

## Assessment of Antibacterial Activities of Leaves Extract of Some Citrus Fruits Against Pathogenic Bacteria

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### Abstract

**Objectives:** Study was planned to assess the Antibacterial Activities of leave extract of some Citrus Fruits against Pathogenic Bacteria.

**Methods:** The fresh leaves of Citrus grandis, Citrus reticulata, and Citrus limon plant were collected. The plants were recognized phenotypically and confirmed for taxonomic character by P.C.S.I.R Laboratory Lahore. Microbial Strains including strains of Staph. aureus and Salmonella typhi, which were taken from clinical Laboratory of Fatima Jinnah Medical University Lahore. The antimicrobial activity of the powdered form of Citrus leaves was tested against the strains of bacteria through disc diffusion method and the zone of inhibition was noted.

**Results:** Antibacterial activities by ethanol extracts of C. lemon and C. reticulata were observed with inhibition zone 2.75 mm and activity index 0.675 against S. aureus. However, low antibacterial activities of C. paradisi were noted against S. aureus and S. typhi. On the other hand, low antibacterial activity of C. lemon and C. reticulata was observed against S. typhi.

**Conclusion:** The results obtained in the study show that the leaves of C. lemon and C. reticulata exhibit higher antibacterial activity as compared to C. paradisi, which may be due to the presence of large amounts of flavonoid and phenolic compounds.

**Key words:** Citrus fruits, antibacterial activity, S. aureus, Salmonella typhi

### Introduction

Medicinal plants are used to treat the ailment of several microbial and non-microbial diseases and also for the formation of new drugs.<sup>1</sup> These become popular due to the limited aptitude of antibiotics and other drugs used for treatment of diseases. The affordability, reliability, accessibility, and low toxicity of herbs used as medicine made them

quite favorable and useful worldwide for health care.<sup>2,3</sup> According to WHO, about 80% of people worldwide use herbs as medicine for health care.<sup>4</sup>

Infections due to micro-organisms are the major health problem that may lead to disabilities and death worldwide in all age groups. Among the pathogenic bacteria, Escherichia coli, Staph aureus and Salmonella typhi are highly prevalent and cause infection while resisting many drugs.<sup>5</sup> Staphylococcus aureus is a main reason for food poisoning through the secretion of enterotoxins.<sup>6</sup> Salmonella typhi causes a water and food borne disease and is related to typhoid fever and problems of gastrointestinal tract. These bacteria form colonies in the epithelium of intestine by the phagocytosis using proteins of effector type, resulting in the reorganization of the cytoskeleton of host.<sup>7</sup> In general, micro-organisms have many genetic modes of actions to attain resistance against antibiotics. This has increased the need for antimicrobial herbs for the treatment of diseases.

The genus of Citrus links to the family Rutaceae has a

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number of biological metabolites active against gram negative and gram positive bacteria.<sup>5</sup> Citrus sinensis (Orange), Citrus paradise (Grapefruit), Citrus limon (Lemon) and Citrus aurantium (Sour orange) are some fruits of genus Citrus. Precisely, the Citrus peels are a possible source of important metabolites of plant.<sup>9</sup>

Lemon leaf has antispasmodic and sedative properties. These can be used to treat neurological problems such as nervousness, insomnia and palpitation. The alkaloid form of citrus plants shows antibacterial and anticancer properties using the extract of its root, stem, leaves, and flowers.<sup>10,11</sup>

Bacterial cells attack the lipid bilayer of the membrane of the cell and alter the activity of cell enzymes. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found in vitro to have antimicrobial properties and these may inhibit the bacterial growth by a diverse mode of action as compared to antibiotics.<sup>12</sup>

The resistance due to microorganisms is rapidly increasing and this creates a hurdle against the proper treatment of infectious ailments. Additionally the side effects of antibiotics is also a big problem. Thus, there is a need to find the antibacterial activity of herbs, which might become an important part in treatment of diseases.

**Objectives:** The purpose of this study was to evaluate antimicrobial activity of leaf extract of Citrus plants against the strains of Staphylococcus aureus and Salmonella typhi.

## Methods

### Collection of plant samples:

The fresh leaves of Citrus grandis, Citrus reticulata, and Citrus limon were collected in the local market of Lahore city. The plants were recognized phenotypically and confirmed for taxonomic character by P.C.S.I.R Laboratory Lahore. Duration of study was March 2016 to August 2016. Leaves of plants were washed thoroughly, dried and homogenized to form a powder and stored in an airtight glass bottle at 4°C.

### Preparation of leaves extract of Grapefruit/

### orange fruit / Lemon fruit:

Leaves were dried in shade and powdered in a mechanical blender. Five hundred grams of powder was soaked in 500 mL of 99 % ethanol (Merck, Germany) as a solvent in a ratio of 1:4 (w/v) and incubated at 37°C for a period of 24 hours and later filtered by Whatman filter paper. Ethanol was allowed to evaporate and the extract was concentrated using evaporator and stored at 4°C until used.<sup>13</sup>

### Preparation of sample:

Samples for antimicrobial activity were prepared by dissolving 100 mg of each extract in 1 ml of dimethyl sulphoxide (DMSO).

### Microbial strain used:

Microbial Strains including strains of Staph. Aureus and Salmonella Typhi were taken from the clinical Laboratory of Fatima Jinnah Medical University Lahore. Sub-culturing of strains was carried for further use. The stock cultures were kept on the medium of nutrient broth medium with 10% glycerol at -20°C.

### Preparation of bacterial suspension:

Colonies of strains of bacteria Staphylococcus aureus and Salmonella typhi were transferred to the nutrient broth and were incubated at 37°C for 24 hr and preserved in sterile flasks until their use.

### Antibacterial activity by agar well diffusion method:

The antimicrobial activity of metabolites of Citrus leaves was applied against the strains of bacteria by using the disc diffusion method or Kirby-Bauer method. Bacterial strains were allowed to grow separately in plates of agar (Merck, Germany) for four hour at 37°C. Spreading of inoculum was carried out on the agar plate for getting an even growth of bacteria. Disc labeled as test was loaded with extract of citrus leaves of three species. Disc loaded with 10 µL of 80% methanol was taken as control. Incubation of plates was done at 37°C for a period of 24 hours. After the period of incubation, the antibacterial activity was found by measuring the diameters of the zone of growth inhibition in mm of micro-organism<sup>6</sup>. The positive control and negative control wells were

filled with Ampicillin ( 4 µg/ml) and DMSO respectively.

**Activity index for each leaf extract was calculated by the following formula (Dharjia):**

Activity index (AI)= Inhibition Zone of the sample/ Inhibition Zone of the standard.

**Statistical analysis:**

Data was analyzed by SPSS 20. Variables were expressed as mean ± standard deviation for each microbial strain. Student ‘t’ test was applied for comparison and p values were taken as significant at p < 0.05.

**Results**

Most Antibacterial activities of ethanol extracts of C. lemon and C.reticulata were observed with inhibition zone 2.75±0.09, AI was 0.675 against S.aureus. However, low antibacterial activities of ethanol extracts of C. paradisi was observed with inhibition zone 1.0± 0.01, AI was 0.25 against S.aureus. On the other hand, low antibacterial activities of ethanol extracts of C. lemon and C.reticulata was observed with inhibition zone 1.2±0.02, AI was 0.50 against S. typhi. Very low antibacterial activity of ethanol extracts of C. paradisi was observed with inhibition zone 1.0±0.01, AI was 0.32 against S.typhi

**Discussion**

About 80% of the population of the world relies on medicine derived from plants for healthcare and it is believed that these herbal medicines have no side effects. In the United State, roughly 25% of allopathic drugs contain one or more ingredients from plants.<sup>3</sup> Medicine derived from plants has strong antimicrobial effects and may offer a harmless and economical treatment against infections caused by viruses, bacteria or fungi.<sup>5</sup>

According to our study, maximum antibacterial activities by ethanol extracts of C. lemon and C.reticulata were observed against S.aureus. Our study is supported by another study, which reported that extracts of plants, especially citrus fruit plants are more active against Gram-positive microbes like S.aureus as compared to Gram-negative and seem to be a significant alternative to antibiotic resistance and managing the disease<sup>16,17</sup>. It is proposed that the leaves of citrus limon are potential sources of antioxidants. The activity of antioxidants may depend on their concentration and amount. The activity of antioxidants may be due to flavonoid and phenolic compounds present in extract.<sup>18</sup> It is suggested that phenolics and flavonoids were higher in other parts of citrus plant in comparison with citrus juice, which is why the peels are more effective than juice.<sup>16</sup>

A group of workers also studied the antibacterial role of extract of citrus lemon and of Citrus aurantium (sour taste orange). According to their study, both of these showed a significant effect on food borne micro-organism.<sup>18</sup> It is reported that the extract of all citrus fruits showed very high antibacterial activity against S.aureus.<sup>19</sup> Our results fall in line with another study which found that extracts of leaves of Citrus paradisi do not show antibacterial activity against S. aureus, E. coli and Salmonella typhi.<sup>20</sup>

We observed low antibacterial activities of ethanolic leaf extracts of C. lemon and C.reticulata, against S. typhi. A study also found that Gram-negative bacteria showed resistance to the extract. The reason may be the difference in the structure of cells of Gram negative and Gram positive bacteria. Study stated that the outer side of cell membranes of Gram-negative bacteria act as a barrier towards microorganism from entering in the cells.<sup>16</sup>

A study demonstrated that medicinal herbs including

**Table 1:** Antibacterial Activity (Zone of Inhibition) of Extracts of Citrus Fruits

Leaf Extract	Concentration (mg/ml)	Zone of Inhibition of Staph aureus (mm)	Zone of Inhibition of S typhi (mm)	Activity Index (AI) Of Staph aureus	Activity Index (AI) of S typhi
Citrus lemon	100	2.75 ±0.09	1.2 ± 0.02	0.675	0.48
Citrus reticulate (orange),	100	2.75 ± 0.08	1.3 ± 0.02	0.675	0.52
Citrus paradisi (grape fruit)	100	1.0 ± 0.01	0.8 ± 0.01	0.25	0.32
Dimethyl sulfoxide	1 µl/ml	-	-	-	-
Ampicillin	10 (µg/ml)	4 ± 0.19	2.5 ± 0.06		

citrus plants show bacteriostatic effects on the enzymes which are related to production of energy. Besides, they have the ability to alter the permeability of cell walls and protein denaturation.<sup>6</sup>

**Limitation of study:** A need to find the toxicity of these citrus fruits and use other solvents for extracting polar compounds that may have bactericidal activity.

## Conclusion

The results obtained in the study show that the leaves of *C. lemon* and *C. reticulata* exhibit higher antibacterial activity as compared to *C. paradisi*, which may be due to the presence of large amounts of flavonoid and phenolic compounds. However, further research is required to use other solvents that dissolve more polar compounds and give better results than the present one.

## Authors' Contributions

**RK:** Article writing/ Experiment design/conduct

**LA:** Data interpretation/ Literature survey

**SS, SR:** Experiment performance

**AS:** Proof reading

**HA:** Data collection

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