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A comparative evaluation on the antimicrobial activity of selected citrus fruit juices

Chitra
Postgraduate and Research, Department of Zoology, CMS College Kottayam, Kerala, India

Abstract
The study was carried out to lead a comparative analysis on antimicrobial activity of selected Citrus fruits and to identify the antimicrobial compounds present in them. The citrus fruits used for the study were *Citrus aurantifolia, Citrus indica, Citrus limon, Citrus limetta* and *Citrus grandis*. The antimicrobial activity of fruit juices was tested on selected bacterial strains of *Staphylococcus aureus* (Gram positive) and *Escherichia coli* (Gram negative). The result showed that all the citrus fruits used in the experiment exhibited antimicrobial activity, the most effective ones were *Citrus aurantifolia and Citrus limon* while *Citrus limetta* showed less zone of inhibition in comparison to the others.

Keywords: citrus fruits, antimicrobial activity, fruit juices, phytochemical components

1. Introduction
Citrus is a genus of flowering trees and shrubs of Rutaceae family. Plants in the genus produce citrus fruits, including important crops like oranges, lemons, grapefruit, pomelo and limes. The most recent research indicates its origin in Australia, New Caledonia and New Guinea [1]. Some researchers believe that the origin is in the part of Southeast Asia bordered by Northeast India, Burma (Myanmar) and the Yunnan province of China [2]. Citrus fruits have well documented nutritional and health benefits [3]. They can actually help prevent and cure some diseases. Citrus fruit intake has been associated with a 10% reduction in odds of developing breast cancer. [4]

Oranges were historically used for their high content of Vitamin C [5], which prevents scurvy. Citrus fruit intake is associated with a reduced risk of stomach cancer [6], reduce blood pressure and kidney stone formation. Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents [7]. According to world health organization (WHO), any plant which contain substances that can be used for therapeutic purpose or which are precursor of chemo-pharmaceuticals semi-synthetic new drugs is referred as medicinal plant [8]. Citrus fruit in general contain sugar, polysaccharide, organic-acid, lipids, carotenoids, vitamins, minerals, flavonoids, bitter lemonoids and volatile compounds. They had been used in traditional Asian medicines for centuries to treat indigestion and to improve bronchial and asthmatic conditions [9-11]. Citrus varieties are considered to be containing a rich source of secondary metabolites with the ability to produce a broad spectrum of biological activities. The medicinal herbs have the bacteriostatic effects on the enzymatic activity associated with energy production, or they can cause denaturation of proteins, modifying cell wall permeability, or causing the loss of macromolecules. Therefore, it is difficult for the microorganisms to develop resistance against these medicinal herbs [12]. The present study was conducted to draw a comparative analysis on the antimicrobial activity and effectiveness of selected citrus fruits.

2. Materials and methods
The study was conducted in the zoology department of CMS College Kottayam, Kerala, India.

2.1 Fruit collection and extraction
The fruits used for the study were - *Citrus aurantifolia, Citrus indica, Citrus limon, Citrus limetta and Citrus grandis*, all of them were purchased from a vendor shop in Mallappally Market (Pathanamthitta district, Kerala) and were brought to the laboratory for taxonomical identification. The fruits were squeezed manually and were filtered using thin pore size mesh to collect the juice (100% concentration).

2.2 Culture media and strains
The media used was nutrient agar and the strains used were *Staphylococcus aureus* (Gram positive) and *Escherichia coli* (Gram negative). They were bought from Mangalam diagnostic Research center, Kottayam, Kerala.

2.3 Disc preparation
Whatman filter paper number 1 was used to prepare discs of approximately 6 mm in diameter, which were placed in a Petri dish and sterilized in a hot air oven. The loop used for delivering the fruit juice was made of 20-gauge wire and had a diameter of 2 mm. This delivered 0.005 ml of juice to each disc.

2.4 Disc diffusion method
Disc diffusion method for antimicrobial susceptibility testing was performed according to the standard method by Kirby-Bauer [13] to assess the presence of antibacterial activity of the

Citrus juices. A bacterial suspension (which has been adjusted to 0.5 McFarland standards) was used to lawn Nutrient agar plates evenly using a sterile swab. The plates were dried for 15 minutes at room temperature in the laminar airflow and then used for the sensitivity test. The discs, which had been impregnated with juice, were placed on the Nutrient agar surface. Each test plate includes two discs, one impregnated with juice of citrus species and one with distilled water (control). In each plate the discs were placed about equidistance to each other. The plate was then incubated at 37°C for 18 to 24 hours and after the incubation, the plates were examined for inhibition zone. The inhibition zone was then measured using sliding calipers and recorded. The tests were repeated three times to ensure reliability.

2.5 Phytochemical screening

Phytochemical screening was done using standard methods. All the experiment has been repeated in triplicate for final confirmation of the result.

1. Test for saponins: To 1 ml of aqueous extract, few volume of distilled water was added in a test tube. The solution was shaken vigorously and observed for a stable persistent froth for 20 min.
2. Test for sterols and steroids: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpene.
3. Test for the phenolic compounds (Flavonoids): The ethanol extract of 5 ml was added to 1ml concentrated sulphuric acid (H_2SO_4) and 0.5g of Mg. A pink or red coloration that disappears on standing 3 minutes indicates the presence of flavonoids.
4. Tannins: About 1 ml concentrated extract was added in 2 ml of water in a test tube. 2 to 3 drops of diluted ferric chloride ($Fecl_3$) solution was added. A dark green or blue-green coloration indicates the presence of tannins.
5. Test for Glycoside: 2 ml of concentrated H_2SO_4 was added carefully and shaken gently. A reddish brown colour indicated the presence of steroidal ring in an aglycone portion of the glycoside [14].

3. Results

Table 1: Antimicrobial activity analysis of Citrus fruit juices using disc diffusion assay

Citrus fruits used	Mean of the Diameter of inhibition zone (mm)	
	Escherichia coli (Gram negative)	***Staphylococcus aureus (Gram positive)***
1) *Citrus aurantifolia*	16	14
2) *Citrus indica*	8	9
3) *Citrus limetta*	7	8
4) Citrus grandis	14	9
5) Citrus limon	15	15

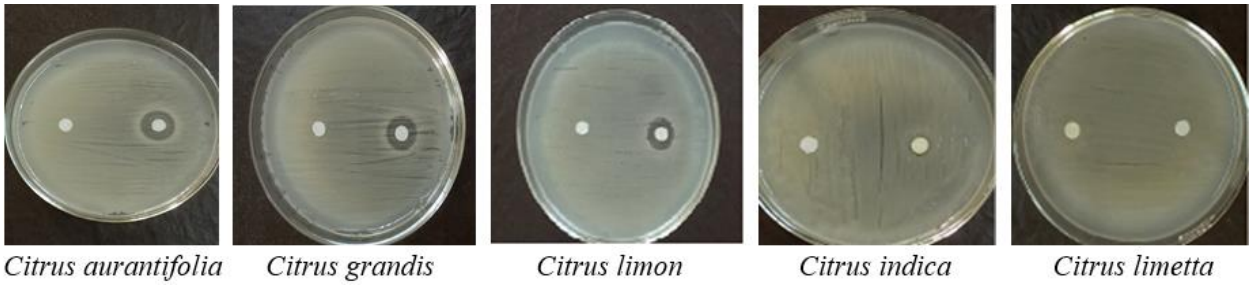

Fig 1: Pictures showing zone of inhibition on Escherichia coli culture plates by selected fruit juices.

In each plate, Right disc is impregnated with citrus juices and Left disc impregnated with distilled water.

Below the picture the name of the fruit is mentioned, which had been used for impregnation.

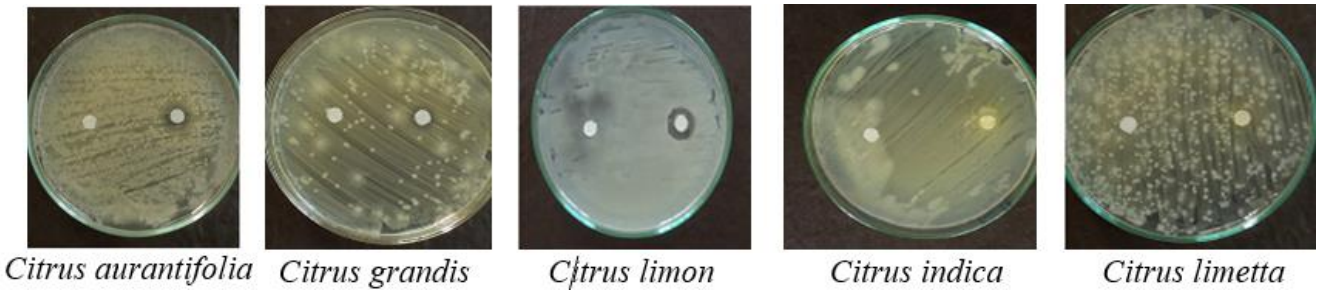

Fig 2: Pictures showing zone of inhibition on *Staphylococcus aureus* culture plates by selected fruit juices.

In each plate, Right disc is impregnated with citrus juices and Left disc impregnated with distilled water.

Below the picture the name of the fruit is mentioned, which had been used for impregnation.

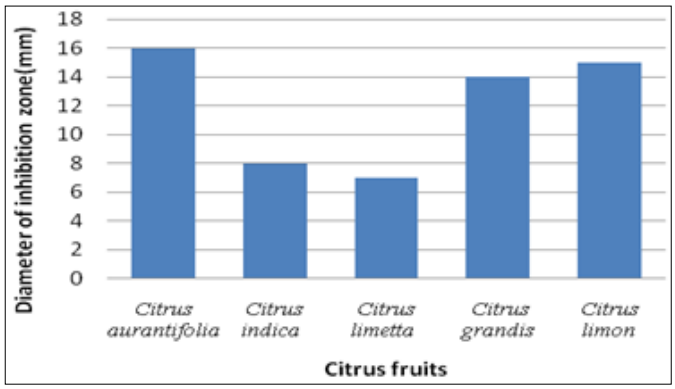

Fig 3: Antimicrobial activity of selected Citrus fruit juices on *Escherichia coli* (Gram negative)

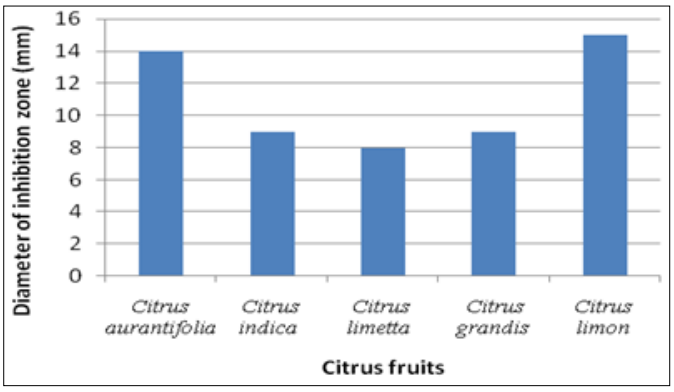

Fig 4: Antimicrobial activity of selected Citrus fruit juices on *Staphylococcus aureus* (Gram positive)

Table 2: Phytochemical screening results of selected citrus fruit juices.

Tests	*Citrus aurantifolia*	*Citrus grandis*	*Citrus limon*	*Citrus indica*	*Citrus Limetta*
1) Saponins	+	+	+	+	+
2) Sterols and Steroids	+	+	+	+	+
3) Flavonoids	+	+	+	+	+
4) Tannins	+	+	+	+	+
5) Glycoside	+	+	+	+	+

+ Sign indicates presence and – sign indicates absence

4. Discussion

The present study investigates antimicrobial activity of selected citrus fruit juices; the fruits used were *Citrus aurantifolia, Citrus indica, Citrus limon, Citrus limetta and Citrus grandis.* All off them exhibited antimicrobial activity on *Staphylococcus aureus* (Gram positive) and *Escherichia coli* (Gram negative) strains but the best inhibition zone was exhibited by *Citrus aurantifolia*, with 16mm and 14mm diameters on *Escherichia coli* and *Staphylococcus aureus* culture plates respectively. Among the five citrus fruits used, bacterial strains were seen showing more resistance towards *Citrus limetta* juice by showing an inhibitor zone of only 7mm and 8mm on *Escherichia coli and Staphylococcus aureus* culture plates. Phytochemical tests indicated the presence of Saponins, Steroids, Flavonoids, Tannins and Glycoside in the juices, which are mostly responsible for these antimicrobial activities. More research needs to be conducted in identifying these biochemical components individually.

5. Conclusion

Plants play a vital role in pharmaceutical industry due the presence of important biochemical components produced by them and extraction of these enables in conducting numerous researches in the field of medicine. It helps in producing new drugs, medicines that can act on wide variety of diseases. Not only the pharmaceutical industry, food industry can also be benefited by using them as preservatives in food without having any side affects, as they are natural and can be cultivated easily in large quantity. Numerous researches have already been carried out on citrus plants and all of them have revelved many of their benefits. Extraction, Isolation and purification of the active components present in them will lead to new discoveries and inventions, which would be beneficial to the whole world.

6. References

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