



Antimicrobial Effect of Black Grape Seed Extract

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Abstract:

Grape is a phenolics rich plants, Black Grape seed extract was reported to have many pharmlologically benefits including antioxidant, antimicrobial, anticancer and anti aging and anti Alzheimer's properties. Black grape seed phenols were extracted by percolation method. Results showed that the minimum inhibitory concentration of phenolic extract had no effect against *K.pneuminae*, However, variable inhibitory effects were developed against pathogenic *P.aeruginosa*, *E.coli*, *Bacillus*, *S.aureus*, *E.faecalis* and *C.albicans*. We found that black grape seed extract (*Vitis vinifera*) was more effective against gram positive bacteria than gram negative bacteria and its effect also against pathogenic *C.albicans*.

Keyword: Black grape seed extract, Antimicrobial activities, MIC.

التأثير ضد المايكروبي لمستخلص بذور العنب الاسود

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الخلاصة:

يمتاز العنب بكونه من النباتات الغنية بالفينولات ، عرف مستخلص بذور العنب الاسود بامتلاكه العديد من الفوائد الصيدلانية منها : التأثير المضاد للاكسدة ، التأثير ضد المايكروبي ، التأثير ضد السرطان ، التأثير ضد الشريخوخة ، التأثير ضد فقدان الذاكرة . تم أستخلاص فينولات بذور العنب الاسود بطريقة السكسوليت . اظهرت النتائج بأن MIC لمستخلص بذور العنب الاسود لا يملك اي تأثير ضد بكتيريا *K.pneuminae* ، في حين أظهر العديد من التأثيرات ضد كل من بكتيريا *P.aeruginosa* ، بكتيريا *E.coli* ، بكتيريا *Bacillus* ، بكتيريا *S.aureus* ، بكتيريا *E.faecalis* و خميرة *C.albicans* . لقد وجدنا ان مستخلص بذور العنب الاسود له تأثير أقوى ضد البكتيريا الموجبة لصبغة غرام مقارنة بالبكتيريا السالبة لصبغة غرام ، كما يمتلك تأثير مضاد لخميرة *C. albicans* الممرضة .

Intrudction:

In the past decade interest on the topic of antimicrobial plant extracts has been growing [1].Plants used for traditional medicine contain awide range of substaneous that can be used to treat chronic as well as infectious diseases. The medical values of plants lie in some chemical substances that produce a definite physiological action on the human body [2].Substantial attention is presently focused on the potential health effects of grapes and grape products [3]. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds [2].Grape fruit contains various nutrient elements such as: vitamins, minerals, carbohydrates, edible fibers and phytochemicals. Polyphenols are the most important phytochemicals in grape because they posses

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many biological activities and health promoting benefits [4]. Grape is a phenol-rich plant [4, 5] and these phenolics are mainly distributed in the skin, stem, leaf and seed of grape rather than their juicy middle section [4]. Polyphenols grape seeds are mainly flavonoids [5]. Most phenolics in muscadines are located in the seeds, gallic acid, catechin and epicatechin are the main phenolic founds in muscadine seed [2,5]. Grape seed extract(GSE) is reported to have many pharmacological benefits including: antioxidant, anti-inflammatory, anticarcinogenic and antimicrobial properties, slowing aging, Alzheimer's disease and other neurodegenerative disorders and diabetes [6-8]. Also GSE shows promise for application in the food industry as an inexpensive novel natural alternative to reduce viral contamination and enhance food safety [3, 7, 9, 10]. Also grape peel were found to exert antimicrobial activities on 11 bacterial species associated with Catfish *Clarias gariepinus* spoilage [11]. GSE high in proanthocyanidins, positively affected the *in vitro* demineralization and / or remineralization processes of artificial root caries lesions, suggesting its potential as a promising natural agent for noninvasive root caries therapy [1,12]. The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants [2]. Since this study considered as one of the first studies in Iraq to test the natural antimicrobial activity of black GSE, the objective of this study were to investigate the effect of GSE against different microbial strains, the antimicrobial activities determined by MIC and HPLC for GSE.

Method:

Samples of Black Grapes: Black grapes (*Vitis vinifera*) were collected from supermarket in May 2012, Grape berry seeds (11gm) were used in this experiment.

Extraction: Washed and dried grape seeds were powdered and extracted in a Soxhlet extractor with hexane (6 hours) for removal of the fatty acid matter (seed hexane extract), then defatted seed powder was extracted for (10 hours) with methanol (seed methanol extract), oven dried, then convert it to a powder form.

Microbial Strains:

Microorganisms: *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Candida albicans* were obtained from Biology department (pathogenic strains), College of Science, University of Baghdad. The microorganisms were grown in Brain Heart Infusion broth (Himedia) at 37°C for 18 hrs. The turbidity of the growth were compared with McFarland standard (tube no. 0.5) to reach approximately 1.5×10^8 CFU/ml.

Determination of Microbial Activities:

The antimicrobial activities were determined by agar diffusion method as described by [13]. Prepared a serial dilution of GSE dissolved in methanol, analyzed were prepared to final concentration of (500, 250, 200, 150, 100, 50, 25, 12.5) µg/ml. The microbial inoculums (prepared as described previously) was uniformly spread using sterile cotton swab on a sterile Mueller Hinton agar (Himedia), added 50 µl of serial dilution (500, 250, 200) µg/ml of GSE to the wells (6 mm diameter holes, 20 mm apart from one another), Methanol was considered as control, incubated for 24 hrs at 37°C, under aerobic conditions. Results was appear as inhibition of microbial growth around the well and measured in mm.

Minimum Inhibitory Concentration (MIC):

The MIC were determined by a liquid micro dilution method as described by [2]. The microbial inoculums (prepared as described previously). Used a serial dilution of GSE (1000, 500, 250, 200, 150, 100, 50, 25, 12.5) µg/ml dissolved in methanol (prepared as described previously), to each well added 50 µl of microbial inoculums. The MIC defined as: The lowest concentration of GSE which inhibited the visible growth after incubation for 18 hrs at 37 °C.

Chemical Analysis (High Pressure Liquid Chromatography):

The sample hydrolysate were separated on FLC (Fast Liquid Chromatographic) column, 3 µm particle size (50 × 4.6 mm I.D.) C-18DB column, Mobile phase were 0.1% TFA:acetonitrile (55:45 V/V), detection UV set at 280 nm and flow rate was 1.5 ml/min.

Results and Discussion:

Chemical analysis of GSE:

The HPLC analysis were applied out and the peaks of GSE figure-1, were identified and quantified by comparison with standards figure-2, and their amounts were also calculated as well table-1.

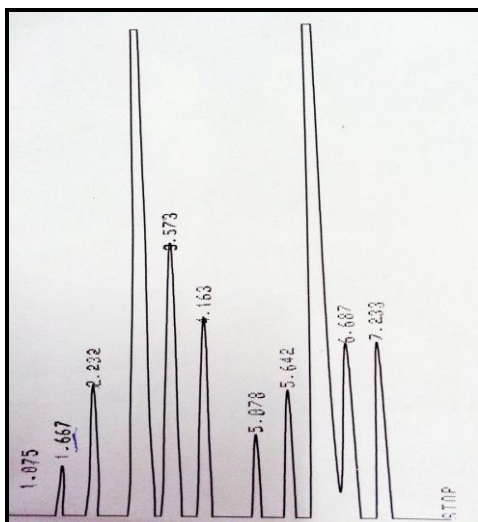


Figure 1- HPLC profile of GSE

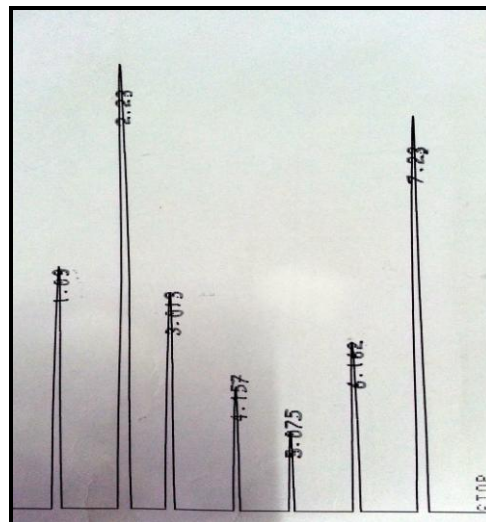


Figure 2- HPLC profile of standards

The HPLC showed that GSE was rich in polyphenolic compounds (Flavonoid): Epicatechin, Catechin, ProcyanidinB2, Epigallocatechin, Procyanidin, ProcyanidinB1, Gallic acid in different concentrations table-1.

Table 1-The types and concentrations of HPLC analysis for GSE

| Phenolic compound | $\mu\text{g/ml}$ |
|-------------------|------------------|
| Gallic acid | 39.41 |
| ProcyanidinB1 | 63,9 |
| Procyanidin | 71.96 |
| Epigallocatechin | 73.77 |
| ProcyanidinB2 | 123.67 |
| Catechin | 193.67 |
| Epicatechin | 220.76 |

The highest phenolic compound concentrations were : Epicatechin, Catechin and Procyanidin 2 respectively .This result agree with other researchers , they showed that the GSE rich in polyphenolic compounds .Flavonoids are widely distrusted in grapes, especially in seeds and stems, and principally contain (+)-catechin, (-)-epicatechin and procyanidin polymers [5,7,14,15]. [4,16] showed that Catechin and epicatechin had antibacterial activity,Acordingly , the antimicrobial activity of GSE could be attributed to these compounds .

Determination of minimum inhibitory concentration (MIC):

The result showed that GSE was more effective against G+ve bacteria than G-ve bacteria table-2, which may be due to the complexity composition of G-ve bacteria.

Table 2- The inhibition zones (mm) of GSE against different microbial strains.

| GSE concentration ($\mu\text{g/ml}$) | <i>C.albicans</i> | <i>E.coli</i> | <i>P.aeruginosa</i> | <i>K.pneumoniae</i> | <i>Bacillus</i> | <i>E. feacalis</i> | <i>S.aureus</i> |
|--|-------------------|---------------|---------------------|---------------------|-----------------|--------------------|-----------------|
| 500 | 16 | 11 | 13 | - | 14 | 12 | 13 |
| 250 | 13 | 9 | 11 | - | 13 | 10 | 12 |

- : No inhibition zone.

This agree with other researchers, they found that G+ve bacteria more effective than G-ve bacteria but with different concentrations. The different in antimicrobial activities of plant extracts depends on the solvents of extraction [11]. Also [4] suggesting that conjugation of phenolic compounds and protein in microorganism especially key enzyme might be major pathway to inhibit the growth of microorganism .Other researcher [2, 17] showed that the partial hydrophobic nature of their phenolic compounds is responsible for the antimicrobial activity .Accumulation and attachment of these phenolics to the bacterial cytoplasmic membrane eventually lead to the cell death.

No inhibitory effect was found against *K.pneumoniae* so we eliminated it from MIC studies. The minimum inhibitory concentrations showed in table-3.

Table 3- The MIC of GSE against different microbial strains.

| Microbial strain | <i>C.albicans</i> | <i>E.coli</i> | <i>Pseudomonas</i> | <i>Bacillus</i> | <i>E.feacalis</i> | <i>S.aureus</i> |
|-------------------------|-------------------|---------------|--------------------|-----------------|-------------------|-----------------|
| MIC($\mu\text{g/ml}$) | 25 | 150 | 250 | 50 | 50 | 20 |

Also GSE was effect against *C.albicans* at 25 $\mu\text{g/ml}$. [4, 8] showed that phenolic compounds in grape displayed potent antifungal activity against the human pathogenic fungi *C.albicans* at concentration of 10-20 μl .

Conclusions:

This study confirms that black GSE had potential effect against different microorganisms, so it can be used as natural alternative microbial inhibitor either in the food industry or as treatment for human infections, but further studies needs to establish its effect in vivo and in vitro. Also use different extraction methods and compared the antimicrobial effect between grape components.

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