# Antimicrobial activity of grape fruit seed crude extract on microbial growth

***Hanna Salem Yossef****

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## Abstract

Antimicrobial and antiyeast activity of ethanolic and aqueous extract of grape fruit seed (*Citrus paradise* ; *Rutaceaa*) was examined against 10 bacterial and 2 yeast strains. The level of the antimicrobial effects was established using an in vitro agar assay and minimum inhibitory concentration (MIC). In general ethanolic extract were more effective on gram positive bacteria than gram negative bacteria and strongest antimicrobial effect against *Streptococcus pyogenes* and *Salmonella entritidis*.

Other tested bacteria and yeasts were sensitive to extract ranging from 4 to 16 mg/ml and more.

**Key words: Grapefruit, antimicrobial, bacteria**

## Introduction

Grape fruit seed extract (GSE) is reported to have powerful antimicrobial activity. However, only a few scientific reports of antibacterial and antifungal in vitro effects could be found in the literature. These studies mostly deal with the preservation of vegetable and fruit[1], peanuts [2,3] beef [4] and chicken [5].An in vitro related to the GSE activity on the intestinal micro flora. The antibacterial efficacy mechanism of action and in vitro toxicity of a commercial GSE investigated recently [6,7]. It has been found that the extract disrupts bacterial membrane and librates the cytoplasmic content within 15 minutes. The latest in vitro investigation showed that the commercial 33% grape fruit water glycerol solution exerted potent antifungal activity against the yeast-like fungi strains and lower activity against dermatophytes and mold [8]. Our study contributes to identification of the antimicrobial and antifungal effects of the self-made ethanolic and aqueous extract of grape fruit seed.

**Preparation of grape fruit seed extract**

Ethanolic and aqueous extract of grape fruit (*Citrus paradise* ; *Rutaceaa*) was prepared from commercially available grape fruits, air dried powdered seed (20gm/100ml of water) aqueous solutions, (20 gm/100ml of 70% ethanol). Ethanolic solution prepared in soxhlet apparatus for 6hrs. After cooling the solvent was removed using rotary evaporator and dry residue was for microbial test. Sterilization of grape fruit extract was done by Millipore filter paper size 0.2µ.

**Microbial strains**

Microbial local strains from different clinical specement were used. (College of Science, Biology Department, Baghdad University).

**Antimicrobial activity**

Antimicrobial activity was based on the agar diffusion method and minimum inhibitory concentration values (MIC). The agar diffusion

*Department of Biology/ college of Science/ Baghdad University

method was performed according to [9]. Testing inoculums with $10^4$-$10^5$ cells (0.1 ml portion) was swabbed on solidified Muller Hinton Agar (Merck) for bacteria and on Sabouraud dextrose agar for yeast. 5 microliter of test solutions were applied in 5mm diameter holes. The same volume of 70% of ethanol was tested as control.

The diameter of the clear zone (inhibition zone) around the hole was measured. Minimal inhibitory concentration (MIC) values were evaluated by the dilution susceptibility test [10]. Test strains were grown in a nutrient broth containing progressively dilution of test extract (1,2,4,8,16,32 mg/ml) and incubated at 37 ˚C for yeast. Last two tubes were free of test extract and served as a growth control in broth and 70% ethanol. The lowest concentration of extract which prevent growth (tube without turbidity) was considered to be MIC.

## Results and Discussion:

**Table (1) The effect of ethanolic GSE on microbial growth.**

| Microorganism | Inhibition zone (mm) (10mg/hole) | MIC mg/ml |
|---|---|---|
| *Staphylococcus aureus* | 13 | 8 |
| *Staphylococcus epidermidis* | 12 | 8 |
| *Streptococcus pyogens* | 17 | 8 |
| *Streptococcus faecalis* | 14 | 4 |
| *Bacillus cereus* | 13 | 8 |
| *Salmonella typhi* | - | 4 |
| *Salmonella entritidis* | - | 2 |
| *Escherichia coli* | - | 16 |
| *Klebsiella sp.* | - | 8 |
| *Pseudomonas aeruginosa* | - | 16 |
| *Candida albicans* strain1 | 10 | 8 |
| *Candida albicans* strain 2 | 11 | 8 |

**Table (2) The effect of aqueous GSE on microbial growth.**

| Microorganism | Inhibition zone (mm) (10mg/hole) | MIC mg/ml |
|---|---|---|
| *Staphylococcus aureus* | 9 | 16 |
| *Staphylococcus epidermidis* | 9 | 16 |
| *Streptococcus pyogens* | 14 | 4 |
| *Streptococcus faecalis* | 11 | 4 |
| *Bacillus cereus* | 12 | 8 |
| *Salmonella typhi* | - | 4 |
| *Salmonella entritidis* | - | 4 |
| *Escherichia coli* | - | 16 |
| *Klebsiella sp.* | - | 16 |
| *Pseudomonas aeruginosa* | - | More than 16 |
| *Candida albicans* strain 1 | 8 | 8 |
| *Candida albicans* strain 2 | 8 | 8 |

It has been established the fully natural ethanolic and aqueous extract of grape fruit seed affects the tested bacteria and yeast remarkably, but exerts less antimicrobial in the literature. These differences may be differ according to the extraction methods and solvent which used in. Our study showed the antimicrobial efficacy of grape fruit extract both in water and in ethanol compared with control which has no effect, but it was more effect with 70% alcohol extraction than in water, may be this return to flavonids content which affected by solvents [11].

This investigation showed that the grape fruit seed extract was active against all gram positive bacteria, but exerted no inhibiting effect on the growth of the tested gram negative bacteria when we use agar plate (Table 1). GSE exhibited the largest zone of inhibition for *Streptococcus pyogenes*

(17mm) and *Streptococcus feacalis* (14mm). GSE showed low activity against the tested strain of *Candida albican* (inhibition zone, 8mm). Grape fruit seed extract contents like flavonids can cause cell membrane disruption and Librates the cytoplasmic contents and cause cell death.

Table (2) showed the antimicrobial effect of aqueous GSE but less effect than ethanolic extraction, The largest inhibition zone for *Streptococcus pyogenes* (14mm) while the highest MIC value was *for Pseudomonas aeruginosa* more than (16mg/ml) the difference of antimicrobial effect between ethanolic and aqueous may be return to solvent which used in extraction.

Our result showed clear differences between antimicrobial effects of self-made GSE and some commercially available preparations reported previously [8,12]. Despite the fact that commercial extracts were formed to be superior to the tested self-made GSE activity against all the tested bacteria and yeast.

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# الفعالية المضادة للجراثيم لمستخلص بذور نبات الكريب فروت (*Citrus paradise*) على نمو الجراثيم

***هناء سليم يوسف****

*قسم علوم الحياة / كلية العلوم/ جامعة بغداد.

**الكلمات المفتاحية :كريب فروت ،مضادات المكروبات ،بكتريا.**

**الخلاصة:**

اختبر لمستخلص الكحولي و المائي لبذور نبات الكريب فروت (*Citrus paradisi*) ضد عشر عزلات من البكتريا و عزلتان من الخمائر. الفعالية المضادة للجراثيم تمت دراستها بطريقة استعمال اطباق الأكار و التركيز المثبط الادنى MIC. بشكل عام وجد ان كلا المستخلصين المائيو الكحولي له تأثير مضاد للجراثيم و ان تأثيره على البكتريا الموجبة لصبغة كرام و قد وجد ان اقوى تأثير هو على بكتريا *Streptococcus pyogenes* و *Salmonella entritidis* و قد وجد ان البكتريا الأخرى و الخمائر هي ايضاً حساسة لتأثير المستخلص بمدى 4mg/ml الى 16mg/ml او اكثر.