



EVALUATION OF ANTIBACTERIAL PROPERTIES OF FRUIT PEEL EXTRACTS OF *CITRUS KARNA* AGAINST HUMAN PATHOGENS

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ABSTRACT

The antibacterial activity of *Citrus karna* fruit peel extracts was evaluated on bacteria strains like *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*. Hexane, chloroform and methanol were used for extraction and the antibacterial activity was performed by agar well diffusion method. The results were compared with ampicillin, a commercial antibacterial agent. Methanolic extract shows the highest antibacterial activity against all the four bacterial strains, whereas hexane extract shows lowest antibacterial activity. Among the tested bacterial isolates *P. aeruginosa* was the most inhibited majorly with the methanol extract. The methanolic extract shows lowest (14mm) zone of inhibition against *B. subtilis*. The chloroform extract displayed inhibitory zones of between 12-15mm while the hexane extract exhibited inhibitory affinity between 11-13mm. The MIC of the plant extracts on the susceptible bacterial isolates was between 37-333 μ g/ml. Present study concludes that fruit peels of *Citrus karna* have a broad spectrum antibacterial activity against human pathogens.

KEYWORDS: Citrus karna fruit peel extracts, Antibacterial activity, zone of inhibition, MIC



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INTRODUCTION

Plants constitute an important source of drugs in modern medicine. Also, there is always a need for new antimicrobial compound because continuous and inappropriate use of antibiotics leads to the problem of microbial resistance. Therefore, it is necessary to search for new or novel compounds to develop new drugs, either synthetic or natural[1]. Also, because of the realization of health hazards and toxicity associated with indiscriminate use of synthetic drugs and antibiotics, there has been considerable resurgence in the use of herbs and herbal drugs all over the world. Recent researches suggest that medicinal plants increase the immune power of human body thereby preventing infection. These and various other advantages of herbal therapeutics over synthetic medicines has attracted the attention of researchers to study the various aspects of these drugs, their physiological impact on human health and their pharmacological and microbiological aspects[2]. During last decade intensive studies have been done with extracts and biologically active compounds isolated from plant species used for natural therapies or herbal medicine [3]. Antimicrobial properties of herbs, spices and their derivatives such as essential oils, extracts and decoctions have been established in the past three decades[4]. Secondary metabolites or phytochemicals such as phenols, flavonoids, alkaloids, terpenoids, and essential oils are proved to be responsible for the antimicrobial activity of plants. These secondary metabolites are not only essential for the plant itself, but play an important role in plant's defense system and give protection against pathogens and herbivores. There are many reports which suggest that secondary metabolites possess antimicrobial activity[5]. Phenol and polyphenol group of compounds consist of thousands of diverse molecules with heterogenous structure with the common feature of having one or more

phenol ring. Phenolic compounds are synthesized in plants by shikimic acid pathway. The site and numbers of hydroxyl groups on the phenol ring is related to their toxicity to microorganisms, hence increased hydroxylation results in increased toxicity. Several workers have reported that phenolic compounds such as gallic acid, coumarins, polyphenols, caffeic acid, cinnamic acid, pyrogallol, eugenol *etc.* show antimicrobial activity against virus, bacteria and fungi [6]. Increase in the emergence of new bacterial strains that are multi-resistant coupled with the non-availability and the high cost of new generation antibiotics have resulted in increase morbidity and mortality [7,8]. Thus, the aim of this study is to investigate the antibacterial activity of *Citrus karna* fruit peel extracts against four human pathogens.

MATERIALS AND METHODS

Plant Material

The fruits of *Citrus karna* were collected from the Horticulture fields of SHIATS, Allahabad in the month of January, 2013. The plant material was identified and authenticated in the post graduate department of Horticulture, SHIATS, Allahabad. The peels were removed manually and dried in sunlight. The dried peels were ground into a coarse powder in a mixer.

Preparation of extracts

50g of dried and powdered plant material were extracted first with 200 ml of hexane, followed by chloroform and then with methanol by using a soxhlet extractor for 5 h at a temperature not exceeding the boiling point of the solvents[9]. The extracts are filtered and then concentrated to dryness. Each extract was transferred to glass vials and kept at 4° C before use.



Figure 1
Extract preparation by Soxhlet apparatus.

Test organisms

The organisms used comprises of two gram-positive bacteria (*S. aureus* and *B. subtilis*) and two gram-negative bacteria (*E. coli* and *P. aeruginosa*). The test organisms were obtained from the Research Laboratory of Microbiology and Fermentation Technology SHIATS, Allahabad, India.

Test for purity of extract

The extract obtained was tested to ensure its purity by streaking it separately on to the sterile plates containing nutrient agar. The plate was incubated at 37°C for 24 hours and then examined for possible growth of contaminants, the absence of which confirms the purity of the test extract [10].

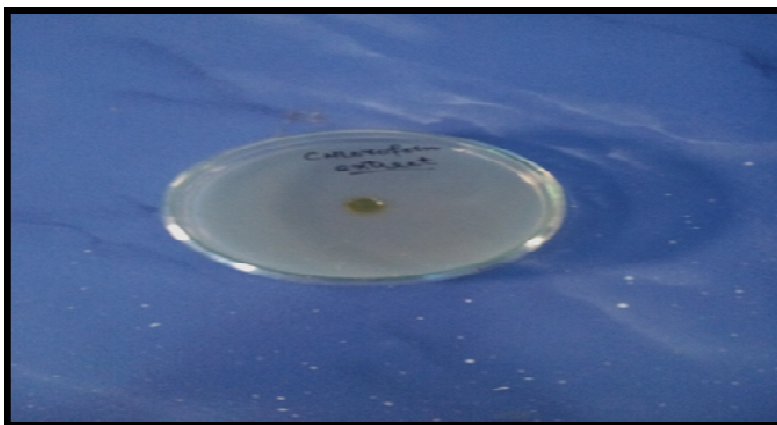


Figure 2
Showing purity of test extract.

Antibacterial assay

Antibiogram was done by agar well diffusion method [11, 12] using plant extracts and commonly used antibiotics. The test quantity of specific extracts was dissolved in dimethylsulphoxide (DMSO), depending upon the solubility of the extracts. The dissolution of the organic extracts was aided by 1% (v/v) DMSO, which did not affect the growth of

microorganisms, in accordance with our control experiments. The surface of media was inoculated with bacteria from a broth culture. Wells or cups of 5 mm size were made with sterile borer into agar plates containing the bacterial inoculums. 20µl volume of the sample extracts of concentration 2.0 mg/ml was poured into a well of inoculated plates. Ampicillin (10mg/ml) was used as a positive control which

was introduced into the well instead of plant extract. After 18 h of incubation at a specific temperature the plates were examined and the diameters of the inhibition zones were measured to the nearest millimeter. Antibacterial activity was recorded if the radius of zone of inhibition was greater than 4mm [13]. The antibacterial activity results were considered as inactive if < 4.5 mm; 4.5-6 mm as partially active ; while 6.5-9 mm as active and greater than 9mm as very active [14].

Minimum Inhibitory Concentration assay

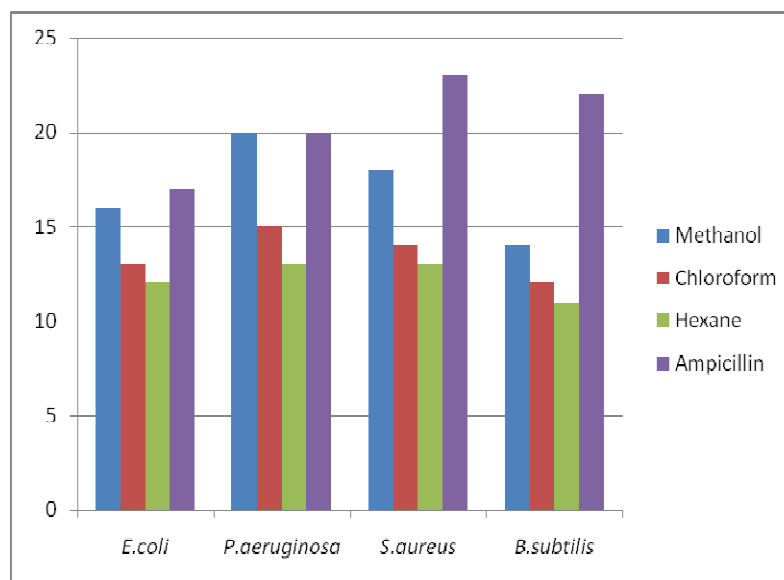
Minimum Inhibitory Concentration of fruit peel extracts was also determined [15, 16]. Dilution of the fruit peel extracts of *Citrus karna* was prepared in sterile nutrient broth to achieve a decreasing concentration ranging from 1000µg/ml to 12µg/ml in sterile tubes labeled 1 to 5. Each dilution was seeded with 10µl of the standardized bacterial inoculums (10^8 - 10^9) CFU/ml. The inoculated culture tubes were incubated at 37°C for 24hrs. A set of tubes containing only seed broth (*i.e.* without plant extract) was kept as control. The lowest dilution of the plant extract that retained its inhibitory effect resulting in no growth (absence of turbidity) of a microorganism was recorded as the MIC value of the extract. After incubation,

10µl of content of each test tube was transferred with a loop on to nutrient agar media. Agar plates were incubated for 24 hours at 37°C. The lower concentration that did not permit any visible growth when compared with the control was considered as the MIC.

RESULTS AND DISCUSSION

The antibacterial activity reveals that the methanolic extract of *Citrus karna* fruit peels is highly active against both Gram positive and Gram negative bacteria. The methanolic extract shows the highest zone of inhibition (20mm) against *P. aeruginosa* and lowest (14mm) zone of inhibition against *B.subtilis*. On the other hand hexane extract shows lowest antibacterial activity against both Gram positive and Gram negative bacteria as compared to other two extracts. Among the tested bacterial isolates *P. aeruginosa* was the most inhibited majorly with the methanol extract. The chloroform extract displayed inhibitory zones of between 12-15mm while the hexane extract exhibited inhibitory affinity between 11-13mm. Graph 1 summarizes the microbial growth inhibition of all the three extracts of *Citrus karna* fruit peels.

Graph 1
Antibacterial activity (mm) of fruit peel extracts of *Citrus karna*



*0 to 45 Zone of inhibition in mm; Borer size=5mm

Ampicillin used as a positive control showed wide zones of inhibition against all the test organisms while dimethyl sulphoxide (DMSO) negative control shows no zones of inhibition. It was completely resistant to all the test organisms. The MIC which is the lowest concentration of a plant extract that still retained

an inhibitory effect against the growth of a microorganism was assessed by using broth dilution method. The MIC values of various extract of *Citrus karna* fruit peels for different pathogenic bacteria ranged from (12 to 1000µg/ml) are shown in table 1.

Table 1
MIC values of *Citrus karna* peel extracts for different bacterial strains in µg/ml.

Bacterial Species	Methanol extract	Chloroform extract	Hexane extract
<i>E.coli</i>	111µg/ml	333µg/ml	333µg/ml
<i>P.aeruginosa</i>	37µg/ml	37µg/ml	111µg/ml
<i>S.aureus</i>	37µg/ml	111µg/ml	111µg/ml
<i>B.subtilis</i>	111µg/ml	333µg/ml	333 µg/ml

CONCLUSION

From the present investigation, it may be concluded that methanolic extract of *Citrus karna* fruit peels has potent antibacterial activity against both Gram positive and Gram negative bacteria. However, further studies are needed to isolate the active molecule responsible for antibacterial activity. The study thus may lead to the formulation of a potent antimicrobial agent from natural sources.

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