Journal of Pharmacognosy and Phytochemistry 2015; 4(3): 278-281

Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2015; 4(3): 278-281
Received: 16-07-2015
Accepted: 18-08-2015

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Evaluation on antimicrobial activity of fruit peels of selected citrus species against human pathogenic microorganisms

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Abstract
The antibacterial activity on dried fruit peels of Citrus species were evaluated on bacteria strains like *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Proteus mirabilis* and *Pseudomonas aeruginos*a. Methanol, chloroform, acetone and water extracts were used for the present study. The in vitro antibacterial activity was performed by agar disc diffusion method. The study reveals that all the fruit peels extracts were active against *E.coli* and *Staphylococcus aureus* followed by the other bacteria. Among all the extracts, *Citrus aurantium* showed highest activity against all the tested human pathogenic bacteria. But, in the case of methanol extract of fresh peel of *Citrus limon* showed highly significant activity against all the tested organisms. *Pseudomonas aeruginosa* and *Proteus mirabilis* are highly sensitive to the *Citrus limon* extracts when comparing to the other extracts of fruit peels. Considering about the maximum sensitivity effect of various bacteria against different fruit peels extracts, it becomes very clear that the bacteria *Staphylococcus aureus* and *Escherichia coli* were highly sensitive. The result of the study shows that the organic and water extracts were more potent against both the Gram- positive and gram -negative bacteria used for the present study. This study support to a certain extent, the recycling of fruit waste and utilizing it in a number of innovative ways.

Keywords: *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa.*

1. Introduction
Citrus is the one of the most important commercial fruit crops grown in all continents of the world [1].Citrus fruits are mainly used by juice processing industries, while the peels are generally wasted. Since the juice yield of citrus is less half of the fruit weight, very large amounts of by product wastes, such as peels are formed every year [2]. Peel waste are highly perishable and are seasonal, is a problem to the processing industries and pollution monitoring agencies. There is always an increased attention in bringing useful products from waste materials and citrus waste are no exceptions. By product recovery from fruit wastes can improve the overall economics of processing units. Besides this, the problem of environmental pollution also can be reduced considerably. The citrus peels are rich in nutrients and contain many phytochemicals; they can be efficiently used as drugs or as food supplements too. Since there is an increase in the number of antibiotic resistance pathogens, there is always a search of an alternative drug. One of the possible solutions is the development of new drugs to meet the challenge of antimicrobial resistance [3]. Citrus peels if proved to have antibacterial activity; they can be also used in same food industry which generates large peel wastes as food preservatives [4]. In the present study the fruit peel of four important plants such as *Citrus maxima, Citrus limon, Citrus sinensis* and *Citrus aurantium* belonging to the family Rutaceae were selected for assessing their antibacterial properties.

2. Materials and methods
2.1. Collection of plant material
The fruits of *Citrus maxima, Citrus limon, Citrus sinensis*, and *Citrus aurantium* were bought from sakthan market Thrissur, after their identification by Mr. Jacob Abraham Pulikal, Associate Professor, Department of Botany, St. Thomas' college, Thrissur.

2.2. Extraction of the plant material
The fruit peels of the plants were separately washed in tap water, dried in shade and powdered in a mechanical grinder. The powdered parts of the peels were stored separately at 2-4 °C. The shade dried and finely ground samples weighing about 20g were extracted with chloroform,

90% acetone and 90% methanol using soxhlet apparatus. Also water extracts were taken using water bath. The screening for antibacterial activity was carried out after evaporating the solvents using solvent recovery apparatus and reconstituting the residue in the respective solvents (10ml).

2.3. Isolation and characterization of human pathogenic bacteria

Pus, blood and urine sample collected from polyclinic were placed on Blood Agar for the isolation and the Petri plates were incubated at 37° C for 24 hours. After incubation culture characters and colony morphology was observed [5]. Each one of the isolates were characterized separately and identified as *Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli, Proteus mirabilis and Pseudomonas aeruginosa.* The streak-plate method is employed for getting a pure culture [6].

2.4. Antibacterial assay by disc-diffusion method

In this method, a Petri plate containing an agar growth medium is inoculated uniformly over its entire surface [7]. Paper discs impregnated with various extracts of Citrus species are placed on the surface of the agar. Incubate the Petri plates, inverted at 37 °C for 24 hours. During incubation, the antimicrobial agents diffuse from the disc, from an area of higher concentration to an area of lower concentration. An effective agent will inhibit the bacterial growth, and measurements can be made of the size of the zones of inhibition around the discs. The zone size is affected by such factors as the diffusion rate of the antimicrobial agents and the growth rate of the organisms [8].

2.5. Statistical analysis

Standard deviation of the data were found out, significance levels were found to be with one way ANOVA test using MSTATC software.

3. Results and discussion

Effect of water, methanol, chloroform, acetone extract of *Citrus maxima, Citrus limon, Citrus sinensis*, and *Citrus aurantium* against human pathogenic organism**.**

Table I: Activity against *Staphylococcus aureus*

Sample	Water extract (A)		Methanol (B)		Chloroform I		Acetone (D)	
	Average Zone in mm	P value	Average Zone in mm	P value	Average Zone in mm	P value	Average Zone in mm	P value
Citrus maxima-A	8.33	<0.01	10	<0.01	10.33	<0.01	11	<0.01
Citrus limon – B	14.33	<0.01	13.33	<0.01	14.67	<0.01	15.33	<0.01
Citrus sinensis – C	10.33	<0.01	11.33	<0.01	11.33	<0.01	13.33	<0.01
Citrus aurantium – D	12.67	<0.01	15	<0.01	13.33	<0.01	15.33	<0.01
Gentamicin	21.67	<0.01	21.33	<0.01	21.67	<0.01	23.33	<0.01

Table II: Activity against *Pseudomonas aeruginosa*

Sample	Water extract (A)		Methanol (B)		Chloroform I		Acetone (D)	
	Average Zone in mm	P value	Average Zone in mm	P value	Average Zone in mm	P value	Average Zone in mm	P value
Citrus maxima-A	7.67	<0.01	8	<0.01	8	<0.01	9.33	<0.01
Citrus limon – B	10.33	<0.01	10.67	<0.01	8.33	<0.01	9.33	<0.01
Citrus sinensis – C	8.67	<0.01	9	<0.01	9.33	<0.01	7.67	<0.01
Citrus aurantium – D	8.67	<0.01	7.67	<0.01	9.67	<0.01	9.33	<0.01
Gentamicin	-	-	-	-	-	-	-	-

Table III: Activity against *Proteus mirabilis*

Sample	Water extract (A)		Methanol (B)		Chloroform I		Acetone (D)	
	Average Zone in mm	P value	Average Zone in mm	P value	Average Zone in mm	P value	Average Zone in mm	P value
Citrus maxima-A	11.33	<0.01	10.33	<0.01	8.33	<0.01	9.33	<0.01
Citrus limon – B	13.33	<0.01	12.67	<0.01	10.33	<0.01	15.67	<0.01
Citrus sinensis – C	10.33	<0.01	12	<0.01	9.67	<0.01	10.67	<0.01
Citrus aurantium – D	13.67	<0.01	13.33	<0.01	9.33	<0.01	11.67	<0.01
Gentamicin	18	<0.01	18	<0.01	19	<0.01	18.67	<0.01

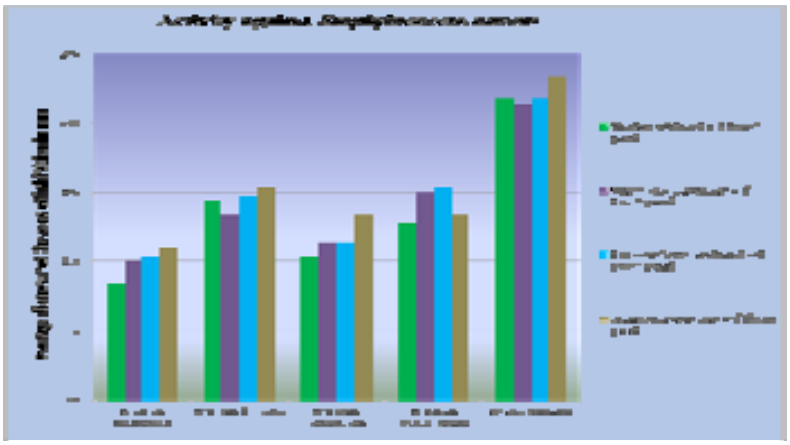
Table IV: Activity against *Klebsiella pneumoniae*

Sample	Water extract (A)		Methanol (B)		Chloroform I		Acetone (D)	
	Average Zone in mm	P value	Average Zone in mm	P value	Average Zone in mm	P value	Average Zone in mm	P value
Citrus maxima-A	11	<0.01	9.33	<0.01	10	<0.01	10.33	<0.01
Citrus limon – B	12.33	<0.01	11.67	<0.01	13.33	<0.01	15.67	<0.01
Citrus sinensis – C	13.33	<0.01	13.33	<0.01	12	<0.01	13.67	<0.01
Citrus aurantium – D	14	<0.01	13.67	<0.01	13	<0.01	16.33	<0.01
Gentamicin	11.33	<0.01	10.67	<0.01	11.67	<0.01	12.33	<0.01

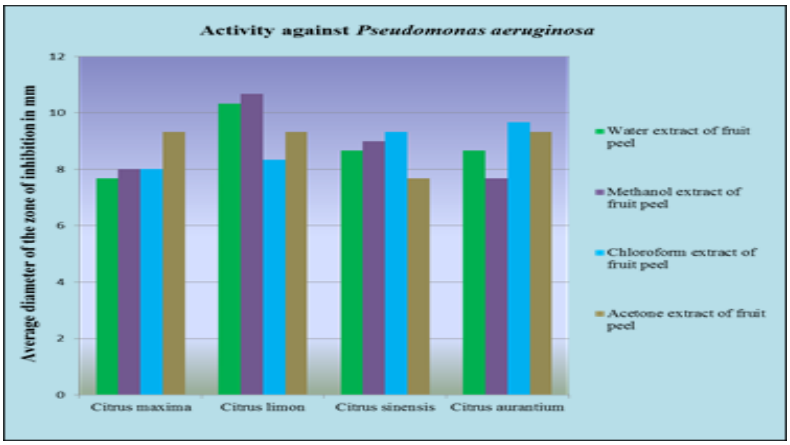
Table V: Activity against *Escherichia coli.*

Sample	Water extract (A)		Methanol (B)		Chloroform I		Acetone (D)	
	Average Zone in mm	P value	Average Zone in mm	P value	Average Zone in mm	P value	Average Zone in mm	P value
Citrus maxima-A	10	<0.01	9	<0.01	10.67	<0.01	13.33	<0.01
Citrus limon – B	15.33	<0.01	12.67	<0.01	15.67	<0.01	15	<0.01
Citrus sinensis – C	15.33	<0.01	11.33	<0.01	14	<0.01	15.33	<0.01
Citrus aurantium – D	16.67	<0.01	14.33	<0.01	16.67	<0.01	16.67	<0.01
Gentamicin	15.33	<0.01	15	<0.01	15.67	<0.01	16	<0.01

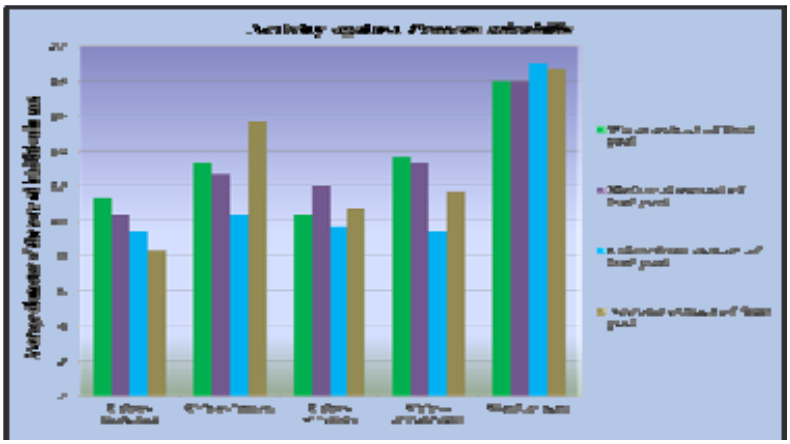
Graph on antibacterial activity of citrus species against micro-organisms



Graph I



Graph II



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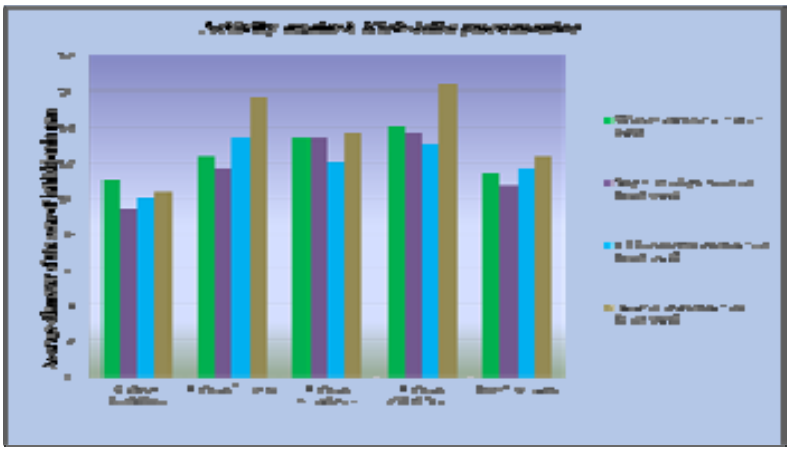
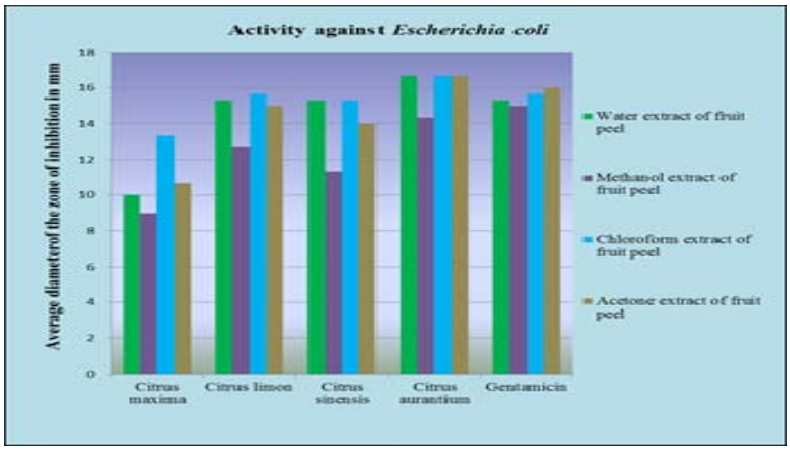


Graph- IV



Graph- V

The present study indicates that both the organic and water extracts of fruit peels of all the four plants under study were effective against all the tested human pathogenic organisms. Among *E.coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae* showed highly significant activity against all the fruit peels extracts. But *Pseudomonas aeruginosa* showed little resistant to all the extracts, except the methanol extract of fruit peel. Also the fruit peel extracts showed significant activity against *Proteus mirabilis*.

4. Conclusion

The result of the present study supports the recycling of fruit waste. Thereby, yielding new products and meeting the requirements of essential products required in human, animal and plant nutrition as well as in the pharmaceutical industry. The study also reveals the fact that the use of this natural drug in the optimum dosage can replace the use of antibiotics.

5. Future scope

The antibacterial activity of all the citrus fruit peel may be due to the presence of antioxidant bioactive components in its fruits peel. So by future studies it is possible to clear out the exact chemical nature of the compounds responsible for their action against the respective bacteria. If this goal is achieved soon, we can design formulations with less expense and great accuracy of specific chemical compounds from these plant parts, or we can attempt for their synthetic analogues.

6. References

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