

# Comparative study of antibacterial activity of ripen and unripen Indigenous *Citrus union* of Assam, India

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## ABSTRACT

Citrus fruits are acidic fruits of family Rutaceae. They contain healthy nutritional content that are important for the body. Other than nutritional content they also have antioxidant, antimicrobial, insect repellent, medicinal and many other properties. Microorganisms are responsible for many of the diseases. They are present everywhere in the environment. Different synthetic drugs are available in the market which can be used against microbes. But, microorganisms become resistant to the available drugs. Alternative medicine or biocompounds are required which can replace the function of synthetic drugs. Citrus in different forms i.e extract in different solvents have strong antibacterial and antifungal properties. The extract of simple distilled water also has significant result. Citrus is eaten mainly in ripen form. Citrus juice may be consumed directly after extraction of the juice. This study was performed to check the antimicrobial activity of juice in ripen form. Unripen juices were also study to compare the activity with the ripen juices. Significant result was found in both the cases.

**Keywords :** Pharmaceutical; Flavanoids; Polyphenols; Antimutagenic; MIC

## 1 INTRODUCTION

Though different types of antibiotics are produced by pharmacological industries, resistant to antibiotics by micro organisms has been increased now a days. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agent[1]. It is cultivated mainly for its alkaloids, which are having anticancer activities and 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 [2]. Citrus flavonoids have a large spectrum of biological activity including antibacterial, antifungal, antidiabetic, anticancer and antiviral activities [3], [4]. Flavonoids can function as direct antioxidants and free radical scavengers, and have the capacity to modulate enzymatic activities and inhibit cell proliferation [5]. In plants, they appear to play a defensive role against invading pathogens, including bacteria, fungi and viruses [6]. Flavonoids are generally present in glycosylated forms in plants, and the sugar moiety is an important factor determining their bioavailability. Preparation from peel, flowers and leaves of bitter orange (*Citrus aurantium* L.) are popularly used in order to minimize central nervous system disorders [7].

In addition, the fiber of citrus fruit also contains bioactive compounds, such as polyphenols, the most important being vitamin C (or ascorbic acid), and they certainly prevent and cure vitamin C deficiency-the cause of scurvy [8].

Citrus (Lemon) juice exhibit significant antimicrobial affect and bioactivity is associated with minerals and other active compounds. Hence these citrus fruit juices have been used for maintaining good health for a long period of time. Even use of this juice shows great impact on therapeutical treatment. Microbes are all around us and because their resistant power to many antibiotics, it is now become difficult to control them. Out of which *E.coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus* etc become very much resistant to common antibiotics. The fruit juices can inhibit those microbes due to presence of some active compounds produced during secondary metabolism of the plant. For instance, of two million people who acquired bacterial infection in United States of America (USA) hospitals annually, 70% of the cases involved the strains that are resistant to

at least one antibacterial agent [9]. The emergence of antibiotic-resistant microorganisms had swiftly reversed the advances of previous fifty years of research on antibiotics [10]. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments [11]. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances e.g. the phenolic compounds which are part of the essential oils, as well as tannin [12]. Essential oils are more effective in controlling biofilm cultures due to their better diffusibility and mode of contact [13]. The essential oils and other extracts of plants have evoked interest as sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases [14], [15].

The effect of essential oils from *C. aurantium*, *C. limon*, *C. paradisi* and many other plant oils and extracts was studied and found that the minimum inhibitory concentrations (MIC) were between 5-2% (v/v) [16]. The peel of Citrus fruit is a rich source of flavanones and many polymethoxylated flavones, which are very rare in other plants [17]. The antimicrobial abilities of essential oils, among which citrus oils, are also shown to be a particularly interesting field for applications within the food and cosmetic industries [18]. It has also been used as an anti-diabetic agent [19].

Although lemon juices are commonly used for antimicrobial study, juices of oranges, limes, grapefruit etc can also be used for the same purpose. Citrus fruit is widely known for its medicinal impact due to presence of flavenoids, flavenones and other compounds which are rare in other plants hence increased their use.

## **2 MATERIALS AND METHODS**

### **2.1 Collection of samples**

Fresh ripen and unripen samples of *Citrus uniuon* were collected from local market of Guwahati, Assam, India.

### **2.2 Juice extraction**

The fruits were washed thoroughly in normal tap water, distilled water and then dried completely in open air. The juices were extracted by cutting the fruits in half and carefully squeezing to extract juices. The collected juices were filtered through 4 fold muslin cloth. The filtrate was stored at -20°C for further study.

### **2.3 Microbial cultures**

Microbial pure cultures including *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* were collected from the microbiology laboratory, College of Veterinary Science, Khanapara, Ghy-22, AAU, Assam, India. Microbes were sub cultured in Nutrient broth. Experiment was performed using 24 hr grown bacterial cultures at 37°C.

### **2.4 Evaluation of antimicrobial property**

Antimicrobial study was performed to detect antimicrobial property of the juice for different cultures by agar plate diffusion method. Nutrient agar plates (diameter 15cm) were prepared with desired pH. 1mL of the inoculum of each microbe was spread followed by boring 1.0 cm well with the help of a sterile cork borer. Dilution of the juices was made to prepare the extracts for the present study. The concentration of the juices

used for the present study are original (100% pure juice), 500 $\mu$ L/mL, 250 $\mu$ L/mL, 125 $\mu$ L/mL, 62.5 $\mu$ L/mL, 31.25 $\mu$ L/mL respectively. 400  $\mu$ L of each of the juice extracts were added to the wells. At the center well Streptomycin sulphate (1mg/mL) was added. Plates were incubated at 37°C for 24 hrs.

### 2.5 Determination of minimum inhibitory concentration ( MIC)

MIC or Minimum Inhibitory Concentration can be defined as the lowest concentration of an antimicrobial substance that can degrade or inhibit the growth of the microorganisms. The antimicrobial effect of the juice was determined by measuring the zone of inhibition (cm). The zone of inhibition was compared with those produced by the commercial antibiotic streptomycin sulphate (1mg/mL).

## 3 RESULTS AND DISCUSSION

### 3.1 Antibacterial plates



*E. coli*

*B. subtilis*

*S. aureas*

*P. aeruginosa*

fig1 : Antibacterial plates for ripen *C. unio* for different concentrations of juices.



*E. coli*

*B. subtilis*

*S. aureas*

*P. aeruginosa*

fig2: Antibacterial plates for unripen *C. unio* for different concentrations of juices.

### 3.2 Antibacterial effect of streptomycin

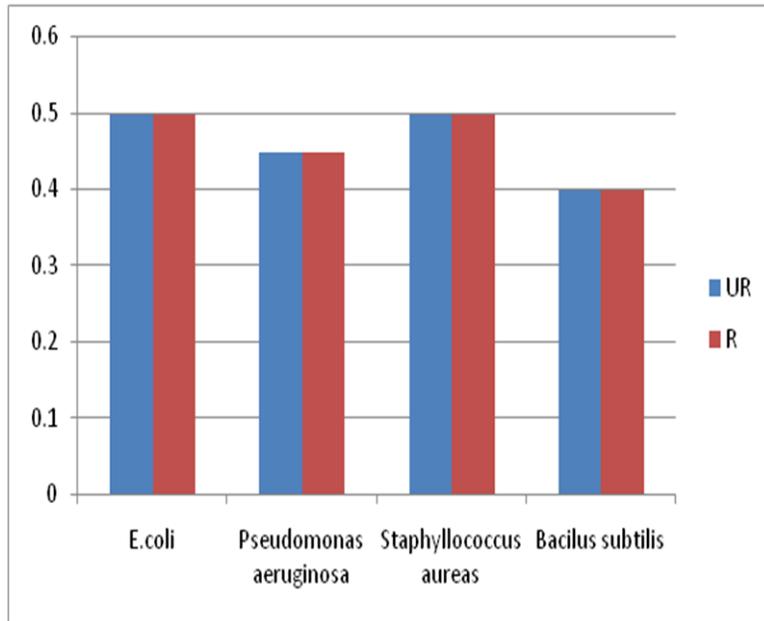


FIG3: ANTIBACTERIAL ACTIVITY OF STREPTOMYCIN USED AS CONTROL (x axis represents name of microbes and y axis represents distance in cm).

The effect of streptomycin (control) against different microorganisms was found to be in the range 0.5 cm to 0.4 cm. The largest zone of 0.5 cm was recorded for both *E.coli* and *Staphylococcus aureus* followed by *Pseudomonas aeruginosa* as 0.5 cm. The least was recorded as 0.4 cm for *Bacillus subtilis*.

HOWEVER, THE VALUES WERE FOUND TO BE SAME FOR BOTH RIPEN AND UNRIPEN JUICES AGAINST THE CONTROL STREPTOMYCIN. IT INDICATES THAT THE ANTIBACTERIAL ACTIVITY OF BOTH RIPEN AND UNRIPEN ARE SIMILAR FOR THIS PARTICULAR ANTIBIOTIC.

### 3.3 ANTIBACTERIAL EFFECT OF DIFFERENT EXTRACTS

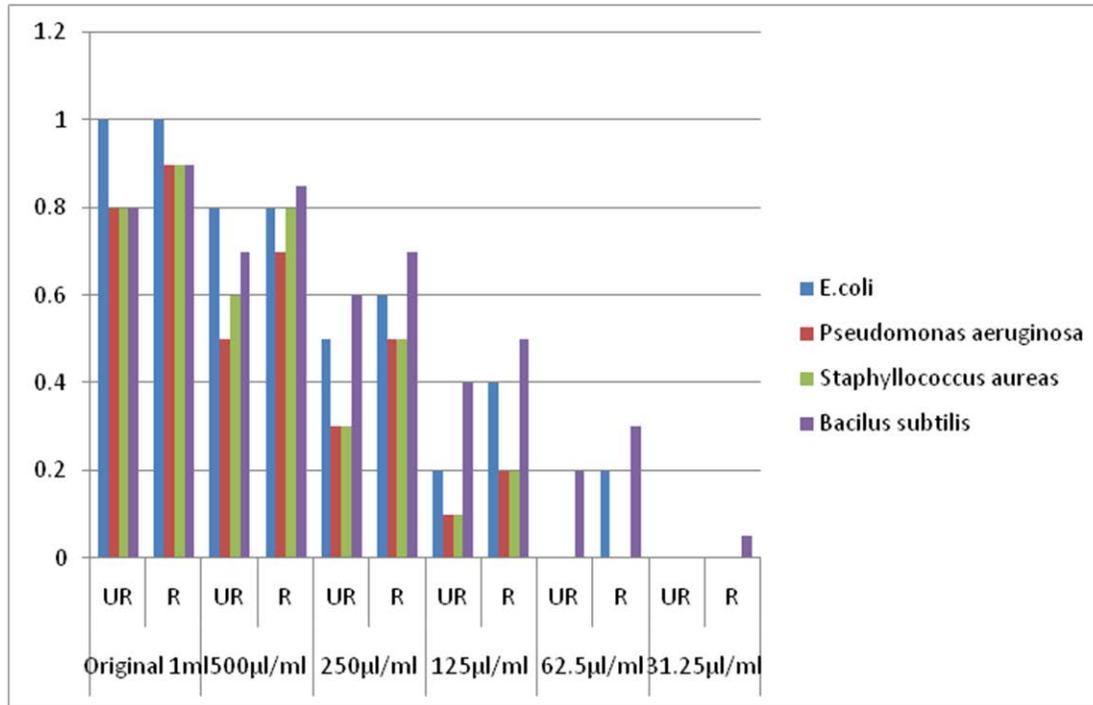


Fig4: Antimicrobial activities of ripen and unripen juices of *C. union* against four different microorganisms (x axis represents concentration of juice and y axis represents distance in cm).

The antibacterial effect was also found to be strong at different concentrations of juices when compared with the control. The zone of inhibition was recorded in the range 0.05 to 1.0 cm for the concentrations of 100% original juice, 500 µl/ mL, 250 µl/ mL, 125 µl/ mL, 62.5 µl/ mL and 31.25 µl/ mL.

For unripen original juice the maximum zone was observed as 1.0 cm for *E. coli* followed by 0.8 cm for all other microbes. For ripen original juice the values were recorded as 1.0 cm for *E.coli* followed by 0.9 cm for all other microorganisms.

For unripen 500µl/mL concentration, the maximum zone was observed as 0.8 cm for *E. coli* followed by 0.7 cm for *Bacillus subtilis*, 0.6 cm for *Staphylococcus aureus*, 0.5cm for *Pseudomonas aeruginosa*. For ripen 500µl/mL concentration the values were recorded as 0.85 cm for *Bacillus subtilis* followed by *Staphylococcus aureus* and *E. coli* as 0.8 cm. For *Pseudomonas aeruginosa* the zone was 0.7 cm.

For unripen 250µl/mL concentration, the maximum zone was observed as 0.6 cm for *Bacillus subtilis* followed by 0.5 cm for *E. coli*, 0.3 cm for *Staphylococcus aureus* and *Pseudomonas aeruginosa*. For ripen 250µl/mL concentration the values were recorded as 0.7 cm for *Bacillus subtilis* followed by *E. coli* as 0.6 cm. For *Pseudomonas aeruginosa* and *Staphylococcus aureus* the zone was 0.5 cm.

For unripen 125µl/mL concentration, the maximum zone was observed as 0.4 cm for *Bacillus subtilis* followed by 0.2 cm for *E. coli*, 0.1 cm for *Staphylococcus aureus* and *Pseudomonas aeruginosa*. For ripen 125µl/mL concentration the values were recorded as 0.5 cm for *Bacillus subtilis* followed by *E. coli* as 0.4 cm. For *Pseudomonas aeruginosa* and *Staphylococcus aureus* the zone was 0.2 cm.

For unripen 62.5µl/mL concentration, the zone was observed as 0.2 cm for *Bacillus subtilis*. For other microorganisms the zone was not observed. It indicates that for all microorganisms other than *Bacillus subtilis* the previous concentration (125µl/mL) is MIC. However, MIC for *Bacillus subtilis* is 62.5µl/mL.

For ripen 62.5 $\mu$ L/mL concentration the values were recorded as 0.3 cm for *Bacillus subtilis* followed by *E. coli* as 0.2 cm. For *Pseudomonas aeruginosa* and *Staphylococcus aureus* the zone was observed. So, the MIC for *Pseudomonas aeruginosa* and *Staphylococcus aureus* is 125 $\mu$ L/mL.

At 31.25 $\mu$ L/mL the was seen only for *Bacillus subtilis* as 0.05 cm. This concentration is also the MIC for this microorganism.

## CONCLUSION

The antimicrobial activity reserach is of great demand. Different types of microorganisms are there in the environment which can cause life threatening disease. Inspite of the synthetic drugs if we can make or extract some bioproduct that can replace the available drugs of the market, the resistance of the microbes against these synthetic onse can be controlled. The research was done to use Citrus juice for the same. If we use only the simple juice, the antibacterial activity is found. Since, fruits are consumed by almost every one. It is eaten for nutrition, freshness etc. Now it is clear that it also has a great antimicrobial activity. The Citrus selected for the present study is indigenous to North Eastern states of India. Much work is to be done to explore the same in different areas.

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