# EVALUATION OF ANTI-MICROBIAL ACTIVITY OF CITRUS AURANTIUM AGAINST SOME GRAM POSITIVE AND NEGATIVE BACTERIAL STRAINS

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#### Abstract

The present investigation was carried out to evaluate the antibacterial activities of Citrus aurantium Linn. Dried leaf powder of Citrus aurantium was successively extracted with petroleum ether, chloroform and ethanol using Soxhlet and macerated to form water extract. All extracts were screened for its antibacterial using agar well diffusion method. The extracts showed antimicrobial activity were subjected to minimum inhibitory concentration (MIC) assay by two fold dilutions method. Petroleum ether, chloroform, ethanol and water extract exhibited in-vitro antibacterial activity.

Key Words: C. aurantium, antimicrobial activity, MIC

#### Introduction

Herbs and plants used as spices with antimicrobial activity have been widely used both traditionally and commercially to preserve and increase the shelf life and safety of foods [1, 2]. So many natural substances may have fundamental role in the host plant/pathogen relationship: the essential oils produced by different plant species are in many cases biologically active, empowered with antimicrobial, allelopathic, antioxidant and bio-regulatory properties. The antimicrobial abilities of essential oils, among which citrus oil, are also shown to be a particularly interesting field for applications within the food and cosmetic industries [3].

The peel of Citrus fruits is a rich source of flavanones and many polymethoxylated flavones, which are very rare in other plants [4]. These compounds, not only play an important physiological and ecological role, but are also of commercial interest because of their multitude of applications in the food and pharmaceutical industries. Citrus aurantium contains synephrine alkaloids and para-octopamine [5, 6, 7] these molecules are usually cited on product labels as active ingredients. Synephrine is a part of many cold/allergy medications. Most weight loss and energy supplements, which contain Ma Huang, also contain the active compound of Citrus aurantium. Flavonoids, including limonene, hesperidin, neohesperidin, naringin, and tangaretin, are present in bitter orange peel, flowers, and leaves. The flavonoid content of bitter orange is noted as being higher in the flowers than the leaves. Bitter orange also contains the furocoumarins bergapten and oxypeucedanin [8, 9, 10].

#### 2. Material and Methods

### 2.1 Plant Material:

The leaves of *Citrus aurantium* were collected from the local areas of Shirpur, Karnataka, India. The plant material was authetified by the department of Pharmacognosy, KLE College of Pharmacy, Karnataka.

## 2.2 Preparation of Extracts:

Collected leaves were cleaned and shade-dried. The dried leaves were pulverized by a mechanical grinder and passed through a 20-mesh sieve. A powdered leaf (500 g) was successively extracted with petroleum ether, Chloroform and ethanol using a Soxhlet apparatus and water extracted by cold maceration. The extraction was carried out for 24 hrs at room temperature with mild shaking. The extracts were filtered and concentrated at

35°C, and the weight of each residue was recorded and percentage yield was calculated [11].

#### 3. Screening for Antibacterial activity:

The antibacterial activity was carried out by employing 48 h cultures of Bacillus subtulis, Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia. Activity of the above mentioned extracts was tested separately using Agar-Well diffusion method. The medium was sterilized by autoclaving at 120°C (15 lb/in2). About 30 ml of the medium (nutrient Agar Medium) with the respective strains of bacteria and fungi was transferred aseptically into each sterilized petri plate. The Plates were left at room temperature for solidification.

Each plate, a single well of 6 mm diameter was made using a sterile borer. The extracts were freshly reconstituted with suitable solvents (Dimethyl Sulphoxide) and tested at various concentrations. The samples and the control (0.1ml) were places in 6-mm diameter well. Antibacterial assay plates were incubated at  $37\pm2^{\circ}$ C for 24 h. Standard disc (6 mm diameter) with Gentamicin (5µg/ml) was used as a positive control for antibacterial activity. Each experiment was carried out in triplicates, and diameter of the zone of inhibition was measured. Observations and results are shown in Table 1. The extracts that showed antimicrobial activity were subjected to minimum inhibitory concentration (MIC) assay by serial two fold dilution method [12]. MIC was interpreted as the lowest concentration of the sample, which showed clear fluid without development of turbidity; Observations and results are shown in Table1

Plant extracts	Zone of Inhibition (mm)			
	Staphylococcus aureus	Bacillus subtulis	Escherichia coli	Klebsiella pneumonia
Ethanol Extract	13±0.23	11±0.11	12±0.27	13±0.12
Chloroform	14±012	12±0.06	13±0.14	12±0.14
Water Extract	12±0.08	13±0.14	12±0.09	14±0.21
Petroleum Ether extract	16±0.21	No	No	13±0.17

Table 1: Minimum inhibitory Concentration of Citrus aurantium leaf extracts

\* Gentamicin disc (25  $\mu$ g) as a positive reference standard; Values are mean inhibition zone (mm)  $\pm$  S.D of three replicates

### 4. Result and Discussion

The antimicrobial assay showed that Chloroform, ethanol and water extracts of C. aurantium leaves exhibited in-vitro antibacterial activity against Gram positive and Gram-negative bacteria, whereas significant activity was not observed with petroleum ether extract. The lowest MIC values were observed for ethanol extract, chloroform extract, water extract and petroleum ether extract against the bacteria. The results reveal that extracts of C. aurantium leaves were significantly effective against both Gram-positive and Gram-negative organism. Preliminary phytochemical screening of the extracts showed the presence of Alkaloids, carbohydrates, flavonoids, terpenoids, sterols and tannins. Thus further work can be carried on the isolation procedure for finding out the exact moiety responsible for the biological activity.

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