ORIGINAL ARTICLE

A Study of Antimicrobial Effects of Citrus Paradisi 'Grape Fruit'

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ABSTRACT

Objectives: In the current study, antimicrobial action of citrus paradisi grape fruit against common pathogens was evaluated. Staphylococcus aureus is a gram positive coccus, forming grape-like clusters on agar culture.

Methodology: Extracts were prepared with two different concentrations: 25 mg extract/1ml DMSO and 50 mg extract/1ml DMSO. The antibacterial activities were determined by using different concentrations of the prepared extracts. Mueller Hinton agar plates were carefully inoculated with 10 µl of bacterial spread, and plates were then properly labeled. The discs were placed on agar plates by means of a plain forceps. 20 µl of prepared extracts with concentration of 50 mg extract/1ml DMSO were added on the discs of half of the plates, while 10 µl of each extract was added on the discs of remaining plates. These plates were then incubated at 37oC for one day. Same procedure was repeated with 25 mg extract/1ml DMSO

Results: The extracts utilized against Bacillus Cereus were labeled as CF-04, CF-12, CF-13 and CF-14. Inhibition in CF-14 was observed to be highest, compared with other sections, showing the activity of ciprofloxacin to be effective in this culture.

Discussion: Current study evaluated the antioxidant and antimicrobial potential of extracts of different parts of citrus paradisi plant prepared in ethyl acetate solution. This study demonstrated the antimicrobial effects of parts of grape fruit plant against six common bacterial strains and highly positive zone of inhibitions were observed.

Conclusion: In all these prepared extracts, CF-10 showed maximum zone of inhibition. The prepared extracts utilized against K pneumoniae were CF-02, CF-04, CF-10 and CF-14, which were prepared from pulp, peel, leaves and stem of citrus paradisi respectively. In all these prepared extracts, CF-14 showed maximum zone of inhibition.

Introduction

Citrus Paradisi belongs to genus 'citrus', belonging to family Rutaceae plants. Grape fruit is a well-known fruit belonging to Rutaceae family of plants. Grape fruit is a large citrus fruit, having a yellow outer peel and a sour, ripened and thick pulp. Its tree is also lager than other citrus fruit trees, averaging about 16 to 20 feet, but can extend to 43 to 50 feet height. The leaves are shady, dull green, slightly long and lean. Although the fruit is yellow-peeled, but some fruits exhibit pink color of the peel. The pulp is quite sour and varies from yellowish-white to light or dark pink in appearance. [1]

Historically, citrus Paradisi fruits have been considered healthful, providing multiple health benefits. Citrus paradisi grape fruit contains a rich source of vitamin C (Hs Lee 1999). The pulp has abundant, insoluble pectin fiber (Cerda et al 1988) and the pink hue is rich in well-known potent antioxidant 'lycopene' (Lee 2000). Lycopene is a well-known protective agent against prostatic cancers and possesses a potential ability to reduce the growth of tumor cells (Cassileth 2010) Research study on benefits of citrus paradisi grape fruit juice documented that the juice extracts of grape fruit reduce plasma cholesterol levels in many individuals (Platt 2000). The seeds are also significant as they contain small amounts of antioxidants (Armando et al 1997). The citrus paradisi grape fruit seed extract has been considered a useful antimicrobial agent, with documented activity against bacteria and fungi both.

High content of vitamin C in citrus paradisi (grape fruits) exerts strong antioxidant effects on human metabolism and, it has been documented that they increase rapid recovery from common cold caused by variety of bacteria and viruses (Strohl 2009). A study conducted in 2007 highlighted an approximate 30% decrease in the risk of breast cancer in post-menopausal women. This benefit was observed to be due to inhibition of hepatic P-450 enzyme CYP34A responsible for metabolizing estrogen (Monroe et al 2007). Grape fruit has abundant β carotenes, which are powerful, natural antioxidants against development of GIT cancers and macular degeneration (J Fiedor 2014). The anti-inflammatory functions of citrus paradisi have been attributed to presence of flavanones in their fruits, which reduce blood cholesterol levels, thus reducing the risk of development of coronary artery diseases (JM Assini et al 2013)

There has been an increasing resistance of common bacteria to antibiotics now a days, due to extensive use of various antimicrobial drugs, owing to the genetic capability of acquiring resistance to these antibiotic drugs by gram positive and gram negative bacteria.

This brings in focus the antimicrobial properties of plant extracts containing bioactive constituents which can potentially kill the bacteria. Some well-known phytochemicals which are effective in bacterial killing are anthocyanins, polyphenols, tannins and carotenoids. Phytochemicals are potent plant-derived chemicals which help prevent chronic illnesses in human beings (Huaidong Du et al 2016).

Methodology

Preparation of extracts: Extracts were prepared with two different concentrations: 25 mg extract/1ml DMSO and 50 mg extract/1ml DMSO. Extracts of grape fruit were prepared by dipping about freshly obtained 700 grams of grape fruit pulp in 1000 ml of ethyl alcohol in a glass container. Dried leaf, stem and peel powders were also dipped in 1000 ml of ethyl alcohol separately. Each solution was finely filtered by utilizing Whatman's filter paper, ethyl alcohol gradually evaporated by rotary evaporators at 90 rpm at room temperature, and kept in separate flasks. The residues were collected in medium-sized pyrex falcon tubes.

Preparation of specific media: Mueller Hinton growth medium was used for bacteria under study in this research. This medium required meat infusions, starch, agar and casein acid hydrolysate mixture.

Growth of bacterial strains: MacConkey's agar was freshly prepared for growth of Staphylococcus aureus, E coli, Salmonella typhi, while EMB agar was used for growth of Klebsiella pneumoniae, Bacillus cereus and pseudomonas aeruginosa.

Preparation of DMSO solution: By using electric balance, 25 mg of all extracts were carefully weighed, added in glass Eppendorf tubes, and in each glass tube, 1ml of DMSO was added, put on vibrating vortex till a homogenous mixture was obtained. DMSO was used as a negative control in the current study.

Disc Diffusion technique for anti-bacterial activities in extracts: This technique is utilized for testing the sensitivity of various bacteria in specific growth medium and the resistant to specific antibiotic is observed. The antibacterial activities were determined by using different concentrations of the prepared extracts. Mueller Hinton agar plates were carefully inoculated with 10 μl of bacterial spread, and plates were then properly labeled. The discs were placed on agar plates by means of a plain forceps. 20 μl of prepared extracts with concentration of 50 mg extract/1ml DMSO were added on the discs of half of the plates, while 10 μl of each extract was added on the discs of remaining plates. These plates were then incubated at 37°C for one day. Same procedure was repeated with 25 mg extract/1ml DMSO

Anti-bacterial activity of prepared extracts using agar well-diffusion method: Mueller Hinton agar plates were freshly prepared and inoculated with 10 μl of bacterial spread on entire culture plates. By using a sterile, wooden cork, wells were punched within the agar, and 10 μl of plant extracts with concentration of 50 mg/DMSO 1ml, were added into the wells, and 20 μl of extracts with concentration of 50 mg/DMSO 1 ml was added in the well of remaining half plates. These plates were then placed in an incubator for one day at 37°C.

For detection of active phytochemicals in plant extracts, biochemical tests employed were Molish's test, Ninhydrin test, Alkaline reagent test, Froth's test, Salkowski test and Keller Killians test.

Results

The extracts utilized against staphylococcus aureus were labeled as CF-04, CF-12, CF-13 and CF-14. Inhibition in CF-14 was observed to be highest, compared with other sections, showing the activity of ciprofloxacin to be effective in this culture. The extracts utilized against E coli were CF-04, CF-12, CF-13 and CF-14 and it was observed that CF-12 showed highest inhibition, the region of ciprofloxacin, compared with other parts of culture. The extracts utilized against Klebsiella pneumoniae were labeled as CF-04, CF-12, CF-13 and CF-14. Inhibition in CF-14 was observed to be highest, compared with

other sections, showing the activity of ciprofloxacin to be effective in this culture. Similarly, the extracts utilized against Klebsiella pneumoniae were labeled as CF-04, CF-12, CF-13 and CF-14. Inhibition in CF-14 was observed to be highest, compared with other sections, showing the activity of ciprofloxacin to be effective in this culture. The extracts utilized against Pseudomonas aeruginosa were labeled as CF-04, CF-12, CF-13 and CF-14. Inhibition in CF-12 was observed to be highest, compared with other sections, showing the activity of ciprofloxacin to be effective in this culture. The extracts utilized against Bacillus Cereus were labeled as CF-04, CF-12, CF-13 and CF-14. Inhibition in CF-14 was observed to be highest, compared with other sections, showing the activity of ciprofloxacin to be effective in this culture.

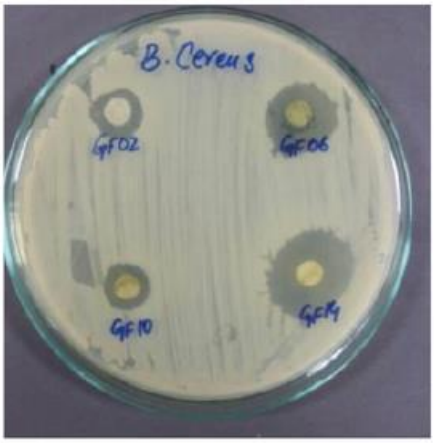
Table I: Anti-bacterial activity of prepared citrus paradise extracts

Bacterial strains	**Prepared extracts**					
10 µl	**20 µl extracts**					
	Positive Control	**Negative Control**	**CF-04**	**CF-12**	**CF-13**	**CF-14**
Staph aureus	30 mm	0 mm	18	26	28	30
Salmonella typhi	20 mm	0 mm	14	28	30	32
Bacillus cereus	18 mm	0 mm	12	18	12	18
E coli	20 mm	0 mm	14	26	22	28
P aeruginosa	02 mm	0 mm	20	32	34	36
K pneumonia	20 mm	0 mm	18	30	26	34

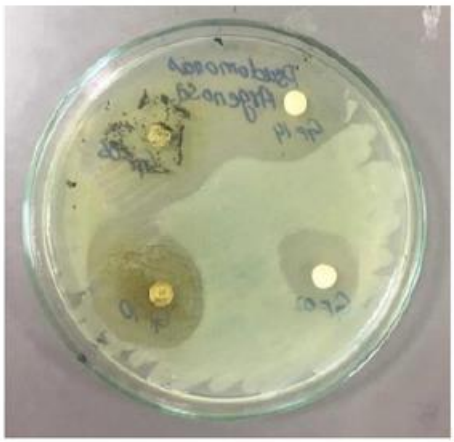
Bacterial strain	Extracts					
(10µl)	25mg/1mL DMSO with 10µl extract					
	Positive control	Negative control	GF 02	GF 06	GF10	GF14
S. aureus	35mm	0mm	20	30	25	32
S. typhi	21mm	0mm	16	30	32	37
B. cereus	20mm	0mm	11	14	16	14
E. coli	21mm	0mm	11	27	20	25
P. aeruginosa	0mm	0mm	21	38	32	37
K. pneumonia	21mm	0mm	19	31	28	36

Bacterial strain	Extracts					
(10µl)	25mg/1mL DMSO with 10µl extract					
	Positive control	Negative control	GF 02	GF 06	GF10	GF14
S. aureus	35mm	0mm	20	30	25	32
S. typhi	21mm	0mm	16	30	32	37
B. cereus	20mm	0mm	11	14	16	14
E. coli	21mm	0mm	11	27	20	25
P. aeruginosa	0mm	0mm	21	38	32	37
K. pneumonia	21mm	0mm	19	31	28	36

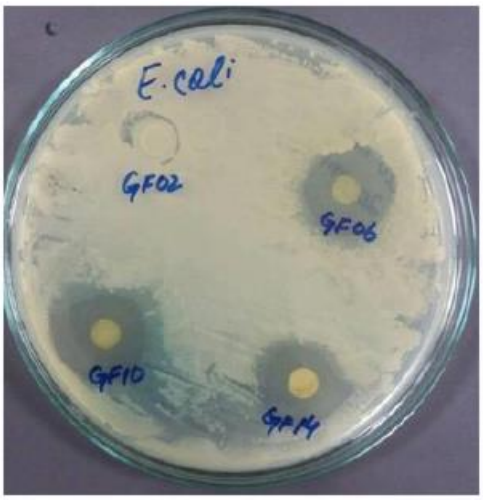
Bacterial strain (10µl)	Extracts (25mg/1mL DMSO with 10µl extract)					
	Positive control	Negative control	GF 02	GF 04	GF10	GF16
S. aureus	35mm	0mm	13	8	17	13
S. typhi	21mm	0mm	0	0	11	13
B. cereus	20mm	0mm	0	0	16	9
E. coli	21mm	0mm	0	0	10	11
P. aeruginosa	0mm	0mm	0	0	0	0
K. pneumonia	21mm	0mm	0	14	8	11



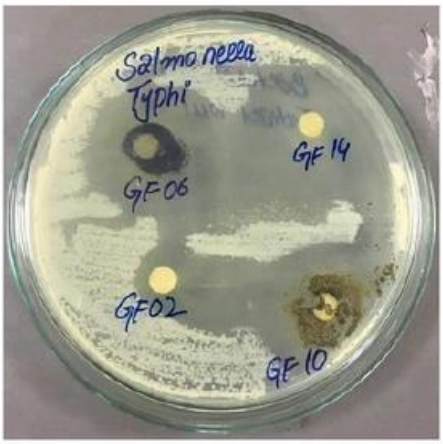
Antibacterial activity of *Citrus paradisi* against B. cereu



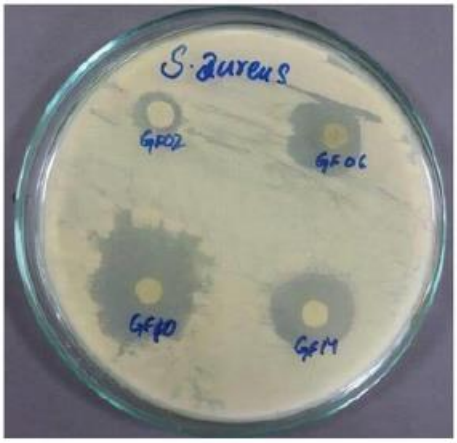
Antibacterial activity of *Citrus paradisi* against *P. ae*



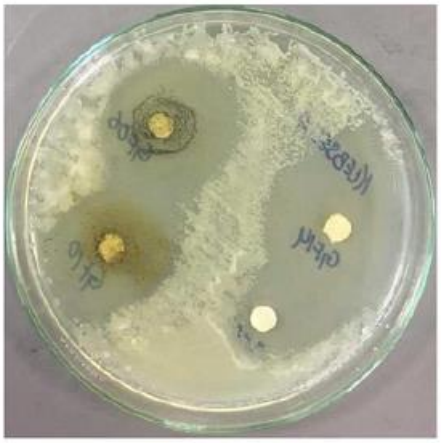
Antibacterial activity of *Citrus paradisi* against *E. coli*



Antibacterial activity of *Citrus paradisi* against *S. ty*



Antibacterial activity of *Citrus paradisi* against *S. aureus*



Antibacterial activity of *Citrus paradisi* against *K. p*

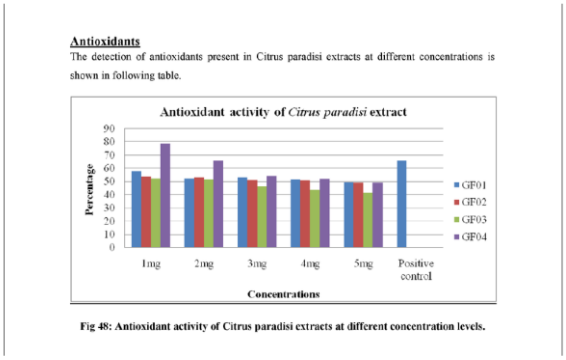


Fig 48: Antioxidant activity of Citrus paradisi extracts at different concentration levels.

Discussion

Regular consumption of grape fruit helps maintain healthy life-styles. Citrus paradisi grape fruit has low calories, beneficial for health due to abundant ascorbic acid (vitamin C) content and plenty of potassium and dietary fibers (Stump 2006). Current study evaluated the antioxidant and antimicrobial potential of extracts of different parts of citrus paradisi plant prepared in ethyl acetate solution. This study demonstrated the antimicrobial effects of parts of grape fruit plant against six common bacterial strains and highly positive zone of inhibitions were observed. A separate study conducted on the antimicrobial effects of grape fruit seed extracts documented that purified seed extracts of grape fruit showed effective antibacterial activity against both gram positive and gram negative bacteria. These seed extracts were non-toxic to human tissue cells and killed the bacteria by opening the cell wall components and liberating the cytoplasmic contents of bacteria, even at dilute concentrations of the seed extracts (John P Heggers et al 2004). Grape fruit seed extracts significantly reduced the population of human pathogens such as Salmonella typhi (Wentao Zu et al 2007).

Conclusion

Overall, the current study has highlighted the presence of antimicrobial agents in different parts of Citrus paradisi grape fruit plant, which can be used effectively in a variety of antibiotics for prevention of acute and chronic bacterial infections. More details study is needed to check the effectiveness of these antimicrobial activities on clinical stage.

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