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RESEARCH ARTICLE

Study the Effect of the Seed Grape *vitis vinifera* Plant Extract on Some Pathogenic Bacteria and Fungi

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Abstract

Aqueous extract of grape seed was prepared for the purpose of studying its effect in inhibiting the growth of some pathogenic bacterial and fungal isolates. *Candida albicans*, *Escherichia coli*, *Staphylococcus aurus*, *Klebsiella pneumoniae*, and Proteus *mirobillis*. The effect of extract concentrations was studied and the effect varied with the concentration on these isolates. The effect of the same extract on *C.albicans* was studied. The result was also show the higher concentration of the extract appeared the greater inhibitory effect on *C. albicans*. The effect of some antibacterial and antifungal agents was also studied. The inhibitory effect varied according to the species of bacteria.at the other hand nystatin showed higher inhibitory effect with inhibition zone 8, 9, 10mm.

Keywords: *Fungi, Pathogenic Bacteria, Seed Grape vitis vinifera, Plant Extract.*

Introduction

Grape berry *vitis vinifera* is essentially an independent biochemical factory [1, 2, 3], beyond the primary metabolits essential for the plant survival sugar specially glucose, proteins, lipids and minerals, the plant has ability to synthesize all other berry component Flavour and aroma compounds [2, 4, 5]. The seed extract was synthesized by the plant in response to stress, including disease and ultraviolet light, as phytoalexin was first reported in skins of grapes by Creasy and Coffee [6, 7, 8, 3].

Secondary metabolites are more limited in the plant kingdom and mostly accumulated by plant cells in smaller quantities than primary metabolites and synthesized in specialized cells at particular development stages making their extraction and purification difficult [9, 10, 11, 12]. These secondary metabolite or products exert in general a profound physiological effect on the mammalian system and, thus are known as active principles of plant [13].The seed extract exhibit a wide range of antifungal and antibacterial properties also anti-inflammatory and antioxidant effects [14, 15, 16]. The aim of this study was to evaluate the potential of seed extract extracted from Grape berry *vitis vinifera* against some pathogenic fungi and bacteria and compared it with some antifungal and antibacterial agent.

Material and Methods

Plant Material and Extraction

The Grape berry *vitis vinifera* seed were purchased from local market. The seed were air dried and powdered; and kept at 4°C until further investigations.

Preparation of Plant Extracts

This experiment was conducted according to the method [17, 18, 19, 20] with some modification. After drying the grape seeds completely in the shade was converted to a fine powder using an electric mill and then weighed (50) grams of dry powder and placed in a glass container (1000) ml was added Distilled water mechanism and completed the volume to (1) liters then add (3) ml of ethyl alcohol absolute to prevent fungal growth. B. It was left for half an hour in the

horizontal vibrator shaker horizontal and at a medium speed and the samples were left to settle for an hour was filtered with three layers of gauze to separate large plankton and then use centrifuges (centrifuges) and speed (3000) rpm for a quarter of an hour to separate small plankton, then Evaporation of water from the extract under low pressure and at a temperature of (40) using rotary evaporation device and placed in an incubator (Incubator) at a temperature of (37 C) and then the extract of the extract powder in a dark bottle and (8C) in moisture-free conditions Until used [9].

Concentrations Preparation

Stock solutions were prepared by mixing 2 g of the dried extract with 20 ml Ethylene glycol, and then it was sterilized with Millipore membrane filter (0.22 µm). Then different concentrations of(0.1,0.2,0.3,0.4) mg ml^{-1} were prepared by mixing known volume from the stock solution with Ethylene glycol using the following equation:

C1V1=C2V2

C1= Concentration of stock solution.

V1= Volume that obtained from stock solution.

C2= Final concentration.

V2= Final volume.

Bacterial and Fungal Isolates Collection

All fungal isolates were obtained from the laboratories of Biotechnology department / College of Science / University of Baghdad. *Candida albicans*, *Escherichia coli*, *Staphylococcus aurus*, *Klebsiella pneumoniae*, and Proteus *mirobillis*.

Anti-bacterial and Antifungal Assay

Effect of different concentrations of plant extract and fungal extract on the inhibition of bacterial and yeast *C. albicans* species.

Method of Agar Weal Diffusing Method

The experiment done according to [10] pure colonies grown on the nutrient agar medium to the center of the nutrient broth, incubated at 37° C for 18-24 hours, then diluted with normal saline. With the standard control tube (McFarland) which is equivalent to (1.5 × 108 cells / ml) and then transferred (0.1) ml of diluted bacterial suspension to the center of nourishing nourishes N. A spread on the surface of the dish using a sterile cotton swab and incubated for 30 minutes at 37 ° C for the purpose of studying the inhibitory activity of plant extracts on bacterial growth, digging a diameter of (6) mm by adding (0.1) ml of each concentration (0.1, 0.2, 0.3) mg / m Of vegetable extract and incubated at a temperature (37 m) for a period (16-14 hours) [11, 21, 22, 23, 24].

Test method for sensitivity of bacteria and yeast to plant extract and antibiotics according to the following:

- Pour (25) ml of nutrient agar for each plate.
- spreading the inoculum (0.1) ml by a spreader of the yeast inoculum containing (1.5×10^8) cells / ml, compared with a standard solution of turbidity fixed, and then let the dishes to dry at room temperature.
- Drilled with a diameter of (5) mm in the cultivated medium by sterile cork piercer (sterile cork-borer).

Samples were planted directly on the appropriate cultivation media, including nutrient agar medium) and bacterial species screening by biochemical tests [25, 26, 27, 12]. The method of spreading bacteria on Nutrient agar medium by sterile swabs for the antibacterial Ciprofloxacin did not work dig in this method, but we put the tablets directly. As for the nystatin we did a drill by cork borer and put 100 ml of each dilution and nystatin only for the yeast *C. albicans*, while the Ciprofloxacin only for bacteria *E. Coli*, *S.aurus*, *K.pnemonia*, and *Proteus mirobillis* [28, 29, 30].

The effect was measured using a ruler and the inhibition zones were calculated by comparison. The inhibitory areas changed from the effect of the extract or the concentration of the extract. The dishes where no inhibition occurred did not concentrate the extract [31, 14].

Results and Discussion

The antifungal activity of grape seed extract was studied against gram positive bacterial strains such as *S. aureus* and other gram negative bacteria and one strain of yeast *C. albicans* using agar weal diffusion method. Grape seed extract showed high inhibitory activity of *E. coli*, *S. aurus*, *K. pnemonia* and

Proteus *mirobillis*. With inhibition zone 16, 5.14 and 11 mm / ml, respectively, at a concentration of 0.4 mg / ml. However, the compound showed little or no inhibitory activities against the negative bacteria of gram stain and yeast cells. The results also show that grape seed extract with four concentrations (0.1%, 0.2%, 0.3%, 0.4%) against four bacteria showed high inhibitory activity of three species of *S. aurus*, *K. pnemonia*, and *Proteus mirobillis*. And 7 mm / mL respectively at a concentration of 0.4 ml. There was no activity against *E. coli* bacteria. Only the high concentration caused its inhibition with a diameter of 1 mm. We conclude from this that *E. coli* bacteria are very strong than other types of gram-negative. At 0.1 mg / ml, the compound showed no activity against only one type of bacteria, *S. aurus*. As shown in Table (1) and Figure (1), it shows the effect of the extract on *S. aurus* bacteria.

Table 1: Inhibitory effects of grape *Vitis vinifera* against some pathogenic bacteria

Isolates	)mg/ml(Concentrations			
	0.4	0.3	0.2	**0.1**
E.coli	1	-	E.coli	**-**
S.aurus	7	6	S.aurus	**4**
K.pnemonia	7	6	K.pnemonia	**-**
Proteus mirobillis	7	6	Proteus mirobillis	**4**

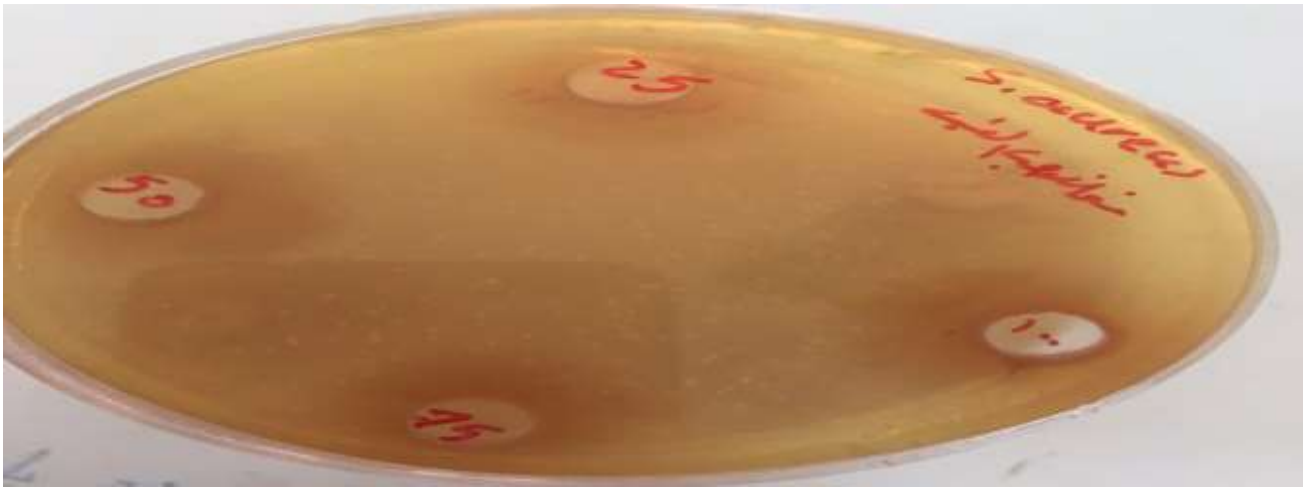

Figure 1: Inhibitory effects of grape *Vitis vinifera* against *S. aurus* cultured on nutrient agar 37c, 48 hrs

The effect of the extract on *C.albicans* was high and its inhibition effect as it increased with the increase concentration, as shown in Table (2) and Figure (2).

Table 2: Inhibitory effects of Grape *vitis vinifera* against *C.albicans*

Fungal isolate	**)mg/ml(Concentrations**			
	0.4	0.3		**0.1**
	9	8	*C.albicans*	**6**

Figure 2: Inhibitory effects of Grape *vitis vinifera* against *C.albicans* cultured on SDA, **37°C, 48hrs**

The effect of the antibiotic Ciprofloxacin was studied on four isolates of bacteria, where the highest inhibitory ability of the antibiotic against *P. mirobills* was 16 mm, and the lowest inhibitory ability of the same antibiotic on the *S. areus* reached 8 mm as shown in the Table (3) and Figure (3) showed the effect of the antibiotic against the bacteria *P. mirobills*. The antibacterial ability higher has been shown against studied gram negative bacteria.

Table 3: Inhibitory effects of antibacterial agent against some pathogenic bacteria

Isolates	Results mm	Concentration	Antibiotic
K.pnemonia	9	10	**Ciprofloxacin**
E.coli	11	10	**Ciprofloxacin**
S. areus	8	10	**Ciprofloxacin**
P. mirobills	16	10	**Ciprofloxacin**

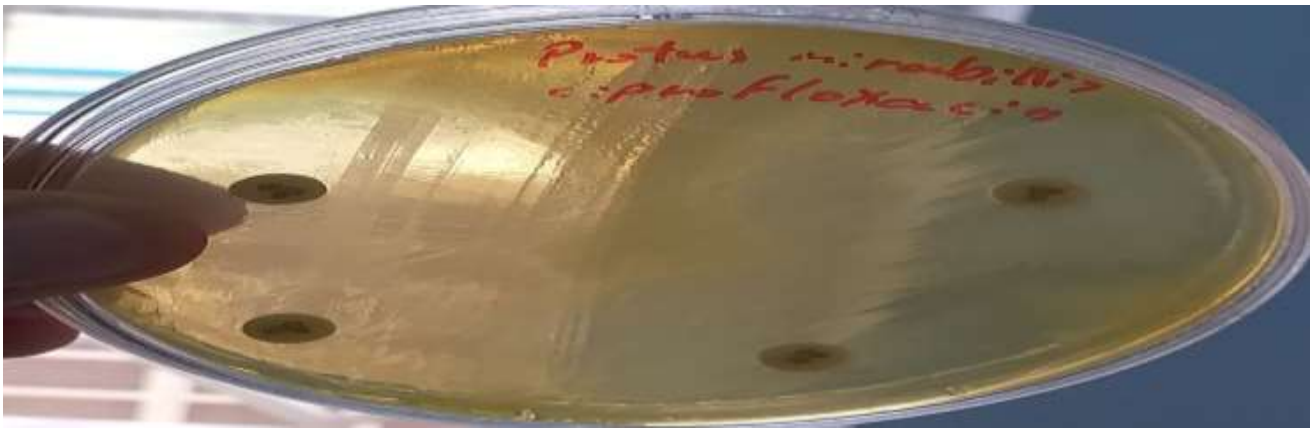

Figure 3: Inhibitory effects of Ciprofloxacin against *P. mirobills* cultured on nutrient agar 37°C, 48hrs

The results showed in Table (4) that the effect of nystatin had a greater inhibitory effect as the concentration increased as shown and used three concentrations (0.1,0.2, 0.3) in Figure (4).

Table 4: Inhibitory effects of antifungal agent Nystatin against *C. albicans*

Fungal isolate	Results	)mg/ml(Concentrations
Candida	8	**0.1**
	9	**0.2**
	10	**0.3**

Figure 4: Inhibitory effects of antifungal agent Nystatin against *C. albicans*

Grape seed extract contains active substances that in one way or another have affected bacterial strains and yeast cells [32, 33, 34].The effect of a multi-proanthocyanidin compound extracted from grape seeds has a strong effect on the bacterium gram positive affected by the and has a weak effect on the gram negative bacteria. No effect of the compound on the yeast has been shown [35].

Absorbance partially purified multi-proanthocyanidin compound of grape seeds has a high inhibitory capacity on the bacterial strains of a finer dye, the highest against *S. aureus Staphylococcus* (16 mm) and the lowest against the bacterium *Pseudomonas aeruginosa* (17 mm) for 5.2 mg / ml. One of the molecular mechanisms is the formation of complexes with proteins by non-

specific forces such as hydrogen bonds and hydrophobic influences as well as the formation of covalent bonds [36]. Phenolic compounds have a role in reducing the virulence of pathogenic bacteria by failing to bind the intestinal toxin of *Helicobacter Pylori* to host cell receptors (intestinal membrane) by forming a complex after treatment with grape seed extract [16]. A polypanthousandin compound had a role in inhibiting the growth of anaerobic bacteria causing gingivitis *fusobacterium nucleatum* [17]. *Prophyromonoas gingivalis* which have more antioxidant activity than vitamin C, E based on TEAC measurements Trolox equivalent antioxidant capacity [37].

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