

The in vitro Antibacterial Activity of Citrus Aurantifolia var Acidica Fruits

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Abstract: In this study the in vitro antibacterial activity of Citrus aurantifolia fruits (CA) is being reported. Three extraction methods were tried as: Hot water (HWCA) cold water (CWCA), and alcoholic (ACA). The screening of Antibacterial activity (AA) was done by agar diffusion technique (ADT). Minimum inhibitory concentration (MIC) Studies were made by agar dilution technique (ADT). the AA and MIC studies were conducted on Muller –Hinton agar (MHA). The in vitro AA from highest to the lowest effective were as follows, ACA, CWCA, and HWCA respectively. The MICs were 3.125, 6.25, and 12.5 mg / ml for S. aureus, Proteus vulgaris, Pseudomonas aeruginosa and E.coli respectively K. pneumonia was unaffected by ACA, CWCA, and HWCA. Such finding may be due to capsule permeability masking effect. **Discussion:** In the present study the results indicates that ACA can be recommended on an in vitro basis as antibacterial agent in topical pharmaceutical preparation and as canned food preservative.

Keyword: Citrus aurantifolia, S. aureus, E.coli, ADT, antibacterial agent

1. Introduction

Botanical word consist of array of feed, poisonous, economic and medicinal plants (1-2). Among these plants Citrus aurantifolia var acidica (Nomi Busrah) was a subject of several investigation to uncover its biological potential. Watery extracts of this fruit showed a prostogen like effect (3) and the present study was at reporting its in vitro antibacterial effect. 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 pneumonia (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 skin diseases (4). The most important biologically active constituents of the C. aurantium fruits are phenethylamine alkaloids octopamine, synephrine, tyramine, N-methyltyramine and hordenine. It is rich in vitamin C, flavonoids and volatile oil. Synephrine is a primary synthesis compound with pharmacological activities such as vasoconstriction, elevation of blood pressure and relaxation of bronchial muscle. whose fruit extracts have been used for the treatment of various diseases such as gastrointestinal disorders, insomnia, head aches, cardiovascular diseases (5). 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 (6).

In present study antimicrobial activity of the three extraction: Hot water (HWCA) cold water (CWCA), and alcoholic (ACA) extracts of leaves of Citrus aurantifolia Linn was studied.

2. Materials & Methods

One, gram positive and four, gram negative clinical isolates were identified as in Baron *et al* (7). Watery hot and cold as well as alcoholic extracts were performed according to (8). Stock solutions of 150 mg/ml and their serial double dilutions were done.

Agar diffusion technique (ADT) was used for screening antibacterial activity (2,9).

Agar dilution technique (Adt) was conducted using Muller – Hinton agar plates in accordance with (10) to determine minimum inhibitory concentration (MIC).

3. Results & Discussion

Alcoholic control showed invitro antibacterial effect of up to 2mm inhibition zones (IZ) While saline control revealed null activity (table1).

Table 1: Judgment of the in vitro antibacterial activity of C.aurantifolia

Parameters	
Inhibition Zones (mm)	Conclusion
0 - 9	resistant
10 -30	Sensitive
0-2-	Control saline and alcoholic

The hot water extract (HWCA) gave inhibition zones IZ ranges from 2 to 10 mm, cold water (CWCA) extracts showed IZ ranges of 3 to 13 mm and alcoholic extracts (ACA) presents IZ ranges of 7 to 15 mm using ADT of different extracts showed different IZ size which may be an indication for different active component. The minimum inhibitory concentration MIC range were; 50. Thus, on summing up one may state:

- 1) The in vitro antibacterial activity from highest effective were as: ACA, CWCA then HWCA.
- 2) The ACA was effective against S. aureus, P. vulgaris, P. aeruginosa and E.coli with MIC of 3.125, 6.25, 6.25 & 12.5 mg/ml respectively 200 for HWCA, 25-75 for CWCA

and 3.125-100 mg/ml for ACA (table 3) *S.aureus* were sensitive to all test extracts *P.vulgaris* and *P.aeruginosa* as well as *E.coli* were sensitive to ACA alone. *K. pneumonia* was resistant to all test extracts. ACA was the most effective among the others. ACA can be an antibacterial agent (AA) in vitro effective against gram positive and gram negative bacteria (table 1,2,3). ACA on in vitro basis may be recommended so for as food preservative mouth wash and an ingredient in skin topical preparation (9)

- 3) *K. pneumonia* was resistant all test extract may be due to capsule.
- 4) ACA may be recommended on an in vitro basis in topical pharmaceutical preparation and as food preservative.

Table 2: The in vitro antibacterial screening mm inhibition zones (IZ) for different extract of *C.aurantifolia* var *acidica* (150mg/ml) against five types of bacteria

Bacteria	IZ		
	Hot water extract (HWCA)	Cold water extract (CWCA)	Alcoholic extract (ACA)
<i>S.aureus</i>	10	13	15
<i>E.coli</i>	3	3	7
<i>K.pneumonia</i>	4	3	10
<i>P.vulgaris</i>	6	6	12
<i>P.aeruginosa</i>	6	8	10

Table 3: Minimum inhibitory concentration (MIC) mg/ml for different extract of *C.aurantifolia* var *acidica* against five types of bacteria.

Bacteria	Hot water extract (HWCA)	Cold water extract (CWCA)	Alcoholic extract (ACA)
<i>S.aureus</i>	50	25	3.125
<i>E.coli</i>	75	50	12.5
<i>K.pneumonia</i>	200	125	100
<i>P.vulgaris</i>	100	50	6.25
<i>P.aeruginosa</i>	125	75	6.25

4. Conclusion

In the present study the results indicates that the extracts of *C.aurantifolia* leaves possess good great possible of bioactive compounds and are useful for rationalizing the use of this plant in primary health care and a good antibacterial and antifungal activity. The results suggest that the extract of *C.aurantifolia* were significantly effective against *S.aureus*, *P.vulgaris*, *P.aeruginosa* and *E.coli* as well as *K. pneumonia*. The in vivo experimental may be helpful in determining the actual potential usefulness of this plant for the handling of causal organisms of infectious diseases.

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