of Antimicrobial activity*:* The antibacterial activity was determined by using the cultures of the bacteria. Activity of the different extracts were tested separately using Agar-Well diffusion method. Muller Hinton agar plates and broth was prepared. The medium was sterilized by autoclaving. The agar plate surface was inoculated by spreading a volume of the microbial inoculum over the entire agar surface. The plates were kept for some time for hardening and then they were punctured with a sterilized borer. Different solvent extracts were introduced separately into the labelled wells. Respective solvent controls were also run simultaneously. The bacterial culture plates were kept for 24 hours and the zone of inhibition formed around the wells was measured.

OBSERVATIONS AND RESULTS

The aqueous extract of the pulp of *Citrus maxima* recorded the highest diameter of zone of inhibition of 27 mm against *Staphylococcus aureus.* The methanolic extracts of the leaf, seed, fruit peel and bark of *Citrus maxima* exhibited antibacterial activity against *Escherichia coli* and *Klebsiella pneumoniae* but no zone of inhibition was noticed with *Staphylococcus aureus*. The methanolic extracts of the pulp of *Citrus maxima* showed considerable antibacterial activity. Similarly the aqueous pulp and seed extracts of *Citrus aurantium* showed significant anti bacterial activity against all the three bacteria. Except the aqueous pulp and seed extracts of *Citrus maxima* the extracts of leaf, peel and bark showed no zone of inhibition for all the three bacteria.

The aqueous extracts of the peel, bark and leaf of *Citrus aurantium* did not show any anti bacterial activity. However, no significant difference was observed in the zone of inhibition of the three microorganisms. It is noticed that the pulp extracts both aqueous and methanolic had the highest potential against the selected pathogenic bacteria.

Results are summarized in Table 1 and Table 2

DISCUSSION

The anti bacterial activities of three citrus leaves extracts *Citrus microcarpa*, *Citrus aurantium*, and *Citrus maxima* against *S. aureus* but not against *E.coli* was reported by Rich Milton R Dubey *et al*., 2016. The present study showed anti bacterial activities of the extracts of *Citrus maxima* and *Citrus aurantium* against *E. coli*, *S. aureus* and *K. pneumoniae*. Madhuri *et al*., 2014 observed anti bacterial efficacy of peel extract of *Citrus sinensis* and *Citrus aurantium* and reported that *K. pneumoniae* exhibited higher susceptibility to peel extracts while *Bacillus cerves* was least affected. In this study peel extracts of both methanolic and aqueous showed considerable anti bacterial activity against *E. coli, K. pneumoniae* and *S. aureus*. The methanolic and aqueous pulp extracts of *Citrus maxima* showed maximum zone of inhibition. Between the bacteria selected *K. pneumoniae* showed a higher sensitivity to the different plant extracts as compared to *E.coli* and *S. aureus*. This signifies potent anti bacterial activity of the extracts. The study of Swarnamoni Das *et al.,* 2013 revealed the anti bacterial activity of the ethanolic extracts of *Citrus maxima* leaves against *E. coli* and *Pseudomonas aeruginosa* . Present findings of the anti bacterial activity of methanolic leaf extracts of *Citrus maxima* and *Citrus aurantium* are comparable to the findings reported by Prasad Venugopal, 2012 in *Citrus aurantium*. The anti bacterial activity of the plant extracts can be attributed to the different phytochemicals present in the Citrus plant parts. The present study can pave way to locate compounds in Citrus plants to develop safer antimicrobial agents in this era of anti microbial resistance.

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Table 1 Anti microbial activity of Methanolic extract-Zone of inhibition

	Citrus maxima			***Citrus aurantium***		
Sample	*E.Coli*	*K.pneumoniae*	*S.aureus*	*E.Coli*	*K.pneumoniae*	*S.aureus*
Pulp	21mm	26 mm	25mm	12mm	10 mm	-
Leaf	8 mm	15 mm	-	8 mm	6 mm	6 mm
Seed	8 mm	12 mm	-	6 mm	6 mm	-
Fruit Peel	10mm	13 mm	-	8 mm	8 mm	6 mm
Bark	5 mm	10 mm	-	-	6 mm	-

Table 2 Anti microbial activity of aqueous extract-Zone of inhibition

	Citrus maxima			***Citrus aurantium***		
Sample	*E.Coli*	*K.pneumoniae*	*S.aureus*	*E.Coli*	*K.pneumoniae*	*S.aureus*
Pulp	18mm	21 mm	27mm	8mm	12 mm	8 mm
Leaf	-	-	-	-	-	-
Seed	16mm	18 mm	20	8 mm	6 mm	6mm
Fruit Peel	-	-	-	-	-	-
Bark	-	-	-	-	-	-

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