Inhibitory Action of Aqueous Extract of Anethum graveolens on Staphylococcus aureus

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Abstract—Tenisolates of bacteria were collected from Rezgari hospital in Erbil city (KRI). The identity of isolates were emphasized as they demonstrated culturally, microscopically and some biochemical properties. Among these isolates, only two isolates (S3 and S7) characterized as Staphylococcus aureus. The susceptibility of S3 and S7 isolates against five different antibiotics (Amp, Amc, Amx, Cip, Tet) was screened, and isolate (S3) was resistant to (Amp and Amc), while isolate (S7) was resistant to (Amc). Furthermore, the antibacterial effect of aqueous extract of Anethum graveolens on (S3) was screened, the minimum and maximum inhibition zone at 9000µg/100µg and24000µg/100µgwas 24mm and 32mm in diameter, respectively.

Index symbols: KRI: Kurdistan Region of Iraq, Amp: Ampicillin, Amc: Ampiclox, Amx: Amoxillin, Cip: Ciprofloxacin, Tet: Tetracycline, S3: Sample 3, and S7: Sample 7.

Keywords: Antibacterial activity, Antibiotic resistant, *Anethum graveolens*, *Staphylococcus aureus*.

1. INTRODUCTION

In the last few decades, many bacterial species have developed resistance to antimicrobial agents that have been commonly used to treat them (1). *Staphylococcus aureus* is one of the pathogens known to rapidly develop resistance to antimicrobial agents as new antibiotics are introduced (2).*S. aureus* is a gram-positive, spherical bacterium approximately 1µm in diameter. Its cells form grape-like clusters, since cell division takes place in more than one plane (3).*S. aureus* colonized mainly the nasal passage, skin, oral cavity and gastrointestinal tract. It is often appears golden yellow on media and hemolytic on blood agar, facultative anaerobic, catalase positive and oxidase negative. It also can grow at 15-45°C and NaCl concentration as high as 15 percent (4). S. aureus known as clumping factor, which reacts with fibrinogen to cause organisms to aggregate, and extracellular *staphylococcus* reacts with prothrombin to form staphylothrombin (5).

Control of antibiotic resistant bacteriaand the treatment of infections caused by them is a major problem worldwide (6 and 7).Elderly, medicinal plants have been used as treatment of various human ailments. A revolution came in the medicinal world with the discovery of antibiotics for treatment of bacterial infections.Various plants were documented as enrolled in development of novel drugs by monitoring and evaluating their antimicrobial activity. It is believed that, crude extract from plants have higher efficiency than isolated components due to synergistic effect (8).

Dill (*Anethum graveolens*) also known as shapt or dill-weed and Sowa, the name came from Greek word aneeson or aneeton which means strong smelling. It is an annual herb with the height about 1.5 meter (9 and 10). It is indigenous to southern Europe. It is an annual herb growing in the Mediterranean region, central and southern Asia. Now dill is cultivated around the world (11,12 and 13). Traditionally, dill used as an aromatic herb and spice in many countries such as India. Moreover, it is used as an anti-convulsion, anti-emetic, anti-cramp especially in children, as a wound healer and to increase the appetite and strengthen the stomach (14 and15).Biological activity of *A. graveolens* oil displays an important role in anti-microbe, anti-bacteria and anti-fungi (16and 17).

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2. MATERIAL AND METHODS

2.1 Plant extraction

2.1.1 Bacteria under study

Ten isolates were collected from Rezgari Hospital in Erbil city. The isolates were microscopically, morphologically demonstrated,and they were characterized by performing some biochemical tests.

2.1.2Collection of plant

Anethum graveolens was obtained from Market, and the leaves were washed with tap water. Furthermore, it was washed with distilled water and left for air drying until completely dried. Then, the plant was converted to powder form and kept in polyethylene sacks in refrigerator at 4°Cfor further use.

2.1.3 Preparation of aqueous extract

The aqueous extract of A. graveolens was prepared according to (18), with slight modification. Sixty gram of ground dried plant was added to 600ml of sterilized distilled water, and heated until boiling point with stirring for three hours. Then, the extract was filtered by both muslin cloth and filter paper (Whatman NO.1), the product evaporated to dryness and stored in refrigerator at 4°C.

2.2 Preparation of inoculum

Two to three colonies from pure culture of isolated bacteria were transferred into test tube which contain 5ml nutrient broth, and incubated overnight at 37°C.

2.3 Stock solution preparation of plant extract

2.4gm of aqueous extract of *A. graveolens*was dissolved in 10ml sterilized distilled water.

2.4 Susceptibility to antimicrobial agents

The two isolates (S3 and S7) was subjected to antibiotic susceptibility test, using cup plate agar diffusion, consisted of nutrient agar enriched with Amp, Amc, Amx, Cip and Tet. Then, they were poured to plate and solidified. After that,S3 and S7were inoculated and streaked on prepared medium(19).

2.5 Antibacterial activity test of plant extract

Susceptibility of identified *S. aureus* (S3) to aqueous extract of *A. graveolens* was measured by the cup plate agar diffusion method on sterilized nutrient agar. After solidification, 0.1ml of bacterial suspension was inoculated and left for about 30 minutes. Cups of 8mm diameter were made in the medium using sterile cork borer. Nine different concentrations of aqueous extract were added into cups, after 24hours incubation at 37°C, the diameter per inhibition zone were measured (20).

3. RESULTS AND DISCUSSION

Ten isolates were collected from Rezgari hospital in Erbil city. Morphological and microscopical characteristics of isolates were demonstrated. Morphologically, among ten collected isolates only two isolates (S3 and S7) were gave features of S. aureus as colonies appeared circular, large, golden yellow in color on manitol salt agar,while they had β-hemolytic property on blood agar (21). The two isolates were appeared purple and cocci shape under microscope.

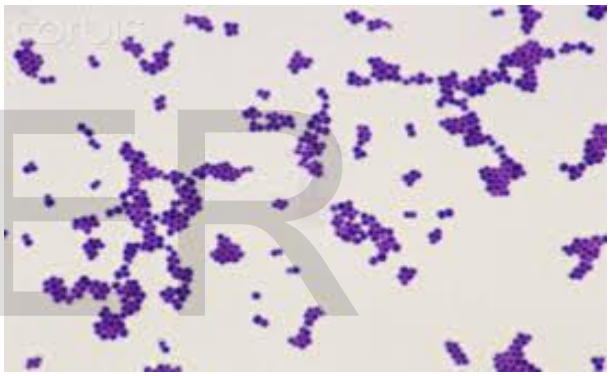

Fig. 1. S. aureus, cluster in arrangement, purple color and cocci shape.

Furthermore, several biochemical tests were performed and isolate S3 and S7 were identified as S. aureus, due to catalase positive, oxidase positive, gelatinase negative and non-motile (22)as shown in the following table.

TABLE 1: RESULTS OF SOME BIOCHEMICAL, MORPHOLOGICAL AND CULTURAL INDICATORS.

S. aureus isolates	Manitol Salt Agar	Blood agar	Gelatinase	Catalase	Oxidase	Motility	Gram Staining
1	No change	α-hemolysis	–	+	–	–	
2	No change	β-hemolysis	–	+	–	–	
3	*S. aureus*	β-hemolysis	–	+	–	–	Purple, cocci
4	No change	ϒ-hemolysis	–	+	–	–	
5	No change	ϒ-hemolysis	–	+	–	–	
6	No change	ϒ-hemolysis	–	+	–	–	
7	*S. aureus*	β-hemolysis	–	+	–	–	Purple, cocci
8	No change	β-hemolysis	–	+	–	–	
9	No change	ϒ-hemolysis	–	+	–	–	
10	No change	ϒ-hemolysis	–	+	–	–	

The table shows that only isolate S3 and S7 changed color of MSA medium from pink to golden yellow as indicator to differentiate S. aureus from other species. they were also β-hemolysis, (gelatinase and oxidase) negative and catalase positive. They were non-motile and purple cocci shape microscopically.

The susceptibility of isolates (S3 and S7) to five different common antibiotics was tested,it showed that S3 was resist to (Amp and Amc) and sensitive to (Amx, Cip and Tet). While S7 was resist to Amc and sensitive to remained antibiotics (table 2).

TABLE 2: RESISTANCY OF S. AUREUS (ISOLATE S3 AND S7).

Antibiotics	Concentration µg/ml	*S. aureus* isolate (S3)	*S. aureus* isolate (S7)
Amp	30	R	S
Amc	30	R	R
Amx	30	S	S
Cip	30	S	S
Tet	15	S	S

R: Resistant S: Sensitive

According to the previous studies, the main reason ofS. aureus resistancy to antibiotics is the presence of β-lactamase enzyme (23), which fights antibiotics by deactivating β-lactam binding proteins (24).

Antibacterial activity of aqueous extract of Anethum graveolens against (S3) of S. aureus was performed as shown in (table 3), and it was demonstrated that plant extract had ability to inhibit growth of this bacterium at differentconcentrations. In first five concentrations (375µ/100µ, 750µ/100µ, 1500µ/100µ, 3000µ/100µ and 6000µ/100µ), no effectwas appeared. More ever, with the use of higher concentrations,they were suppressedgrowth of S3.The resistancy of S. aureus (S3) was decreased by providing higher concentration of aqueous extract of A. graveolens.

TABLE 3: EFFECT OF AQUEOUS EXTRACT *OF A. GRAVEOLENS* ON *S. AUREUS.*

Concentration of *A. graveolens* extract µg/100µl	Inhibition zone (mm)
375	-
750	-
1500	-
3000	-
6000	-
9000	24
12000	27
18000	30
24000	32

-= *no effect*

Furthermore, the minimum inhibitory zone was 24mm in diameter at 9000µ/100µ,while the highest effect was recorded at 24000µ/100µ andproduced inhibition zone was 32mm.

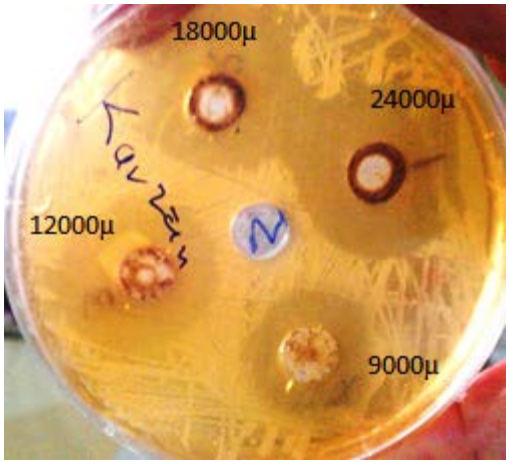

Fig. 2 Antibacterial activity of *A. graveolens*extract on *S. aureus* (S3) at different concentrations.

Activity of Anethum aqueous extract on S aureus may returns to the presence of chemical compositions such as essential oil, terpenoil, oxypencedanin, and falcarindiol (25,26 and 27). In this study, it was concluded that Anethum graveolens can be used as an antibacterial agent against *S. aureus*, due to possess of antimicrobial property in it is structure.

4. Conclusion

Isolate S3 and S7 were identified as S. aureus and antibiotic susceptibility test was performed. As a result, S3 was resist to (Amp and Amc) and sensitive to (Amx, Cip and Tet). While isolate 7 was resist to Amc and sensitive to other antibiotics. Furthermore, the effect of aqueous extract of *A. graveolens*on*S. aureus* (S3) was appeared at 9000µg/100µl, 12000µg/100µl, 18000µg/100µl and 24000µg/100µl, which was 24mm, 27mm, 30mm and 32mm in diameter, respectively. In conclusion, despite of using dill (*A. graveolens*) as a tasty diet, it is beneficial to inhibitinfectious microorganisms such as *S. aureus*.

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References

[1] Swartz, M. N. Use of antimicrobial agents and drug resistance. *New England Journal of Medicine*, vol. 337, no. 7, pp. 491-492,(1997).
[2] Lowy, F. D. Antimicrobial resistance: the example of Staphylococcus aureus. *The Journal of clinical investigation*, vol. 111, no. 9, pp. 1265-1273,(2003).
[3] Crossley, KB. and Archer, GL. The Staphylococci in human disease. Churchill Livingstone,(1997).
[4] Todar, K. Todar's online textbook of bacteriology. Madison, Wis, USA: University of Wisconsin-Madison Department of Bacteriology.(2006)
[5] Von Eiff, C., Becker, K., Machka, K., Stammer, H., and Peters, G. Nasal carriage as a source of Staphylococcus aureus bacteremia. *New England Journal of Medicine,* vol. 344, no. 1, pp. 11-16,(2001).
[6] Cutler, R. R., and Wilson, P. Antibacterial activity of a new, stable, aqueous extract of allicin against methicillin-resistant Staphylococcus aureus. British journal of biomedical science, vol. 61, no. 2, pp. 71-74, (2004).
[7] World Health Organization. Antimicrobial resistance global report on surveillance. 2014 summary.
[8] Jana, S., and Shekhawat, G. S. In vitro Extracts of Indian Medicinal Herb: Anethum grave-olefins.(2010).
[9] Abed, K. F. Antibacterial activity of essential Oil of some Medicinal plants From Saudi Arabia. *Saudi Journal of Biological Sciences*, vol. 14, no. 1,pp. 53-60,(2007).
[10] Jana, S., and Shekhawat, G. S. Anethum graveolens: An Indian traditional medicinal herb and spice. Pharmacognosy reviews, vol. 4, no. 8, pp. 179- 184,(2010).
[11] Wren R C. Potter's new cyclopedia of botanical drugs and preparations. Saffron Walden, CW Daniel. 1988.
[12] Leung A Y, Foster S. Encyclopedia of common natural ingredients used in food, drugs and cosmetics. New York, John Wiley and Sons. 1996.
[13] Yazdanparast R. and Bahramikia S. Evaluation of the effect of Anethum graveolens L. crude extracts on serum lipids and lipoproteins profiles in hypercholesterolaemic rats. *DARU*, vol. 16, no. 2, pp. 88-94,(2008).
[14] Ishikawa, T M., Kudo, M.. and Kitajima J. Water-soluble constituents of dill. Chem Pharm Bull; 55:501-507.(2002).
[15] Kaur, G J. and Arora, D S. Bioactive potential of Anethum graveolens, Foeniculum vulgare and Trachyspermum ammi belonging to the family Umbelliferae - Current status. *Journal of Medicinal Plants Research*,vol. 4, no. 2, pp. 87-94,(2010).
[16] Bader, N., Arshad, M. and Farooq, U. Characteristics of Anethum graveolens (Umbelliferae)) seed oil: Extraction, composition, and antimicrobial activity. *International Journal of Agriculture and Biology*, vol. 10, pp. 329-332,(2008).
[17] Tian, J., Ba, X., Zeng, H., Huang, B., He, J. and Wang,Y. Invitroand in vivo activity of essential oil from dill (Anethum graveolens L.) against fungal spoilage of cherry tomatoes. *Food Control*, vol. 22, pp. 1992-1999,(2011).
[18] Angaji, E. B. S. A., and Angaji, S. M. Antimicrobial effects of four medicinal plants on dental plaque. *Journal of Medicinal Plants Research*, vol. 3, no. 3, pp. 132-137,(2009).
[19] Salah, H. F. Effect of some medicinal plant extract on antibiotic resistance by plasmids of Escherichia coli isolated from different sources. (2007). (Doctoral dissertation, M. Sc. Thesis, College of Education, University of Salahaddin).
[20] Kivanc, M., and Kunduhoglu, B. Antimicrobial activity of fresh plant juice on the growth of bacteria and yeasts. *Journal of Qafqaz University*, vol. 1, no. 1, pp. 27-35,(1997).
[21] Kuroda M., Ohta T., Uchiyama I., Baba T., Yuzawa H., Kobayashi I., Cui L., Oguchi A., Aoki K., Nagai Y., Lian J., Ito T., Kanamori M., Matsumaru H., Maruyama A., Murakami H., Hosoyama A., Mizutani-Ui Y., Takahashi NK., Sawano T., Inoue R., Kaito C., Sekimizu K., Hirakawa H., Kuhara S., Goto S., Yabuzaki J., Kanehisa M., Yamashita A., Oshima K., Furuya K., Yoshino C., Shiba T., Hattori M., Ogasawara N., Hayashi H. and Hiramatsu K. Whole genome sequencing of methicillin-resistant Staphylococcus aureus. Vol. 357, pp. 1225-1240.(2001).

[22] Rods, G. P. UK Standards for Microbiology Investigations.(2014).

[23] Basha, N. S., Rekha, R., Komala, M., and Ruby, S. Production of Extracellular Anti-leukaemic Enzyme Lasparaginase from Marine Actinomycetes by Solidstate and Submerged Fermentation: Purification and Characterisation. *Tropical Journal of Pharmaceutical Research*, vol. 8, no. 4,(2009).

[24] Klein, E., Smith, D. L., and Laxminarayan, R.. Hospitalizations and deaths caused by methicillin-resistant Staphylococcus aureus, United States, 1999-2005. Emerging infectious diseases, vol. 13, no. 12, 1840. (2007).

[25] Stavri, M., and Gibbons, S. The antimycobacterial constituents of dill (Anethum graveolens). Phytotherapy Research, vol. 19, no. 11, pp. 938-941, (2005).

[26] Cowan, M. M. Plant products as antimicrobial agents. Clinical microbiology reviews, vol. 12, no. 4, pp. 564-582, (1999).

[27] Kaur, G. J., and Arora, D. S. Antibacterial and phytochemical screening of Anethum graveolens, Foeniculum vulgare and Trachyspermum ammi. BMC complementary and alternative medicine, vol. 9, no. 1, (2009).