Antibacterial and Antioxidant activates Alcoholic extract of *Anethum graveolens* against some pathogenic bacteria isolates

Dr. Rashid Rahim Hateet.
College of pharmacy - University of Misan

Abstract

The antibacterial activity of alcoholic extract of Dill (*Anethum graveolens*) .It has been evaluated for antibacterial activity (agar well diffusion method), Antibacterial activity of Dill alcoholic ethanolic extract was assessed on five clinical isolates from both gram-positive and gram-negative bacteria which include *Escherichia coli, Staphylococcus aureus* ,*Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Streptococcus pyogenes.* The results showed broad antibacterial activity against both gram-positive bacteria such as *Staphylococcus aureus* and Gram negative bacteria such as *Escherichia coli, Pseudomonas aeruginosa*, *Klebsiella pneumoniae*.The diameter of inhibition zone were 31.5mm and 24.5mm against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* respectively with wide inhibition zone diameters followed.The current study also included identifying Minimum Inhibition Concentration(MIC) of ethanolic extract was less minimum inhibitory concentration is (50 µg\ml) against *S. aureus* While the highest Minimum Inhibition Concentration(MIC) is200 µg\ml towards both bacterial species *P.aeruginosa* and *Streptococcus pyogenes*. The antioxidant activity was analyzed using2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assays .A verification of non-toxicity of the plant extract against human blood revealed a negative test.

Keywords : Antibacterial ,
bacteria , Alcoholic extract, Antioxidant .

INTRODUCTION

Dill (*Anethum graveolens*), also known as dill-weed, belongs to family *Umbelliferae*, is an annual herb growing to a height of 1.5 m. Dill originates from Mediterranean and West Asia. Its seeds are used in tea, breads, soups, salads and preserves. while its leaves are commonly used in salads and tea, It is cultivated for use as a vegetable and also as a source of essential oil. Its medicinal uses are as an antispasmodic, carminative, diuretic, stomachic and stimulant (Simon *et al*., 1984).The development of resistance to the antibiotics by the pathogens increases mortality and severe effects (Winstanley *et al*.,1997). The increased usage of specific antimicrobials correlates with the increased levels of bacterial resistance to those agents (Mordi and Erah,

2006). At present, the isolation of bacteria susceptible to regular antibiotics and recovery of resistant isolates during antibacterial therapy is found to be a global problem (Muhammad and Muhammad, 2005).There has been also an increasing incidence of multiple resistances in pathogenic microorganisms recent years, due to the indiscriminate use of commercial antimicrobial drugs for the treatment of infectious diseases (Shareef, 2011). There is a quest for new drugs to manage different challenging diseases (Cohen, 1991). Plants are rich in alkaloids and other phytochemical contents and many of them are effectively used to cure a wide range of diseases (Chitravadivu *et al*., 2009). Medicinal plants have been traditionally used for pharmaceutical and dietary therapy in long history. A number of herbs and many relevant prescriptions have been screened and used for treating and preventing various tumors and inflammations as folk practices. Plants remain the basis for development of modern drugs; medical plants have used for years in daily life to treat diseases all over the world and all the researchers are looking for them (Jimene Medina *et al*., 2006). Herbs or herbal extracts contain different photochemical with biological activity that can provide therapeutic effects. Research interest has focused on herbs that possess hypolipidemic, antitumor,antiplatelets , or immune-stimulating properties that may be useful in helping reduce the risk of cardiovascular diseases and cancer (Abu harfeil *et al*., 2000). The extracts from aromatic plants have long been used for medicinal purposes (Pascal *et al* ., 2002).The aim of this work is to conduct a comparative study of the antimicrobial activity and also to measure the minimum Inhibitory concentration (MIC).

MATERIALS AND METHODS

Sample preparation and extraction:

Fresh whole of *Anethum graveolens L*.(leaves and stem) sample was purchased from market in Misan City, Iraq. The sample was obtained from retail spice-sellers in the amount of 1 kg. Grinding was done with grinder MJ-176P in the laboratory. The sample was kept in closed containers after being chopped into small pieces. For the preparation of solvent extract, 20ml of methanol was added into10 g of *Anethum graveolens L*. put in flask with 250ml chopped and the mixture leptat room temperature for 72hrs, after that, the sediments have been filtered through(Wattman No.1). The solvent was evaporated by air convection oven at 38°C. The weight of resulted extract was measured by grams and kept at 4°C until use (Wilson, 1995).

The test microorganism

All clinical bacterial isolates were obtained from AL- Sadder hospital which include *Esherichia coli* from urine, *Staphylococcus aureus* from Ear, *Streptococcus pyogenes* from Throat , *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* from burns has been collected all bacterial isolates were maintenance by culturing on specific media until use.

The antimicrobial activity test:

The antimicrobial activity was performed with the disk diffusion method (Deans and Ritchie, 1987). A test culture of each bacterial strain was prepared in a concentration of (1×10^6) cell per ml depending on the McFarland opacity standard.Mueller Hinton agar plates were inoculated with 0.1 ml of each bacterial solates (1×10^6) cell per ml by spreading method and left inoculated plates for 10min then one well of 6 mm diameter was punched into agar, of each Petri dish. A volume of 10 microliter of extract and dissolved extract in alcohol as a control were inoculated wells. They kept at 30 minute before they were transferred to incubator. The plates were incubated at 37°C for 24 h. the diameter of inhibition zones was measured. Each experiment was done in three replicate.

Micro dilution method of determination of the minimal inhibition concentration (MIC)

The MIC values were determined for the inoculums of the bacteria strains were prepared from 12 Mueller- Hinton broth cultures and suspensions were adjusted to The McFarland opacity standard The 96-well plates were prepared by dispensing into each well 100 µl of nutrient broth and 5 µl of the inoculum. A 100 µl aliquot from the stock solutions of each plants extract was added into the first wells. Then, 100 µl from the serial dilutions were transferred into eleven consecutive wells. The last well containing 195µl of nutrient broth without plants extract and 5 µl of the inoculum on each strip were used as the negative control. The final volume in each well was 200 µl. The plates were incubated at 37°C for 18 to 24 h. The plants extract tested in this study was screened two times against each strain. The MIC was defined as the lowest concentration of the compounds to inhibit the growth of the microorganisms.

Antioxidant activity

The DPPH radical scavenging capacity was measured according to Hanato *et al*. (1988). 1 ml of plant extract was mixed with 0.5 ml of 0.2 mM methanolic DPPH solution. The reaction was allowed to stand at room temperature in the dark for 30 min and the absorbance was recorded at 517 nm against a blank(methanol solution). Dilutions were made to obtain concentrations of 1000, 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.90 and 1.95 µg/ml. Tests were carried out in triplicate. The ability to scavenge the DPPH radical was calculated using the following equation:

Scavenging effect (%) = [(A0-A1)/A0] × 100, where A0 and A1 are the absorbance of the control and the sample, respectively.

Cytotoxicity Studies by Haemolytic Activity

The cytotoxicity was studied by examining haemolytic activity against human red blood cells (RBCs) using Running tap water as positive control. The positive control showed about 100% lysis, whereas the phosphate buffer saline (PBS) showed no lysis

of RBCs as Negative control. When the effect of extract plant were compared with the controls,(Muhammad *et al*.,2012).

Statistical analysis

Statistical analysis of data. Analysis of variance (ANOVA) was used to determine the significance ($p \leq 0.05$) of the data obtained in all experiments.

RESULTS AND DISCUSSION

The antibacterial activity of Dill extract against selected bacterial strains was assessed (Table 1). The results from the agar well diffusion method revealed that the extract showed significant to moderate antibacterial activity toward all strains tested. The maximum inhibition zone was 31.5 mm against *K.pneumoniae*.followed by *Ps.aeruginosa* and *S. aureus*(24.5mm) while the minimum zone of inhibition was found to be 18.5 mm in diameter against *E.coli* ,the antibacterial activity of(dill)perhaps because contain on some compounds as alkaloids, anthraquinones, glycosides, flavonoids, tannins, steroids, phlobatanins, triterpenoids and saponins. Other studies refers to the antibacterial activity of dill for example (Dahiya and Purkayast;2012) , Earlier studies on extract of *A. graveolens* revealed its antimicrobial potential (Aggarwal*et al.*,2001; Pascal *et al* .,2002). The antimicrobial activity of *A. graveolens* against *E. coli*, *Salmonella typhi*, *Bacillus subtilis* and *S. aureus*, has also been reported by Badar *et al* .,(2008). The lack of antibacterial activity in dill extract may also be due to absence or denaturation of some active components of extract which are responsible for the bacteriostatic or bactericidal activities(Ahmad *et al.*,2001). Our results are consistent with the reports of previous investigators (Table 1) (Figure1).The control plate did not exhibit inhibition on the tested bacteria.The antimicrobial potential of alcoholic extract was investigated using the minimal inhibitory concentration (MIC) assay (Table2).The Alcoholic extract showed antimicrobial activity with a MIC of 50 µg/ml against *Staphylococcus aureus*100 µg/ml against *Escherichia coli* and *Klebsiella pneumoniae* while was 200µg/ml against *pseudomonas aeruginosa* and *Strep. pyogenes*. These results are in agreement with those of another study reporting that Dill extract exhibited antibacterial activity against a large number of bacteria reported the MIC (Delaquis *et al* .,2002). It was observed that the scavenging activity of ethanolic extract of Dill at all concentrations from 1000 to 1.95 µg/ml is rather strong (10 -70 %). The extract improved 70% inhibition at higher concentrations, indicating lesser antioxidant capacity than positive control Figure (2) .

Table 1: Antibacterial activity of Dill alcoholic extract determined by agar well diffusion method.

	MIC(µg/ml)				
Bacterial strains	*E.coli*	*S.aureus*	*Strep.pyogenes*	*kleb.pneumonia*	*Ps.aeruginosa*
Extract	100	50	200	100	200

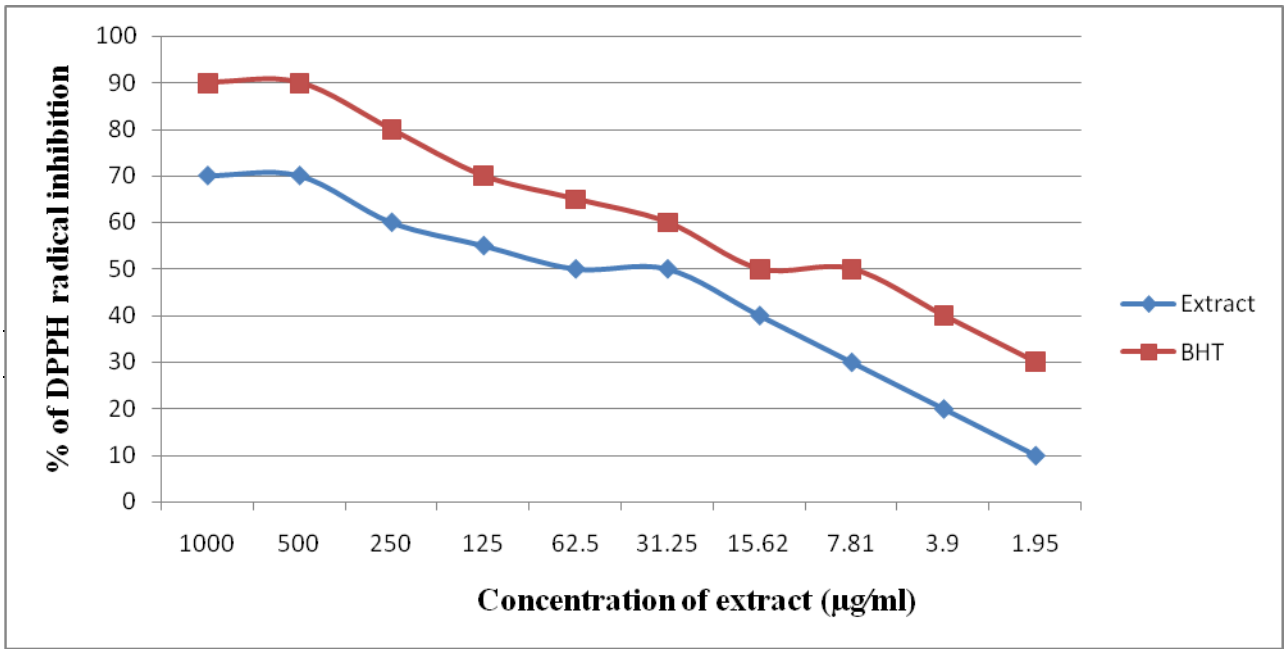
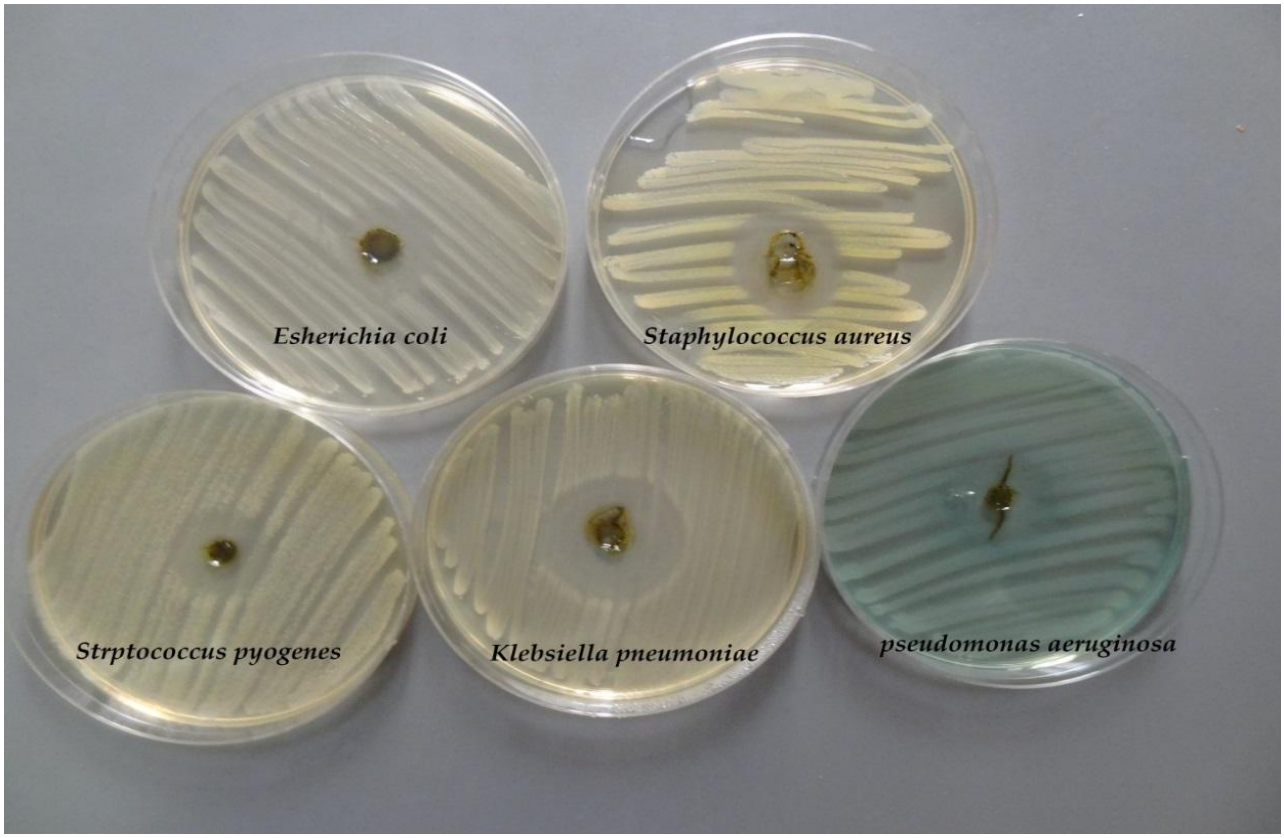


Figure 2:Antioxidant (DPPH scavenging) activity of investigated plant extract presented as percentage of DPPH radicals inhibition


Esherichia coli
Staphylococcus aureus
Strptococcus pyogenes
Klebsiella pneumoniae
pseudomonas aeruginosa

الفعالية الضد بكتيرية والضد تاكسدية للمستخلص الكحولي لنبات الشبنت *Anethum graveolens*ضد بعض العزلات البكتيرية المرضية

د.رشيد رحيم حتيت

كليه الصيدلة – جامعة ميسان

الخلاصة

تضمنت الدراسة الحالية تحضير المستخلص الكحولي الايثانولي لنبات الشبنت*Anethum graveolens* وتقييم فعالية هذا المستخلص تجاه بعض العزلات البكتيرية المرضية باستخدام طريقة الانتشار بالحفر .اذ تم تقييم فعالية المستخلص الكحولي تجاه خمسة انواع من البكتيريا السالبة والموجبة لصبغة كرام وهي *Escherichia coli, Staphylococcus aureus ,Pseudomonas aeruginosa*, *Klebsiella pneumoniae* و*.Streptococcus pyogenes* . وقد اظهر المستخلص الكحولي لنبات الشبنت تاثيرا واضحا تجاة العزلات البكتيرية المختبرة باقطار تثبيط مختلفة فقد بلغ اعلى قطر تثبيط 31.5 ملم تجاه العزلة *Klebsiella pneumonia* تلاه قطر تثبيط بلغ 25.5 ملم تجاه العزلة *Pseudomonas aeruginosa* اما اقل قطر تثبيط فقد بلغ 18.2ملم تجاه العزلة *Escherichia coli* .حددت قيمة التركيز المثبط الأدنى (MIC) وبلغت 50 ميكروغرام /مل تجاه العزلة *Staphylococcus aureus* بينما كان أعلى تركيز مثبط ادنى هو200 ميكروغرام /مل تجاةالعزلة *Pseudomonas aeruginosae*.كما قيمت الفعالية الضدتاكسدية باستخدام 2, 2-diphenyl-1- picrylhydrazyl (DPPH) كما اختبرت السمية الخلوية للمستخلص والذي لم يظهر أي سمية تجاه كريات الدم الحمر عند التراكيز المختبرة

الكلمات المفتاحية: الفعالية الضدبكتيريه , البكتريا المرضية,المستخلص الكحولي ,الفعالية الضد تاكسدية.

REFERENCES:

Ahmad, I.and Beg, A.Z.(2001) Antimicrobial and phytochemical studies on45 Indian plants against multi-drug resistant human pathogens. J.pharmacol. 4:113-123.

Aggarwal KK. Khanuja SPS. Ahmad A. Kumar TRS, Gupta VK, KumarS.(2001) Antimicrobial activity profiles of the two enantiomers of limonene and carvone isolated from the oils of *Mentha spicata* and *Anethumsowa*. FlavFragr J; 17: 59–63.

Abu Harfeil, N.M., A. Maroqa,.and S. Vonkleist 2000. Augmentation of natural killer cell activity invitro against tumor cells by wild plants from Jordan. J. Ethnopharmacol., 71:55-63.

Badar N, Arshad M, Farooq U. (2008).Characteristics of *Anethum* graveolens (umbelliferae) seed oil: extraction composition and antimicrobial activity. Int J. AgriBiol; 10: 329–333

Burt, S.A. 2004. Essential oils: their antibacterial properties and potential applications in foods: a review. Inter. J. Food Microbiol., 94: 223- 233.

Cohen, J.I., Alcorn, J.B., Potter, C.S. 1991. Utilisation and conservation of genetic resources, international projects for sustainable. Agri. Econ. Bot., 45: 190-199.
Chitravadivu, C., Manian, S., Kalaichelvi, K.(2009). Middle-East J Sci.

Res., 4(3): 147-152.

Cohen ML (2002). Changing patterns of infectious disease.Nat.,406:762-767.

Cowan MM (1999). Plant products as antimicrobial agents.Clin.Microb., 22: 564-582.

Dahiya P, Purkayastha S.(2012) Phytochemical analysis and Bacterial efficacy of DILL seed oil against multi-drug resistance clinical isolates. Int J Pharmacy Pharmaceutical Sci. vol 5:60-64.

Delaquis P., K. stanich ,B.Girard, and G.Mazza. (2002) Antimicrobial activity of individual and mix fractions of dill, cilantro, coriander and eucalyptus essential oils. Int. J. Food Microbial. 74 (1- 2):101- 109.

Hatano T, Kagawa H, Yasuhara T, Okuda T, 1988. Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effect. Chem Pharm Bull. 36(6): 2090 -2097.
Hostettmann K, Hamburger M (1991). Antimicrobial effect of some Native plants. Phytochem., 30: 3864-3874.

Harborne, J. B. (1973). Phytochemical Method.Champman and Hall. London, New York.

Jana S, Shekhawat GS (2010) *Anethumgraveolens:* An Indian traditional medicinal herb and spice. Phcog Rev; 4: 179-184.

Jimenez-Medina, E., A.Garcia-lora, ,L.Paco, , I.Algarra., A. collado, and F.Garrido(2006) . A new extract of the plant calendula of ficinalis produces a dual invitro effect: cyto& toxic anti-tumor activity and lymphocyte activiation. BMC. Cancer, 6: 119-132.

Kordali, S., Kotan, R., Mavi, A., Cakir, A., Ala, A., Yildirim, A.(2005) Determination of the chemical composition and antioxidant activity of the essential oil of *Artemisia dracunculus*and of the antifungal and antibacterial activities of Turkish *Artemisia absinthium, A. dracunculus, A. santonicum ,and A.spicigera* essential oils. J. Agri. Food Chem., 53: 9452-9458.

Malika N, Mohamad F, Chakib EA (2004). Antimicrobial activity of natural honey from aromatic and medicinal plants on antibiotic resistant strains of bacteria. Agric. Biol., 6: 289-293.

Mordi, R.M, Erah, P.O. 2006. Susceptibility of common urinary tract solates to the commonly used antibiotics in a tertiary hospital in southern Nigeria. Afr. J. Biotechnol., 5(11): 1067-1071.

Muhammad, H.S., Muhammad, S(2005) The use of Lawsoniainermis Linn. (henna) in the management of burn wounds infections. Afr. J. Biotechnol., 4(9): 934-937.

Pascal JD, Stanich K, Girard B, Mazza G.(2002) Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and *Eucalyptus* essential oils.Int J Food Microbiol; 74: 101–109.

Simon JE, Chadwick AF, Craker LE. (1984). Herbs: An Indexed Bibliography, 1971-1980, The scientific literature on selected herbs and aromatic and medicinal plants of the temperate zone, p:770 . Archon Books, Hamden, CT , The Shoe String Press, Inc., USA.

Shareef, A.A(2011) Evaluation of antibacterial activity of essential oils of *Cinnamomum* sp. and *Boswellia* sp. J. Basrah Res., 37(5): 60-71.

Winstanley, TG ., Limb, D.L., Egginton, R., Hancock, F. (1997) A 10m year survey of the antimicrobial susceptibility of urinary Tract isolates in the UK: the microbe based project. J. Antimicrob .Chemother., 40: 591- 594.

Who H (2001) . Global Strategy for Containment of Antimicrobial Resistance. Availabe on Internet at: www.who.int/emcdocuments.