ORIGINAL ARTICLE

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

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ABSTRACT

Background: Artocarpuslakoocha (A. Lakoocha) is an important medicinal plant which possesses vital and beneficial medicinal values such as steroids, alkaloids, flavonoids and a variety of phenolic derivatives. A. Lakoocha extracts have been clinically utilized in treatment of various chronic, inflammatory disorders such as rheumatoid arthritis, tuberculosis, ulcerative colitis and endocarditis.

Aim: To evaluate the anti-inflammatory effects of A. lakoocha extracts on the expression of atherogenic, pro-inflammatory cytokines IL-5, IL-6, TNF-α and NF-κB, in hyperlipidemia associated atherosclerosis, using rat models.

Methods: Twenty four well-fed rats (Wistar breed), were selected for the current study, weighing average 150-250 grams each and divided into three groups; GROUP I served as healthy control group, fed with normal diet composed of carbohydrates, proteins and fats. GROUP II was hyperlipidemic rats, with atherosclerosis, and considered as disease group..GROUP III rats were fed with extracts of A. lakoocha, extending from day 20 to 60 days.

Results: In the disease group, significantly decreased (P <0.001) expression levels of IL-5 were found in (14.76±2.65) compared with control group (26.54±3.98). IN the group treated with A. lakoocha extracts (22.01±4.53) significantly enhanced (P <0.01) the expression levels of IL-5 were observed. In addition, plasma IL-6 levels were significantly enhanced (P <0.05) in the disease group (44.19±10.90) compared with the control group (32.59 ± 4.51). Treatment of rats with A. lakoocha extract (20.22 ± 2.75) caused significant reduction (P<0.001) in the expression levels of IL-6 as compared with the disease group.Significantly raised (P<0.01) expression levels of TNF-α within the disease group (31.78±4.71) compared with the control group (23.34±2.25) group.

Conclusion: Citrus paradisi grape fruit plant has presence of antimicrobial agents in different parts which can be used effectively in a variety of antibiotics for prevention of acute and chronic bacterial infections

Keywords: Citrus paradise, antimicrobial effect, Artocarpuslakoocha

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 Paradisifruits have been considered healthful, providing multiple health benefits. Citrus paradisigrape fruit contains a rich source of vitamin C [2]. The pulp has abundant, insoluble pectin fiber[3] and the pink hue is rich in well-known potent antioxidant'lycopene'[4]. Lycopene is a well-known protective agent against prostatic cancers and possesses a potential ability to reduce the growth of tumor cells [5]Research study on benefits of citrus paradisigrape fruit juice documented that the juice extracts of grape fruit reduce plasma cholesterol levels in many individuals[6]. The seeds are also significant as they contain small amounts of antioxidants [7].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 antioxidants effects on human metabolism and, it has been documented that they increase rapid recovery from common cold caused by variety of bacteria and viruses[8]. 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[9]. Grape fruit has abundant β carotenes, which are powerful, natural antioxidants against development of GIT cancers and macular degeneration [10]. The anti-inflammatory functions of citrus paradise 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[11].

There has been an increasing resistance of common bacteria to antibioticsnow 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 [12]. 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. It is a normal commensal of nasal flora, and known to cause wide range of toxin mediated and pyogenic diseases in human beings, such as toxic shock syndrome, food poisoning, skin infections including abscesses, carbuncles, cellulitis and impetigo. The toxic shock syndrome toxin enters the blood stream and causes septicemia. Moreover, exfoliatin toxin produced by staphylococcus aureus causes scalded skin syndrome in children leading to separation of epidermal skin layer from the granular layer[13].

Bacillus cereus is a spore-forming bacillus, producing a toxin causing food poisoning following the ingestion of toxin through meat products, milk and vegetable curries. Another toxin acts centrally and leads to persistent vomiting in affected individuals following the ingestion of poultryproducts [14]. E coli is a gram negative rod, an enteric pathogen, considered to be

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the most common cause of urinary tract infection.It is also the most abundant commensal in colon. E coli produces an enterotoxin leading to watery diarrhea, The Shiga toxin produced by E coli may cause blood-tinged diarrhea in adults. This toxin when enters the blood stream leads to life-threatening 'Hemolytic-uremic syndrome' characterized by hemolytic anemia, thrombocytopenia and early death due to acute renal shut down. The uropathic strains of E coli are typically motile and cause urinary tract infections, ascending from ureters up to kidneys, leading to fever, chills, dysuria, flank pains and urinary frequency. The swarming property of E coli resembles the motility of proteus species on agar cultures[15]. Salmonella typhi is a gram negative enteric pathogens, which upon entry in the gastro-intestinal tract, invadesepithelial and sub-epithelial layers of both small and large intestines, causing inflammation and blood-tinged diarrhea. These organisms proliferate in the phagocytes in the payers' patches of small intestines, may disseminate to liver, spleen and cause septicemia. Typhoid fever is a well-known, common infective disease characterized by fever, constipation, leucopenia and typical 'rose spots' on the skin. E coli which do not ferment lactose on nutrient cultures thus forming colorless colonies on MacConkey's agar. E coli are non-spore forming facultative anaerobes on culture medium[16].

Klebsiella pneumoniaeare gram negative rods possessing an anti-phagocytic capsule, causing resistant pneumonia and urinary tract infections in both adults and children. These organisms are lactose fermenters thus producing colored colonies on MacConkey's agar. Klebsiella express two important cell-surface antigens; O antigen and K antigen; both contributing to the pathogenecity of these organisms [17]. Pseudomonas aeruginosa are a gram negative, strictly aerobe rods, causing chronic respiratory tract infections, and cellulitis in patients of burns[18].

MATERIALS AND METHODS

Preparation of extracts: Extracts were prepared with two different concentrations: 25 mg extract/1ml DMSO and 50mg 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 rpmat 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, 25mg of all extracts were carefully weighed, added in glass eppendroff 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-ve 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.

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, woodencork, 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 placedin an incubator for one day at 37^{o}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 1: Anti-bacterial activity of prepared citrus paradise extracts

Bacterial strains 10 µl	**Prepared extracts (20 µl extracts)**					
	+Ve Control	**-Ve 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

Table 2: Antibacterial activity of citrus paradise extracts

Bacterial strain(10µl)	Extracts (25mg/1ml DMSO with 10µl extracts)					
	+Ve Control	-Ve Control	GG 02	GF06	GF10	GF16
S aureus	35mm	0	20	30	25	32
S. typhi	21mm	0	16	30	32	37
B cereus	20mm	0	11	14	16	14
E coli	21mm	0	11	27	20	25
P aeriginosa	0	0	21	38	32	37
K pneumonia	21mm	0	19	31	28	36

Table 3: Antibacterial activity of citrus paradise extracts

Bacterial strain(10µl)	Extracts (25mg/1ml DMSO with 10µlextracts)					
	+Ve Control	-Ve Control	GG 02	GF04	GF10	GF16
S aureus	35mm	0	13	8	17	13
S. typhi	21mm	0	0	0	11	13
B cereus	20mm	0	0	0	16	9
E coli	21mm	0	0	0	10	11
P aeriginosa	0	0	0	0	0	0
K pneumonia	21mm	0	0	14	8	11

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[19]. Current study evaluated the antioxidant and antimicrobial potential of extracts of different parts of citrus paradisi plant preparedin 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 [20].Grape fruit seed extracts significantly reduced the population of human pathogens such as Salmonella typhi[21].

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

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