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Original Research Article

Antioxidant and Antibacterial Effect of *Vitis labrusca, Vitis vinifera* and *Vitis vinifera* Seeds Extract

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Abstract: Grape seeds extract has therapeutic values including antimicrobial activity, antioxidant effect, wound healing and prevention of cardiovascular diseases. This study aimed to evaluate and compare antibacterial activity of different species of grape seed) Vitis *labrusca*, *Vitis vinifera* and *Vitis vinifera)* against some bacterial strains (*Staphylococcus aureus*, *Streptococcus pneumonia*, *Acinetobacter Calcoaceticus*, *Klebsiella pneumoniae* and *Escherichia coli)*. Determine antioxidant effect of grape seed extracts (qualitatively). Antibacterial effects was performed using agar cup cut diffusion method for all bacterial species, followed by using minimum inhibitory concentration MIC for the species showed to be inhibited by grape seeds extracts. Antioxidant assay was done using DPPH scavenging test, methanolic solution of each grape seeds was spotted on TLC paper, sprayed with 0.2 % methanolic solution of diphenyl picryl hydrazyl (DPPH) reagent. Vitamin C was used as positive control. From the results, all grape species didn't have any effect on *K. pneumonia* and *E. coli*, red and black grape seeds showed the highest inhibition zone (20 mm) on *Staph. Aureus* agar plate, green grape had the highest effect on *Sterp. Pneumonia* agar plate (20 mm). The lowest effect was for the red grape seeds extract (13 mm) on *Acinetobacter calciaceticus*. In general the three grape seeds extract had effect on Staph. aureus, *Sterp. pneumonia* and *Acinetobacter calciaceticus*. The red and black grape seed extract was effective against *Sterp. pneumonia* strain at MIC values of 7.8 mg/mL and black grape seeds extract had MIC at 7.8 mg/mL on *Staph. aureus*. However, the test for MIC of seeds extracts for the rest of bacterial species ranged between 15.62 and 87.5 mg/mL. The result showed that black grape seeds extract had the largest spot change in color indicating strong antioxidant effect. The lowest effect was by red grape seeds. From this result black grape showed to be the best grape seeds extract among the three chosen species in its antibacterial and antioxidant efficacy.

Keywords: Staphylococcus aureus, Streptococcus pneumonia, Acinetobacter Calcoaceticus, Klebsiella pneumoniae and Escherichia coli, Grape seeds and DPPH.

INTRODUCTION

Grape seed extract is can be found as a dietary supplement in a liquid form, tablets or capsules. It's generally containing 50 to 100 mg of the extract [1]. Grape seed consist of vitamins, minerals, lipid, protein, carbohydrates and 5–8% polyphenols. The most abundant phenolic compounds isolated from grape seeds are catechin, epicatechin, and procyanidins in addition to dimmers and trimmers [2]. Grape seed proanthocyanidins constitute a complex mixture that consisted of procyanidins and procyanidin gallates [3]. The polyphenols of grape seeds have been known for their advantageous role in human health. The grape seed is shown to exhibit therapeutic values such as antioxidant, antiinflammatory, anti-bacterial, anti-cancer, antiviral, cardioprotective, hepatoprotective, neuroprotective, antiaging and anti-diabetic. The oil extracted from grape seeds is used in cosmetic, culinary, pharmaceutical and medical purposes [4-9]. The polyphenolic fractions and gallic acid derivatives are reported to have antibacterial activity [10], Grape seed proanthocyanidins have been reported to have potent antioxidant effect [11]. Extract of grape seed which were obtained from grapes cultivated in Hasandede, Emir and Kalecik Karasi wine cultivars in Turkey showed concentrations of 2.5%-5% exhibited the most inhibitory effect against a variety of microorganisms including *E. coli*, *K. pneumoniae*, and *S.*

aureus [12]. A similar grape seed extract was tested against 21 gram positive strains and gram negative cocci and showed that gram positive cocci are more susceptible, especially *S. aureus* [13]. Complete inhibition of 43 clinical strains of Methicillin resistant *S. aureus* was noted at concentration of 3 mg\mL crude grape seed extract [14]. In other study performed on grape pomace (skin and seed) which produced during wine production and considered as a waste product of wine industries, antimicrobial activity of grape seed extract (GSE) was determined using agar well diffusion method. It was conducted to determine inhibitory effect of GSE against *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumonia* isolated from urinary tract infection. *S. aureus* and *E. coli* was inhibited to high and least extent respectively by GSE. GSE can be used in the treatment of urinary tract infections [15]. Another study tested the antioxidant activity of grape seed extracts using β-carotene-linoleate model system and linoleic acid peroxidation method. The results showed that different extracts had 65%–90% (scavenging rate) antioxidant activity at 100 ppm concentration, indicating that grape seed extracts might be used as preservation of food products as well as for health supplements and nutraceuticals [16]. The present study aimed to determined antimicrobial activity of three spp. of grape seed extracts (GSE) against chosen bacteria in addition to the qualitative antioxidant effect.

MATERIALS AND METHODS

Collection and Preparation of Grape Seed Extract (GSE)

Fresh grape fruits (*Vitis labrusca* or red, *Vitis vinifera* or black and *Vitis vinifera* or Green grape) were collected in the time between Augusts and October 2019. Seeds of black and green grape were collected house garden in Tripoli, while seeds of red grape were collected from local Libyan market. All seeds were manually separated and collected from its fruits, dried in oven at temperature of 50 Cº (Figure 1). Grape seeds were weighed continuously until weight is fixed. The dried grape seeds were grinded to powder, weighed, labeled and kept in dry place.

Fig-1: Grape seed drying in oven at 50 Cº

Dried seed samples were extracted by cold maceration (Figure 2). Known weights of grape seeds powder were macerated in methanol in a closed container tank and left for 72 hrs x 3 with stirring from time to time. Methanolic seeds extract was filtered using filter paper. All extracts were concentrated using rotary evaporator at 64-65.5 Cº, 150 rpm to obtain the crude extract.

Percentage of yield of all extracts was calculated using this formula:
% of yield = (weight of crude extract / weight of original plant powder) x 100

Fig-2: Grape seeds cold maceration

Antibacterial activity of GSE

Grape seeds crude extracts were tested for their antibacterial activities against *Staphylococcus aureus*, *Streptococcus pneumonia*, *Acinetobacter Calcoaceticus*, *Klebsiella pneumoniae* and *Escherichia coli*. Bacterial strains were obtained from the bacterial stock, Department of Microbiology, faculty of pharmacy, Tripoli University and respiratory disease hospital. Agar cup cut diffusion method was used for all bacterial species, followed by determination of minimum inhibitory concentration (MIC) for strains that were inhibited by grape seeds extracts. Bacterial suspensions were cultured in nutrient broth for 24 hrs, stocked on agar plate by cotton swab. Cuts were made on agar surface. One gram of each seeds extract was weight and dissolved in 1ml of distilled water, then 100 µl of extract was poured in agar cup cuts and the plates were incubated at 37 Cº for 24 hrs. Diameter around cup cuts with no bacterial growth was measured and recorded. MIC test was applied for bacteria with the highest diameters produced by plant seeds extracts

Minimum Inhibitory Concentration test (MIC)

A series of broths are mixed with 2 fold serially diluted antibiotic (Cephalexin) solutions and a standard inoculum is applied (Figure 3). After incubation, the minimum inhibitory concentration MIC was determined as the first broth in which growth of the organism has been inhibited. The more resistant an organism the higher MIC will be. 1ml of each bacterial strain equal to 108 cfu per ml was transferred into set of six test tubes and 1ml of plant extracts was added, then two-folds dilution has been made to obtain a concentration range of 500mg/ml to 6.25mg/ml. after 18 hr of incubation at 37 Cº, 0.1 ml of each concentration were inculcated on to MHA agar using the streak plate method. The plates were then incubated at 37°C for 24 hours. The least concentration that did not show growth of the test was recorded.

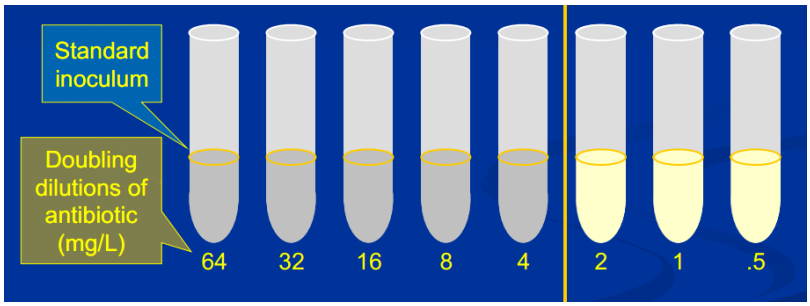

Fig-3: Minimum inhibitory concentration (MIC) procedure for grape seeds extracts

Antioxidant (DPPH) scavenging activity

Methanolic solution of each grape seeds was spotted on TLC plate, left to dry eluted by suitable solvent system and sprayed with 0.2 % methanolic solution of diphenyl picryl hydrazyl (DPPH) reagent. Change in color from violet to yellow considered as a positive result. Vitamin C was used as positive control.

RESULTS AND DISCUSSION

All grape seeds samples were extracted by cold maceration for 72*3, filtrated and concentrated. The percentage of yield was calculated (table 1) for all spices samples using this formula:

% Percentage of yield = (weight of crude extract / weight of original powder) × 100

Table-1: Percentage of yield of the three species of grape seeds

Grape seeds samples	% of yield of grape Seeds
Red grape	12.9 %
Green grape	11.3 %
Black grape	8.2 %

As shown in the above table, red grape seeds percentage of yield were higher than the percentage of yield of other two grape species, although there was no big difference in the % of yield of the three species.

Antibacterial activity of GSE

Grape seeds crude extracts were tested for their antibacterial activities. The results in table 2 showed that all grape seed extracts didn't have any effect on *K. pneumonia* and *E. coli*, red and black grape seeds showed the highest inhibition zone (20 mm) on *Staph. Aureus* agar plate, green grape had the highest effect on *Sterp. Pneumonia* agar plate (20 mm). The lowest effect was for the red grape seeds extract (13 mm) on *Acinetobacter calciaceticus*. In general the three grape seed extracts had effect on *Staph. aureus*, *Sterp*. *pneumonia*, and *Acinetobacter calciaceticus*.

Table-2: Antibacterial effect of three grape seed extracts against different bacterial species

Extract 500mg/ml	*Staph. aureus* (mm)	*Sterp. pneumonia* (mm)	*Acinetobacter calciaceticus* (mm)	*K. pneumonia* (mm)	*E. coli* (mm)
Red grape seeds extract	**20**±0.9	18±0.6	13±0.8	-Ve	-Ve
Black grape seeds extract	**20**±0.6	18±0.4	14±0.5	-Ve	-Ve
Green grape seeds extract	15±0.3	**20**±0.9	15±0.5	-Ve	-Ve
Cephalexin	-Ve	-Ve	-Ve	-Ve	-Ve
All values are expressed as mean ± SD					

As the above mentioned results showed, the three grape seeds extracts exhibited antibacterial effect on the chosen strains. This might be explained due to the presence of phenolic compound in grape seeds.

Minimum Inhibitory Concentration test results (MIC)

MIC test was performed on the micro-organisms which were susceptible to the effects of the grape seed extract, namely *Staph. aureus*, *Sterp. pneumonia* and *Acinetobacter calciaceticus*. This test was performed to measure the lowest concentration of a reagent that inhibits the visible growth of test microbes (MIC) (Table 3). The red and black grape seed extract was effective against *Sterp. pneumonia* strain at MIC values of 7.8 mg/mL and black grape seeds extract had MIC at 7.8 mg/mL on *Staph. aureus*. However, the test for MIC of seeds extracts for the rest of bacterial species ranged between 15.62 and 87.5 mg/mL.

Table-3: Minimum inhibitory concentration MIC of three grape species on different bacterial strains

Bacterial species	*Vitis labrusca* (Red) mg/ml	*Vitis vinifera* (Black) mg/ml	*Vitis vinifera* (Green) mg/ml
Acinetobacter cacoaceticus	62.5±1.2	31.25±1.3	31.25±0.8
Sterp. pneumonia	**7.8**±1.5	**7.8**±0.9	31.25±1.2
Staph. aureus	15.62±0.9	**7.8**±1.0	87.5±1.6
All values are expressed as mean ± SD			

As the tables 2 and 3 showed, grape seeds extract had antibacterial effect on most of the chosen strains. This might be due to the phenolic contents found in grape seed which are partially hydrophobic, and are considered to interact with the bacterial cell wall and lipopolysaccharide interfaces by decreasing membrane stability. The amount of phenolic content in grape seed extract has been directly correlated to the antibacterial properties [17, 18]. Antibacterial activity of grape seed extract is attributed to its content of Stigmasterol, which cause bacterial components degradation by surface interaction and pore formation in the cell wall of bacteria. It might also be accounted to the presence of tannins that has the ability to inactive microbial adhesions, enzymes and cell envelope transport proteins, their complexity with polysaccharide and their ability to modify the morphology of microorganisms [19]. Therefore, this observation is suggestive of the antibacterial effect of grape seed extract. In our study, grape seed extract proved to be bactericidal and was able to produce zones of inhibition ranging from 13-20 mm against chosen bacterial strains with the concentrations of the extract ranging from 15.62 mg/mL and 87.5 mg/mL. Red and black grape seeds extracts showed the highest inhibition zone (20 mm) on *Staph*. *Aureus*, green grape extract had the highest effect on *Sterp. Pneumonia* (20 mm).

Reagor L *et al*. carried out a study to determine the effectiveness of processed grape fruit seed extract as an antibacterial agent against sixty seven distinct biotypes [20]. The results suggested that the antibacterial characteristic of grape seed extract is comparable to that of proven topical antibacterials. One study reported that the structure- activity correlation assays showed that the hydroxyl group of the phenolic compound was effective against *E.coli* and the benzene ring was effective against *S. aureus* [21]. According to Al-Habeb A and Al-Saleh E *et al*. the antibacterial effect of grape seed extract against MRSA is due to disruption of bacterial cell wall membrane in scanning and transmission electron microscopy which could be accounted to the presence of potent polyphenolics in grape seed extract [14].

Antioxidant (DPPH) scavenging activity test results

The result showed that black grape seeds extract had the largest spot change in color. The lowest effect was by red grape seeds (figure 4).

Fig-4: DPPH scavenging activity test of three grape species on TLC in different solvent system, Vit C was used as +ve control. R= Red, B= black, G= Green

As the above figure showed, all grape seeds extracts exhibited a change in DPPH color indicating antioxidant properties. This had been studied extensively by many researchers. Antioxidants such as flavonoids, which act as free radical quenchers [22]. Phenolic compounds from grape seeds have pharmacological and nutraceutical benefits showing antiviral and antimutagenic actions [23] that are closely related to their antioxidant and singlet oxygen quenching ability. Recognition of such health benefits of catechins and procyanidins has led to the use of grape seed extract as a dietary supplement [24, 25]. Besides its antioxidant activity, the grape seed extract proved to act also as antibacterial agent [26, 27].

CONCLUSION

In this study, different grape seeds species were extracted by cold maceration and tested on antibacterial effects on different bacterial strains using cup-cut agar diffusion method and MIC method, in addition to its antioxidant activity using DPPH scavenging activity test, red grape (*Vitis labrusca*) gave the highest percentage of yield (12.9 %) compared to the other two tested grape species. While black grape resulted in the lowest percentage of yield (8.3 %). Black Grape showed to be the most effecting as DPPH scavenger compared to the other two tested species, while the lowest effect was by red grape seeds. In general the three tested grape extracts showed to be effective against *Staph. aureus*, *Sterp. pneumonia* and *Acinetobacter*, while they had no effect on *K . pneumonia* and *E . Coli.* The result of MIC test showed that black grape had the best effect against *Staph. aureus*, *Sterp. Arugenosa* (7.8 mg/ml). finally, black grape extract was the best among the three chosen species in its antibacterial and antioxidant efficacy.

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