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Phytochemical composition and biological activity of Anthum graveolens L. and Vitis vinifera L. seeds extract against pathogenic bacteria.

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Abstract:

In this study ,the biological activity of seeds extraction of ***Anethum graveolens* L.** and ***Vitis vinifera* L.** including methanol extracts for three concentration (25,50,75 mg/ml) against bacteria isolated from patients infected with urinary tract infection *Staphylococcus aureus, Acinetobacter baumanni, Klebsiella pneumonia, Enterobacter* and *Pseudomonas aeruginosa* by using well diffusion method. The results of phytochemical screening of the methanol extract for the ***A. graveolens* L.** and ***V. vinifera* L**. showed that present of flavonoids, tannins alkaloids and carbohydrate.The results showed that seeds extract for ***Anethum graveolens* L.** and ***Vitis vinifera* L**. exhibited antibacterial activity against pathogenic bacteria. The higher diameter inhibition in methanol extract for ***A. graveolens* L.** in the concentration 25 mg /ml , while the higher diameter inhibition in methanol extract for ***V. vinifera* L**. in the concentration 75 mg/ml.

Keywords: *Anethum graveolens, Vitis vinifera,* Phytochemical, methanol extract, Antibacterial.

Introduction

Natural sources are considered as the source of effective compounds which are important for human health **(23).** Plants are one of the natural sources that have great therapeutic value of their use as antimicrobial agents. According to world health organization (WHO) about 80% of the population of developed countries use plant extracts as medicine to treat diseases. **(19).** Plants produce secondary metabolites that have a role in the production of drugs. The medicinal properties of plants were studied due to their pharmacological effectiveness, low toxicity and economic importance. **(3, 28).**

Anethum graveolens L.

(dill) family Apiaceae (Umbelliferae), It is annual and an aromatic herb, used as flavoring and preservative agent and it's found in Mediterranean and West Asia **(7).** The dill plant is rich in essential oil, which is found in herb and seeds the Ayurvedic uses of dill seeds are stomachic, carminative and diuretic. dill herb and seeds have various volatile compounds; carvone responsible for the odour of dill seeds and limonene, α-phellandrene , myristicin, dill ether are the predominant odour of dill herb. Other component found in dill seeds are flavonoids, coumarins, steroids and phenolic acids **(12).** Biological effectiveness of dill such as antimicrobial or antibacterial , anti-inflammatory, antihyperlipidaemic, antioxidative, antihypercholesterolaemic and hypoglycemic **(27, 6, 14, 30, 18).**

Vitis vinifera L.

(grapes), family Vitaceae deciduous woody climber. The plant is widespread in the mediterranean and has medicinal uses in these countries as this plant adapts to their climate **(8,4).** Grapes contains seeds that have benefits to them when eating grapes including containing nutrients such as vitamins (A, B1, B2, B6 and C) , magnesium and have antioxidants properties **(21,32-47)**. Several pharmalogical benefits have been recorded for grape seed extract such as anti-microbial, anti-inflammatory, anti-oxidant , anti-carcinogenic, Alzheimer's disease and diabetes **(5).**

The black grape skin contains phenolic compounds and polyphenols, polyphenols contain simple compounds (monomers) and complex compounds (oligomers and polymers),(20). The phenolic compounds such as anthocyanins, flavanols, phenolic acids and stilbenes (resveratrol) **(9).** These polyphenols have several benefits to human health such as antibacterial, antifungal, decreasing of free radical damage, anti-carcinogenic, inhibition the risk of cardiovascular diseases and anti-inflammatory, etc.**(13).**

For the treatment of human diseases It is necessary to search for anti-microbial resistance drugs from other sources , such as plants, because of the resistance of bacteria to antibiotics **(2).** The aim of this study is to determine the antibacterial activity of seeds extract of the *A. graveolens* and *V. vinifera* against pathogenic bacteria.

Materials and Methods:

Plant material:

The plant materials of *A. graveolens and V. vinifera* (seeds) obtained from local market of Najaf. The identification and authentication of the plants materials was done by Botany Taxonomy Department, Kufa University.

Microbial Strains:

Microorganisms: *Staphylococcus aureus, Acinetobacter baumanni, Klebsiella pneumonia, Enterobacter* and *Pseudomonas aeruginosa.* were obtained from patients infected with urinary tract infection.

Preparation of methanol extracts:

The seeds of *A. graveolens and V. vinifera* plant were cleaned with distilled water to remove the soil then left dried by air. The plant material was divided into small pieces and ground to powder with electric grinder. The dry powder of seeds was weighed using an electric weighting scale. The extraction was prepared by Maceration method with slight modification. A total 20 g of each plant (*A. graveolens and V. vinifera*) seeds powder was steeped in 200 ml of methanol 95% for 3 days to obtained methanol extract, and then filtered through eight layers of gauze. It was further filtered by using filter paper (What man No.1) The solvent was removed by evaporation using rotary evaporator. Then, the weight of the extract was measured and then the methanol extract of *A. graveolens and V. vinifera* was kept in refrigerator until use extract **(11).**

Preparation of Chemical reagents:

1-Detection of Alkaloids

A=Dragendroffs Reagent

Dragendroffs Reagent was prepared by mixing two solution the first solution was prepared by dissolving 0.6 g of bismuth nitrate in 2 ml acetic acid and then 10 ml of distilled water was added. While the second solution was prepared by dissolving 6 g potassium iodide KI in 10 ml of distilled water, then mixed first solution with second solution in addition 7 ml from concentration Hcl and 15ml of distilled water and the final solution was completed by adding 200 ml distilled water **(11).**

B=Mayer s Reagent

Prepared by dissolve 13.5 g of mercury chloride and potassium iodide in 100 ml distilled water, then takes (1-2ml) from it and added to 5 ml of alcoholic extracts **(11).**

2-Detection of Carbohydrates

Benedicts reagent

Prepared by dissolve 137 g from sodium citrate with sodium carbonate in 800 ml distilled water. Filter the solution then 17.3 g of copper sulfate was add dissolved in 100 ml of distilled water. then takes (1-2ml) from it and added to 5 ml of alcoholic extracts **(11).**

3-Detection of tannins

Lead acetate 1% test

Five ml of each extracts were treated with few drops of 1% lead acetate **(15).**

4-Detection of flavonoids

Lead acetate 1% test

The extract was treated with few drops of lead acetate solution **(26).**

Antibacterial assay:

The extracts obtained from *A. graveolens and V. vinifera* (seeds) they were used to study their antibacterial activities, using agar well diffusion method **(10).** Different species of bacteria were selected as target, *Staphylococcus aureus, Acinetobacter baumanni, Klebsiella pneumonia, Enterobacter* and *Pseudomonas aeruginosa.* Mueller–Hinton Agar (MHA) was poured in Petri dishes (Three Replication), after solidification. Suspension of the tested bacteria ($1x10^6$ CFU/ml) was spread on to media plates by sterile cotton swabs after 15 min. Four wells were made in plates with sterile borer (5 mm).

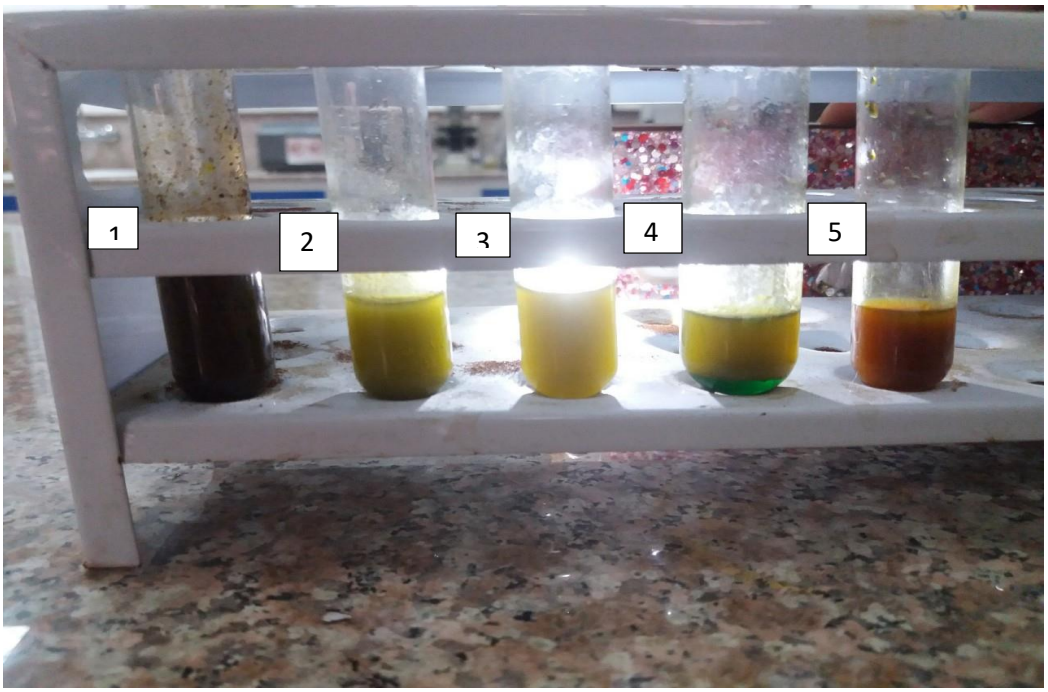
By dissolving the extract with Dimethyl Sulphoxide (DMSO) different concentrations of extracts were prepared at (25, 50, 75 mg /ml).100 μl from each concentration of extracts were loaded in the wells. The plates were left at the room temperature for 10 minutes to allow spreading the extract into the agar. The plates incubated at 37 C^0 for 24 hours. The bacterial growth was determined by measuring the diameters of inhibition zones, measured in millimeters (mm), around each well in plates.

Results and discussion:

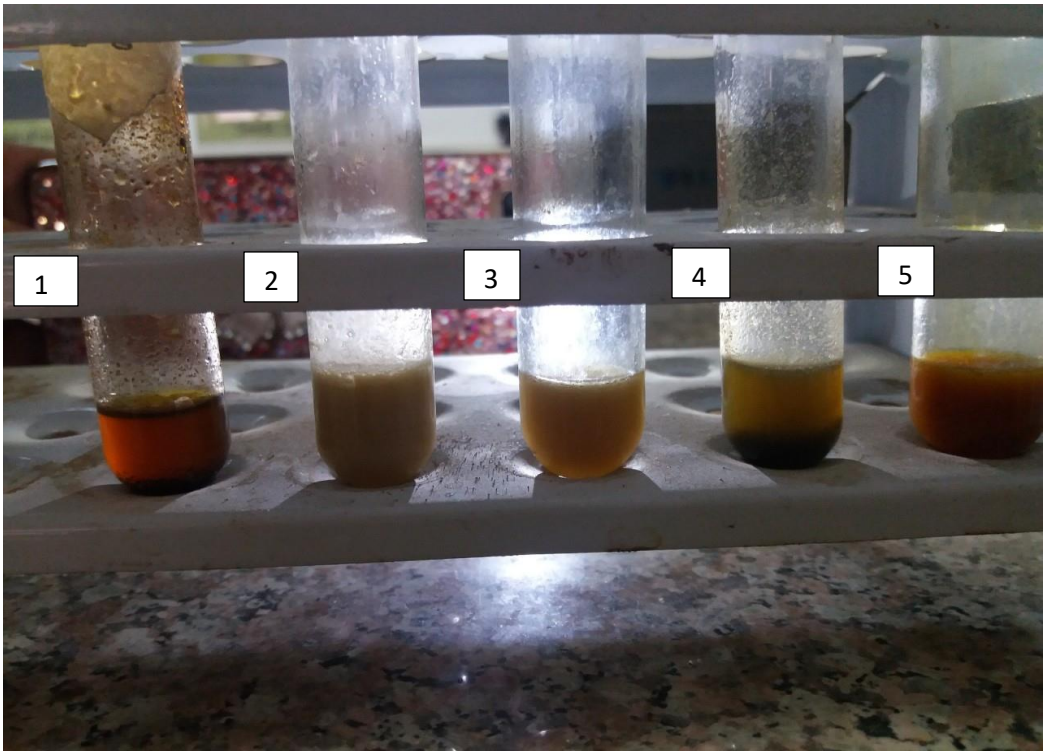
Phytochemical composition detection:

Results showed chemical detection of methanol extract of the *A. graveolens and V. vinifera ,* contains of Flavonoids , tannins, alkaloids and carbohydrates, figure (1,2). table (1). This indicates that the chemical compounds of *A. graveolens* and *V. vinifera* are soluble in methanol solvent.

Figure (1): Phytochemical composition detection of methanol extract of the *A. graveolens* .



Figure(2): Phytochemical composition detection of methanol extract of the *V. vinifera.*



1- Flavonoids Test (yellow green coloured)
2- Tannine Test (white gelatinous precipitate)
3- Mayer's Test (white coloured)
4- Benedict's reagent (green precipitate)
5- Dragendroff's Reagent (red precipitate)

table (1): Phytochemical composition detection of methanol extract of the *A. graveolens* and *V. vinifera* .

	avonoids **est**	**nnine** **est**	**ayer's** **est**	**enedict's** **eagent**	**agendroff's** **Reagent**
graveolens	+	+	+	+	+
vinifera	+	+	+	+	+

Antibacterial activity:

In this study, agar well diffusion method was used to evaluation the antibacterial activity of the *A. graveolens and V. vinifera* methanol extract.

The methanol extract of the *A. graveolens and V. vinifera* seeds showed good antibacterial activity against the *Staphylococcus aureus, Acinetobacter baumanni, Klebsiella pneumonia, Enterobacter* and *Pseudomonas aeruginosa* , inhibition zones and zone diameter showed in table (2,3,4,5, 6).

The maximum inhibition zone diameter was obtained in methanol extract of the *A. graveolens against Acinetobacter baumanni* , *Staphylococcus aureus* and *Klebsiella pneumoniae* with diameter (38.333 ± 5.2387, 37.000 ± 8.7178 and 37.000 ± 4.0415 mm respectively) in concentration 25 mg/ml. while the minimum inhibition zone diameter was obtained in *Pseudomonas aeruginosa* with diameter (21.100 ± 1.3051 and 23.000 ± 2.8868 mm) in concentration (50 and 25 mg/ml respectively). In the present study, bacterial species, showed different degrees of sensitivity to the methanol extract, which may be due to the differences in the structure of cell wall and chemical composition of both types of bacteria **(25).** Higher levels of phenolic compounds in *A. graveolens* plant might be responsible for their biological effects **(1).** The inhibitory effectiveness of phenolic compounds can be explained by the adsorption of cell membranes of these compounds and their interaction with protein especially enzymes causes the inhibition of microbial growth **(16, 29).**

The antibacterial activity of methanol extract of the *V. vinifera* showed highest value of inhibition zone diameter in *Staphylococcus aureus* with diameter (34.000 k± 2.0817, 33.667 ± 7.6884 and 33.333 ± 8.1104 mm) in concentration (75, 25 and 50 mg/ml respectively) and the less value in *Pseudomonas aeruginosa*, *Acinetobacter baumanni* and *Klebsiella pneumonia* with diameter (20.833 ± 5.0854, 21.667 ± 10.525 and 21.500 ± 6.9342 mm) in concentration (25, 75 and 50 mg/ml respectively). phenolic compounds are mainly distributed in the skin, leaf, stem and seed of *V. vinifera* **(29)**. Phenolic compounds are very important and effective against bacteria, gallic acid is one of the most effective compounds against bacteria **(24)**. Other phenol compounds rutin, catechin and quercetin also have antibacterial properties **(31).**

The current study showed that methanol extract of *A. graveolens* and *V. vinifera* seeds contains number of secondary metabolites, table (1) . which have a role in anti-bacterial activity through various mechanisms. Tannins interacts with proline rich protein to be irreversible complex, it results in inhibition of cell protein formation, flavonoids are complex with bacterial cell wall proteins and extracellular-soluble proteins, lipophilic flavonoids damage bacterial cells membranes **(22).**

Table 2: Inhibition zone diameters (mm) of antibacterial activity of *A. graveolens* and *V. vinifera* . against *Staphylococcus aureus* isolated from patients infected with urinary tract infection.

oncentration	*graveolens*	*vinifera*	value
mg/ml	.000 ± 8.7178	.667 ± 7.6884	7885
mg/ml	.000 ± 4.0415	.333 ± 8.1104	9724
mg/ml	.333 ± 4.1767	.000 ± 2.0817	9465

±: mean and stander error.

Table 3: Inhibition zone diameters (mm) of antibacterial activity of *A. graveolens* and *V. vinifera* against *Acinetobacter baumanni* isolated from patients infected with urinary tract infection.

oncentration	*graveolens*	*vinifera*	value
mg/ml	.333 ± 5.2387	.000 ± 5.8595	1423
mg/ml	.167 ± 1.9650	.333 ± 3.9299	0693
mg/ml	.000 ± 2.0817	.667 ± 10.525	3144

±: mean and stander error.

Table 4: Inhibition zone diameters (mm) of antibacterial activity of *A. graveolens* and *V. vinifera* against *Klebsiella pneumoniae* isolated from patients infected with urinary tract infection.

oncentration	*graveolens*	*vinifera*	value
mg/ml	.000 ± 4.0415	.500 ± 8.3716	2499
mg/ml	.600 ± 0.51962	.500 ± 6.9342	1857
mg/ml	.33 ± 3.283	.33 ± 3.283	0000

±: mean and stander error.

Table 5: Inhibition zone diameters (mm) of antibacterial activity of *A. graveolens* and *V. vinifera* against *Enterobacter* isolated from patients infected with urinary tract infection.

oncentration	*graveolens*	*vinifera*	value
mg/ml	.500 ± 2.0207	.667 ± 5.6298	3847
mg/ml	.500 ± 1.4434	.333 ± 2.8480	7333
mg/ml	.033 ± 4.5718	.000 ± 4.0415	8818

±: mean and stander error.

Table 6: Inhibition zone diameters (mm) of antibacterial activity of *A. graveolens* and *V. vinifera* against *Pseudomonas aeruginosa* isolated from patients infected with urinary tract infection.

oncentration	*graveolens*	*vinifera*	value
mg/ml	.000 ± 2.8868	.833 ± 5.0854	7298
mg/ml	.100 ± 1.3051	.667 ± 1.4530	4674
mg/ml	.367 ± 3.5592	.667 ± 3.5277	9551

±: mean and stander error.

Conclusion:

The extract from the seeds of *A. graveolens* and *V. vinifera* have a good antibacterial activity. Hence, it is of medicinal importance and can be used as antibacterial agent.

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