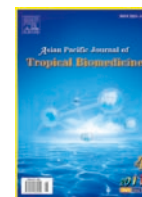




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# Effect of *Citrus hystrix* and *Citrus limon* extracts on antibacterial activity against human pathogens.

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## ABSTRACT

**Objective:** To isolate bacteria from infected human and also screening of antibacterial property against human pathogenic bacteria in two species of Citrus. **Methods:** The present study was isolated microorganism from human pathogens and determined by Agar diffusion method. **Results:** The results showed that the aqueous extract of bark, leaf and fruit peels of the two species did not produce any significant result on tested viz, *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Klebsiella pneumonia* and *Proteus vulgaris* microorganisms. However the methanolic extracts of *Citrus hystrix* fruit peel produced the maximum inhibition zone of 22 mm and 19 mm against *Staphylococcus aureus* and *Salmonella typhi* respectively. *Citrus hystrix* fruit peel extracts has produced the inhibition zone of 15 mm and 11 mm against *Escherichia coli* and *Klebsiella pneumonia* respectively. The fruit peel of *Citrus limon* produced an maximum inhibition zone of 20 mm and 18 mm against *Staphylococcus aureus* and *Salmonella typhi* respectively. The methanol extract of *Citrus limon* produced an maximum inhibition zone of 13 mm and 9 mm against *Escherichia coli* and *Klebsiella pneumonia* respectively. But *Proteus vulgaris* was minimum level of inhibition by all the solvent extract viz., bark, leaf and fruit peels of two species of Citrus. **Conclusions:** The present investigation conclude that the extract methanolic fruit peel extract of *Citrus hystrix* and *Citrus limon* possess antibacterial properties that can be explored as a viable, alternative source to commercially available antibiotic drugs.

## 1. Introduction

Nature has been a source of medicinal agents and an impressive number of modern drugs. Higher plants have been a rich source of medicine because they produce a wide array of bioactive molecules. Each plant and plant parts has one or more substances that can be used for therapeutic purposes and the plants are called medicinal plants. Medicinal plant substances can inhibit growth of pathogens or kill them because they have antimicrobial compound. The basis of sophisticated traditional medicine system and natural products provide excellent leads for new drug development (Newman *et al.*, 2000).

In recent years drug resistance to human pathogenic

bacteria has been commonly reported from all over the world. Microorganisms are present on almost all the body surface both externally and internally. But their disease causing potential depends upon the virulence, *i.e.* ability to cause a disease. The indigenous microflora of human body contains pathogens mainly *Staphylococcus* members. It causes urinary tract infections and many other diseases. During the last 10 years the development of new antimicrobial drugs have been established because the society has been facing one of the most serious public health care over the emergence of infectious bacterial displaying resistance to many drugs. There is a continuous and urgent need to discover the new antimicrobial compounds with diverse chemical structures and novel mechanism of an action because there has been an alarming increase in the incidence of new and re-emerging infectious diseases. Another big concern is the development of resistance to the antibiotics.

*Citrus* is one of the most important commercial fruit

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crops grown in all continents of the world [Tao *et al.*, (2008)]. Importance of *Citrus* is attributed to its diversified use and growing world demand with about 102.64 million tones total world production and probably stands first largest among the produced fruit [NAQVI (2004)]. 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 that is regarded as safe. *Citrus* peels if proved to have antibacterial activity; which generates large peel wastes that can also be used in food industry as preservative. It is well known that essential oils from *Citrus* spp. have pronounced antimicrobial effect against both bacteria and fungi (Lanciotti *et al.*, 2004). *Citrus hystrix* peel oil contained terpinen-4 (13.0%), pinene (10.9%), terpineol (7.6%), 1,8-cineole (6.4%), citronellol (6.0%) and limonene (4.7%) (Waikedre *et al.*, 2010.) It is already reported that ethyl acetate extract of *Citrus hystrix* peel had stronger antibacterial activity (Chanthaphon *et al.*, 2008). The antioxidant activities of *citrus* species are in accordance with their amount of phenolics. *C. hystrix* contained high phenolic content compared to other *Citrus* species, which was responsible for its high antioxidant activity. Several reports showed a close relationship between total phenolic content and high antioxidant activity (Prasad *et al.*, 2005; Amin *et al.*, 2006; Li *et al.*, 2009).

## 2. Materials and methods

### 2.1 Plant material

The bark, leaves and fruit peel of *Citrus limon* (L.) Burm.f. and *Citrus hystrix* DC. were collected from Mayiladuthurai, (11° 6' 35" N, 79° 39' 0" E) Nagapattinam District TamilNadu, India, during April, 2010. Two species of *Citrus* were identified and conformed in Rabinat herbarium, St. Joseph's College, Trichirapalli. India. The collected specimens were identified with the help of Flora of Presidency of Madras (Gamble, 1953). Species identification was confirmed by comparing the collected specimens with the Herbarium of Department of Botany, University of Calicut, Kerala, India. Duplicated voucher specimens have been deposited in the herbarium in Department of Botany, Annamalai University, Tamil Nadu, India.

### 2.2. EXTRACT PREPARATION

The bark, fresh leaves and fruit peels were shade dried separately and ground into fine powder (20<sub>mesh</sub>; ~1 g) with mechanical grinder Thirty grams of bark, leaf and fruit peel powder was then macerated in 100 ml absolute Methanol for 48 h by soxhlet extraction apparatus. The extract was evaporated to dryness at 40 °C in a vacuum using a rotary evaporator and store at 5 °C in a refrigerator. The aqueous

extract, the bark, leaves and fruit peel were shade dried and ground to fine particles with a mechanical grinder. It was then macerated in 500 ml of sterile distilled water for 48 h using a 500 ml conical flask. The conical flask was properly labelled and covered with aluminum foil to prevent contamination. The extracts were then filtered off with sterile filter paper (Whitman No 1). The prepared extract was evaporated to dryness and stored in the refrigerator at 5 °C for further use.

### 2.3. Isolation of microorganisms

The test microorganisms like *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus vulgaris* were isolated from infected human skin, mouth and urine at Government Hospital, Trichirapalli. The samples were plated out on Blood Agar (Himedia) for prepared according to manufacturer's specifications. The isolated microorganism was subcultured and characterized using the methods of Cruickshank *et al.* (1975) and Cowan (1985). They were then stored in agar slants in the refrigerator at 4 °C.

### 2.4. Antimicrobial susceptibility test

The isolated bacterial cultures were maintained on nutrient agar slants at 40C. These bacterial cultures were diluted using Nutrient Broth and diluted bacterial culture (0.2ml) was spread over sterile Nutrient agar plates. About 0.2ml of the plant extracts were applied for the sterile filter paper disc (Whatman No.1, 6mm in diameter) before being placed to the agar plates. Each extracts was tested triplicate. The plates were incubated at 37C for 24 hours. The inhibition zones were recorded. The antimicrobial activities of plant extracts were indicated by clear zones of growth inhibition. The commercial Ampicillin antibiotic disc (1 mg/mL-1, Himedia) was used as control disc.

## 3. Results

Table 1 showed that the results of antibacterial activity of extract from bark, leaves and fruit peel of *Citrus hystrix* and *Citrus limon*.

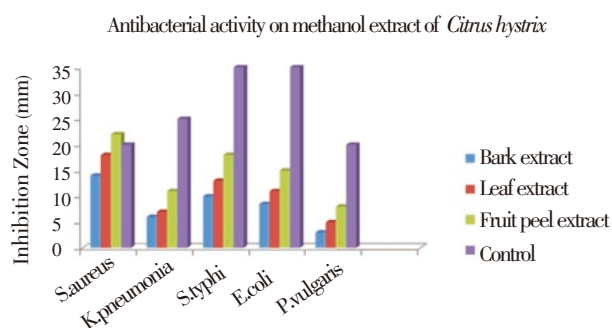
**Table 1**

Antibacterial activity on methanol extracts of *Citrus hystrix* and *Citrus limon* by disc Diffusion method

Plant	Parts	Inhibition zone (mm)*				
		S. aureus	K. pneumonia	S. typhi	E. coli	P. vulgaris
C. hystrix	Bark	14±0.3	6.0±0.2	10±0.2	8.5±0.2	3.0±0.1
	Leaf	18±0.5	7.0±0.2	13±0.3	11±0.3	5.0±0.1
	Fruit peel	22±0.5	11±0.3	18±0.5	15±0.5	8.0±0.2
C. limon	Bark	12±0.3	5.0±0.2	08±0.2	07±0.2	2.0±0.1
	Leaf	15±0.4	7.5±0.2	11±0.3	09±0.3	4.0±0.1
	Fruit peel	19±0.5	9.0±0.3	16±0.5	13±0.5	6.0±0.2

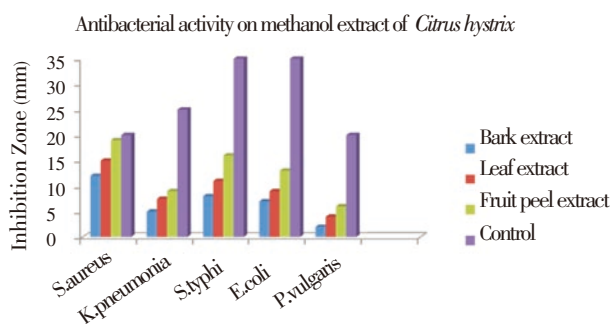
mm\* = Mean of three replicates

The commercial Ampicillin antibiotic disc (1 mg/mL–1, Himedia) was used as positive control. The extractions were carried out using methanol and aqueous solvents. The methanol extracts gave the high activity against all tested bacteria but the aqueous extract has not any antibacterial activity. Methanol extract inhibited the growth of five tested bacteria and the maximum inhibition zone was recorded against *Staphylococcus aureus* and *Salmonella typhi*. The methanol extract of *C. hystrix* fruit peel extract was highest inhibition zone (Tab. 1 & Fig. 1) compared to *Citrus limon* fruit peel extract. However the methanolic extracts have shown (Tab 1 & Fig. 1) fruit peel of *Citrus hystrix* produced an inhibition zone of 19 mm and 22 mm against *Salmonella typhi* and *Staphylococcus aureus* respectively.



**Figure 1.** Antibacterial activity on methanol extract of *Citrus hystrix* by Disc diffusion Method

Extract from fruit peel of *Citrus hystrix* produced highest inhibition zone compared to control against *Staphylococcus aureus*. Whereas the fruit peel extract of *Citrus hystrix* (Tab. 1 & Fig. 2) produced an inhibition zone of 14 mm and 16 mm on *Klbesiella pneumonia* and *Escherichia coli* respectively. Citrus peel extracts showed a significant antibacterial activity against all the test organisms compared to other like leaf and bark extracts. The methanol solvent shows that *Citrus hystrix* and *Citrus limon* fruit peel extract has high degree of antibacterial activity as compared to the leaf and bark extract.



**Figure 2.** Antibacterial activity on methanol extract of *Citrus limon* by Disc diffusion Method

**Table 2**

Positive control use against tested bacteria

Micro organism	Control	Inh. zone (mm)
<i>Staphylococcus aureus</i>		20
<i>Klbesiella pneumonia</i>		25
<i>Salmonella typhi</i>		35
<i>Escherichia coli</i>		35
<i>Proteus vulgaris</i>		20

Control = Ampicillin (1 mg/mL–1)

## 4. Discussion

The methanol extract may have diverse antibacterial agent that has different modes of action or the bacteria may have a special metabolism to overcome or adapt its activity. So, methyl alcohol proves to be a good solvent for the extraction of antibacterial agents from both sources as it has shown better yield as well as antibacterial activity. Aqueous extract showed no any degree of sensitivity against tested pathogenic bacteria and was not able to inhibit the growth of all of the tested pathogens. Ashokumar et al reported that, the difference may be in the phytochemical composition in various part of the plant or may be also due to the extraction method used and or environmental factors or difference in the genotypes of the Citrus plant used [5]. The citrus peel extract exhibited similar or higher antibacterial activity.

The present experimental study concludes that, the methanolic fruit peel extract of *Citrus hystrix* and *Citrus limon* possess antibacterial effect on pathogenic bacteria such as *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Klbesiella pneumoniae*, *Proteus vulgaris*. The results of the present study suggest that the extract methanolic fruit peel extract of *Citrus hystrix* and *Citrus limon* possess antibacterial properties that can be explored as a viable, alternative source to commercially available antibiotic drugs. Further studies are needed to test it on other microorganisms and against various infections, where in the information procured would further serve as a strong evidence for the plant as potent antimicrobial agent.

## Conflict of interest statement

We declare that we have no conflict of interest.

## Acknowledgements

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