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Asian Pacific Journal of Tropical Disease

journal homepage: [www.elsevier.com/locate/apjtd](http://www.elsevier.com/locate/apjtd)

Document heading

## *In vitro* Antimicrobial Activity of *Citrus aurantifolia* and its Phytochemical screening

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## ARTICLE INFO

## Article history:

Received 15 June 2012

Received in revised form 5 July 2012

Accepted 7 October 2012

Available online 28 October 2012

## Keywords:

*Citrus aurantifolia* Linn

Antibacterial

Antifungal

phytochemistry

hydroalcohol

## ABSTRACT

**Objective:** To evaluate the antimicrobial efficacy of *Citrus aurantifolia* Linn (CA) against some microorganisms – bacteria and fungus were *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas spp.*, *Aspergillus niger*, *Aspergillus fumigates*, *Mucor spp* and *Penicillium*. **Methods:** 100  $\mu$ l of 10 mg CA were assessed against eight test microorganisms by agar well Diffusion Method. Gentamicin and Ketoconazole 10 mg/ml were used as standards. A different solvent was used to obtain CA leaf extract by using maceration technique. **Results:** %yield obtained for dried leaf extract of CA with chloroform, ethanol, acetone, petroleum ether and aqueous ethanol was approximately 15%, 18%, 09%, 11% and 24% respectively. Due to its high yield value hydroalcoholic extract of CA was used for estimating the antimicrobial activity and its phytochemical screening. Phytochemical screening of CA plant reveals the presence of Alkaloids, carbohydrates, flavonoids, steroids and tannins. **Conclusions:** The study demonstrates that the hydroalcoholic extract of CA leaf exhibit antibacterial activity on *Klebsiella pneumoniae*, *Pseudomonas sp.*, *Staphylococcus aureus* and antifungal activity among *Aspergillus niger*, *Aspergillus fumigates*, *Mucor species*. These recognized a good support to the use of this plant in herbal medicine and as base for the development of new drugs and phytomedicine.

### 1. Introduction

Today, Traditional structure of medicine is being practiced on many accounts. Medicinal plants are having great impact in the field of curing diseases and as a source of medicines for a wide variety of human ailments [1]. Several up to date research work and practical experience have shown that using medicinal plants is better than allopathic drugs by being safer besides having synergistic effect. Even though large number of medicinal plants is recognized by folklore system of medicine but their active constituents have not yet been investigated. Herbal medicines are prescribed widely even their biologically active constituents are unknown because of their fewer side effects relative low cost and effectiveness.

Human infections, particularly those involving the mucosal and skin surfaces, constitute a major problem, especially

in tropical and subtropical developing countries. These microorganism will cause various diseases such like *Staphylococcus aureus* (pneumonia, impetigo, cellulitis, scalded skin syndrome, mastitis, chorioamnionitis and neonatal sepsis), *Escherichia coli* (diarrhea), *Klebsiella pneumoniae* (pneumonia, thrombophlebitis, urinary tract infection, cholecystitis, diarrhea, upper respiratory tract infection, osteomyelitis, meningitis), *pseudomonas* (urinary tract infections, respiratory system infections, dermatitis, bacteremia, Pneumonia, Necrotising enterocolitis) *Aspergillus niger* and *Aspergillus fumigates* (Aspergillosis, Allergic Bronchopulmonary) and *mucor sp* (skin diseases) [2–4]

*Citrus aurantifolia* (CA) belong to the family Rutaceae and are used in traditional folklore structure of medicine to cure various diseases. *C.aurantifolia* is commonly called as key lime or bitter orange and its ayurvedic properties are Rasa, Guna and Virya. Scores of research studies have been previously done on the plant *Citrus aurantifolia* that exhibits bioactive activities for cold fevers, sore throats, sinusitis and bronchitis, as well as helping asthma. Lime oil is mainly used as antidepressant because it promotes refreshment to the

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tide mind. It can be helpful for rheumatism arthritis, obesity and cellulite and has an astringent and toning action to clear oily skin and acne, helps with herpes, cuts and insect bites [5–7]. During last two decades, the plant has been subjected to extensive Phytochemical, pharmacological and clinical investigations and many interesting findings in the areas of Insecticidal Activity [31], Cardiac diseases, anticancer, Eye conditions, Inflammatory Bowel Disease and Improved lung function [32].

In present study phytochemical screening and antimicrobial activity of hydroalcoholic extracts of leaves of *Citrus aurantifolia* Linn was studied.

## 2. Materials and methodology

### 2.1 Plant Material

The leaves of CA were collected from the city of Thanjavur, Tamil Nadu, India. The plant was identified and authenticated (BSI/SRC/5/23/2011–12/Tech–1053) by Dr. G.V.S.Murthy, Head of office, Tamil Nadu Agriculture University, Botanical Survey of India, Coimbatore, Tamil Nadu, India.

### 2.2 Preparation of Extracts

Collected leaves were cleaned and shade air dried. The dried leaves were pulverized by using electrical grinder and passed through a 20–mesh sieve. A powdered leaf (50 g) was successively extracted with Chloroform, ethanol, acetone, petroleum ether and hydro alcohol using cold maceration method[8]. The extraction was carried out for 72 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.

### 2.3 Test organisms

The test microorganisms used in this study were Bacteria: *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas* spp. and fungus: *Aspergillus niger*, *Aspergillus fumigates*, *Mucor* spp and *Penicillium*. The test organisms were clinical isolates and obtained from the Department of Microbiology, PRIST University, Thanjavur, Tamil Nadu, India.

### 2.4 Preliminary Phytochemical Screening

Hydroalcoholic extracts of CA plant (bark, stem, roots, peel and leaves) were subjected to preliminary phytochemical screening for the presence or absence of various active metabolites [9, 10].

### 2.5 Antibacterial activity

The antibacterial activity of the hydroalcoholic extract was determined in accordance with the agar–well diffusion method. The bacteria was first isolated and grown in a nutrient broth for 18 h before use and standardize the culture to 10<sup>6</sup>cfu/

ml. Mueller–Hinton agar (OXOID) was prepared and bored the wells into the agar using a sterile 4 mm diameter cork borer. 200  $\mu$ l of the standardized cell culture was spread on a MH agar. Approximately 100  $\mu$ l of the hydroalcoholic extract at 10 mg/ml–1 were introduced into the wells, allowed to stand at room temperature for about 2 h and then incubated at 37°C. After 24 h the plates were observed for zones of inhibition. The zone of inhibition was compared with that of control and standard Gentamicin at a concentration of 10  $\mu$  g/ml–1.[11–22]

### 2.6 Antifungal activity

The fungal organisms were first isolated and allowed to grow on a rose bengal agar (RBA) (OXOID) at 25°C for 72 h. The fungi were harvested after sporulation by pouring distilled water on the surface of the plate and later scraped the spores with a sterile glass rod. 100  $\mu$ l of the standardized fungal spore suspension was spread on the Potassium Dextrose Agar (PDA) using a glass spreader. Sterile 4 mm diameter of cork borer was used to bored wells into the PDA. Approximately 100  $\mu$ l of 10 mg *Citrus aurantifolia* extract were introduced into the wells and allowed to stand (1h) for proper diffusion of the extract into the media. The plates were observed for zones of inhibition after 72 h at 25°C and compared with ketocanazole at a concentration of 10 mg/ml.[23–30]

## 3. Results

Various parts of *Citrus aurantifolia* plant (leaves, stem, roots, bark and peel) were used to estimate the presence of active constituents. Preliminary phytochemical screening of Hydroalcoholic extracts of *Citrus aurantifolia* plant was shown in Table 1.

The leaves was extracted by using different solvents such like Chloroform, ethanol, Acetone, Petroleum ether and hydroalcohol and the obtained %yield value was 15%, 18%, 09%, 11% and 24% respectively. In this study hydroalcoholic leaf extract of CA posses' high solubility property and high % yield value hence it is used for evaluating its antimicrobial activity.

The obtained zone of inhibition indicates that hydro CA leaves exhibited in–vitro antibacterial activity against Gram–positive and Gram–negative organisms. Control group represents the diameter of sterile cork borer of 4 mm without any zone of inhibition. All the bacterial strains established some degree of sensitivity to the plant. Among the four organisms, the CA extract showed a higher activity on *Klebsiella pneumonia* and *Staphylococcus aureus*. (Table–2).

The obtained zone of inhibition indicates that CA leaves exhibited in–vitro antifungal activity against *Aspergillus niger*, *Aspergillus fumigates* and *Mucor* spp. Control group represents the diameter of sterile cork borer of 4 mm without any zone of inhibition. Hydro alcoholic extract of CA show antifungal activity on *Aspergillus niger*, *Aspergillus fumigates* and *Mucor* spp (Table 3). The results reveal that extracts of *C.aurantifolia* were significantly effective against *Mucor* spp.

**Table 1**phytochemical screening of Hydroalcoholic extract of *Citrus aurantifolia* Plant.

Phytochemical Test	Leaves	Roots	Stem	Bark	Peel
Carbohydrates ( Molisch's test)	+	+	+	+	+
Proteins (Trichloroacetic acid test)	–	–	–	–	–
Amino acids (Ninhydrine test)	–	–	–	–	–
Alkaloids ( Dragendorff,s reagent)	+	+	+	+	+
Flavonoids (Shinoda test)	+	–	–	+	–
Steroids (Liebermann–burchard test)	+	–	+	–	–
Triterpenoids (salkowski test)	–	+	–	+	+
Tannins (Ferric chloride test)	+	–	–	–	–
Cholesterol	–	–	–	–	–

(+) = Present (–) = Absent

**Table 2**Antibacterial activity of Hydroalcoholic extract of *Citrus aurantifolia* leaf.

Microorganism	Zone of Inhibition (mm)		
	Test samples		
	Control	CA	Gentamicin
Gram +ve/ Gram –ve			
<i>Staphylococcus aureus</i>	04	12	14
<i>Escherichia coli</i>	04	–	10
<i>Klebsiella pneumonia</i>	04	12	15
<i>Pseudomonas spp</i>	04	10	16

**Table 3**Antifungal activity of Hydroalcoholic extract of *Citrus aurantifolia* leaf.

Microorganism	Zone of Inhibition (mm)		
	Test samples		
	Control	CA	Ketoconazole
<i>Aspergillus niger</i>	04	10	15
<i>Aspergillus fumigates</i>	04	12	17
<i>Mucor spp</i>	04	16	21
<i>Pencillium</i>	04	–	18

#### 4. Discussion

Now a day's many research are focused on herbal medicines and their natural compounds. In traditional systems of medicines Citrus fruits having its own importance to treat various human ailments. Citrus juice is used as antidepressant, promoting resistance against various infections and famously used for scurvy disease which is caused due to the lack of Vitamin C. In the present study we evaluate the antibacterial and antifungal efficacy of *Citrus aurantifolia* leaves although these citrus plants are very likely to infestation by scale insects, whitefly and aphids. A different solvent was used to prepare CA extract by using maceration technique. Each solvent having its own capability to soluble various active components in it. Preliminary phytochemical screening of Hydro alcoholic leaf extract of *Citrus aurantifolia* reveals the presence of Carbohydrates, Alkaloids, Flavonoids, Steroids and Tannins. This is well known, since tannins and saponins are important plant metabolites which is majorly responsible for antimicrobial activity (33). Our results shows that CA leaf extract has antibacterial activity against *Klebsiella pneumonia*, *Pseudomonas sp*, *Staphylococcus aureus* and antifungal activity amongst *Aspergillus niger*, *Aspergillus fumigates*, *Mucor* species. The percentage bacterial inhibition of CA

leaf extract was obtained as *Staphylococcus aureus* (85.7%), *Klebsiella pneumonia* (80%) and *Pseudomonas spp* (62.5%) when compared to standard Gentamicin. The percentage fungal inhibition of CA leaf extract was obtained as *Aspergillus niger* (66.6%), *Aspergillus fumigates* (70.5) and *Mucor spp* (76.1%) when compared to standard Ketoconazole. Our result indicates that Hydroalcoholic leaf extract posse's strongest antibacterial activity specifically against *Staphylococcus aureus*.

#### 5. Conclusions

In the present study the results indicates that the hydro alcoholic extracts of *Citrus aurantifolia* leaves possess good antibacterial and antifungal activity, confirming the great possible of bioactive compounds and are useful for rationalizing the use of this plant in primary health care. The results suggest that the extract of *C.aurantifolia* were significantly effective against *Mucor spp* in case of fungi and showed a higher activity on *Klebsiella pneumonia* and *Staphylococcus aureus* amongst bacteria. In vivo information may be helpful in determining the actual potential usefulness of this plant for the handling of causal organisms of infectious diseases. Thus further work can be carried on the isolation procedure for finding out the exact

moiety responsible for the biological activity.

## Acknowledgement

The authors are gratefully thanks to Hon'ble Chancellor Prof. P.Murugesan, PRIST University, Thanjavur.

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