

## **ANTIMICROBIAL ACTIVITY AND THE MEDIAN LETHAL DOSE OF DILL (*Anethum Graveolens* ) EXTRACT .**

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### **ABSTRACT**

Antimicrobial activities of dill (*Anethum graveolens*) extract were studied by agar well diffusion technique against tested organisms (*Staphylococcus aureus*, *Esherichia coli* and *Candida albicans*), which were streaking onto agar plates and incubated for 16-20 hours at 37°C. The results were showed that the extract did not show antibacterial activity, while the extract of dill seeds exhibited growth inhibition of *C. albicans*. The results were also showed that the median lethal dose (LD<sub>50</sub>) of the ethanolic extract of dill in laboratory white mice was about (1486 mg/kg Bodyweight B.W ). The clinical signs during 24 hours after subcutaneous injection of the extract were rapid breathing, dullness, then death.

### **INTRODUCTION**

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 Z-Medina *etal.*, 2006). Herbs or herbal extracts contain different phytochemicals with biological activity that can provide therapeutic effects. Research interest has focused on herbs that possess hypolipidemic, antiplatelets, antitumor or immune-stimulating properties that

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Received for publication Dec. 15 , 2009 .

Accepted for publication May 6 , 2010 .

may be useful in helping reduce the risk of cardiovascular diseases and cancer (Abuharfeil *et al.*, 2000).

The extracts from aromatic plants have long been used for medicinal purposes. The antimicrobial properties of essential oils obtained from aerial parts and seeds of aromatic plants, such as rosemary (*Rosmerinus officinalis*), eucalyptus (*Eucalyptus dives*) and dill (*Anethum graveolens*) are well documented and the antimicrobial efficiency of different plants essential oils has been studied previously, (Soylu, *etal.* 2006; Elgayyar, *etal.*, 2001; Delaquis, *etal.*, 2002; Aurelli, *etal.*, 1992; and Piccaglia, *etal.*, 1993).

Dill (*Anethum graveolens*), also known as shapt is from the family umbelliferae, The major compounds found in the essential oil of dill includes furanocoumarin, oxypeucedanin, xypeucedanin hydrate and falcarindiol, These compounds are reported to have various degrees of antimycobacterium activity (Stavri and Gibbons, 2005). D- carvone in dill extract, have also been shown to possess high antifungal activity against *Candida albicans* (Jirovetz, *etal.*, 2003).

Little work has been published on the antimicrobial properties of dill utilizing bacterial and fungal isolates. This study was conducted to evaluate the antimicrobial activity of extracted dill and its seeds on the growth of *Staph.aureus*, *E. coli* and *C. albicans* with determination of median lethal dose (LD<sub>50</sub>) of dill extract in laboratory white mice.

## MATERIALS AND METHODS

### 1. The plant:

Mature and fresh dill and its seeds were purchased from local vegetable market in Baghdad city and they classified by Botany department, college

of science, university of Baghdad as *Anethum graveolens L.*, family umbelliferae.

2. Plant extraction:

According to the Harborne, *etal.*, 1975, ethanolic extract of dill and its seeds have been prepared as follow, fifty grams of fresh plant leaves and stem have been put in flask with 250ml of 70% ethanol and stirred on magnatic stirrer at room temperature for 72hrs, after that, the sediments have been filtered through cotton gauze then by filter paper. 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. One hundred grams of dill seeds were macerated in a mortar and pestle and then saturated with ethanol 70% in separated flask with frequent shaking. The solvent with dissolved seeds were filtered and the filtrate was allowed at room temperature for 24 hrs for ethanol evaporation. The extracted volatile oils were stored refrigerated at (4 C) .

3. The test microorganism:-

*Staph. aurens*, *E.coli* and *C. albicans* which were isolated from clinical cases in Baghdad hospital and identified in their laboratories. The organisms were cultured on maintenance media.

4. The antimicrobial activity test:

The antimicrobial activity was performed with the agar diffusion method (Deans and Ritchie, 1987). A test culture of each bacterial strain was prepared in Mueller Hinton broth to a concentration of  $1 \times 10^6$  organism per ml. The inoculated agar plates were prepared by mixing 1 ml of test culture in 25 ml of Mueller- Hinton agar. After the agar was solidified,

four wells of 4 mm-diameter were punched into agar, of each Petri dish. A volume of 100 microliter of dissolved extract in ethylene glycol and oil were inoculated into three wells and a pure ethylene glycol was inoculated into the fourth well as a control. To allow the dissolved extract and oil to diffuse into agar plates, they kept at 20°C for 30 minutes before they were transferred to incubator. The plates were incubated at 37°C for 48 h. The anticandidal activity of dill extract and oil were conducted by dispensing 15 ml of sterile sabouraud dextrose agar. The inocula were prepared by addition of 1 ml overnight *Candida* culture to 9 ml of Mueller- Hinton broth to yield  $10^4$  colony forming unit (CFU) per  $\mu\text{l}$  of the inoculum. 100 $\mu\text{l}$  of dissolved dill extract and seeds oil were inoculated into the wells as above using a dimethyl sulfoxide as a control. Antimicrobial activity was recorded as the width (mm) of the clear zone of inhibition surrounding the agar well. The results were reported as positive (+) if there is inhibition of growth and negative (-) if there is no growth inhibition. Triplicates sets of plates were prepared, the mean of three readings was calculated.

5. Estimation of median lethal dose ( $\text{LD}_{50}$ ) of extract:-

A- Experimental animals: Adult swiss albino balb/C mice (25-30 gm B.W) have been used to estimate the subcutaneous median lethal dose of ethanolic extract of dill. The animals were kept in well air conditioned rooms at the animal house of physiology and pharmacology department, college of veterinary medicine, university of Baghdad, given pellets of balanced specially prepared animal feed and water.

- a. Determination of (LD<sub>50</sub>) Pilot Study: Four mice have been used for determination the ranges of lethal doses that used in acute toxicity study, two mice for each selected dose of extract. The selected doses were 1400 mg/kg B.W, and 1800 mg/Kg B.W according to the lethal outcome (death one or two mice) . The range of acute toxicity doses have been selected.
- b. Estimation of (LD<sub>50</sub>) Thirty mice have been divide into five groups six mice each . the extract was subcutaneously administered by use of the following lethal doses:

Group I= 1400 mg/kg B.W

Group 2= 1500 mg/kg B.W

Group 3= 1600 mg/kg B.W

Group 4= 1700 mg/kg B.W

Group 5= 1800 mg/kg B.W

The animals have been watched for 24 hours for the development of toxicity symptoms and lethality. the log dose-probit response curve was done from which the (LD<sub>50</sub>) has been determined (Katzung, 2003).

## RESULTS

### 1) The antimicrobial activity of dill extract:-

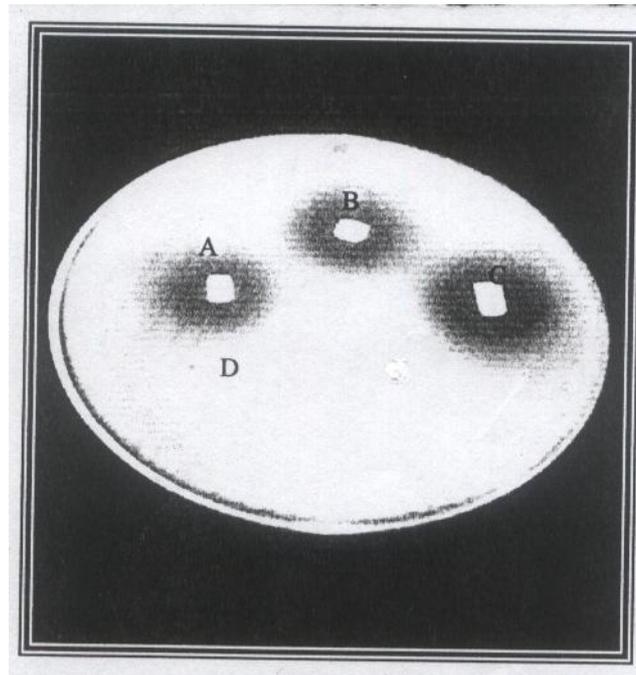
Table (1) shows the invitro activity of dill extract and oil on the growth of *S. aureus*, *E. coli* and *C. albicans*. The leaves and stem extract of the dill did not show any inhibition of growth of test organisms. The extracted seed oil showed that no growth inhibition in *Staph. aureus* and *E.coli*

except *C.albicans* where considerable growth inhibition zone (mm) ranged from 18-20 mm. Table (1) and figure (1).

**Table 1.** *The antimicrobial activity of dill extract and its seed on the growth of test microorganisms .*

Dill		Test microorganisms	The mean diameter of inhibition zone (mm)
Leaves stem extract	Seed oil		
–	–	<i>Staph. aureus</i>	–
–	–	<i>E.col:</i>	–
–	+	<i>C. albicans</i>	19

+ : growth inhibition  
– : no growth inhibition



**Figure 1.** *The inhibitory effect of dill seed oil on the growth of C. albicans.*

**A,B,C:** 100  $\mu$ l of dill seeds oil

**D:** dimethyl sulfoxide as acontrol .

2) The median lethal dose (LD<sub>50</sub>):

## a. Apilot study:

The result of this study revealed that the dose which caused the half of death of the mice was 1486 mg/kg body weight, so the doses which are used for the experiment ranges between 1400 and 1800 mg/kg B.W.

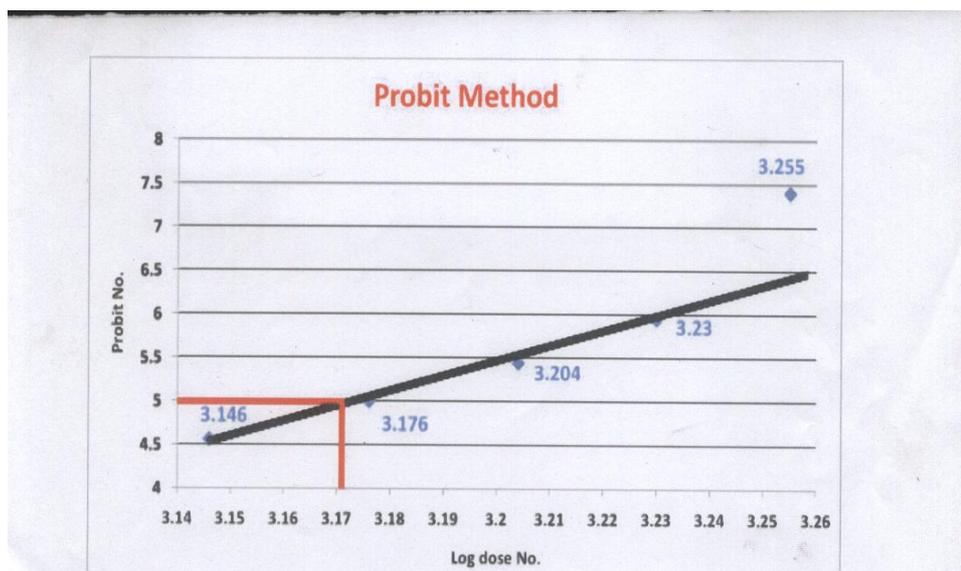
b. Determination of (LD<sub>50</sub>) (probit method):

The mortality percent and conversion to probit number according to the acute toxic doses of dill extract in group 1, 2, 3, 4, and 5 were listed in table (2).

**Table 2.** *Acute toxicity effect of different lethal doses of dill extract in mice.*

Group number	Dose mg/ml	Log dose	Total number	Number of dead animals	Mortality percent %	Probit number
I	1400	3.146	6	2	33.3	4.56
2	1500	3.176	6	3	50.0	5.00
3	1600	3.204	6	4	66.6	5.44
4	1700	3.230	6	5	83.3	5.95
5	1800	3.255	6	6	100.0	7.40

The median lethal dose was measured after logarithm of doses against probit response were plotted from which (LD<sub>50</sub>) was determined by vertical cross link from probit response to the log number dose figure (2). The calculated (LD<sub>50</sub>) has been 1486 mg/kg B.W.



**Figure 2.** Median lethal dose of *Anethum graveolens* in mice by probit method.

## DISCUSSION

The essential oils are natural plant products and their formation and accumulation in plants have been reviewed by Croteau, 1986. The essential oils from aromatic plants are for the most volatile part and thus lead themselves to several methods of extraction such as water and steam distillation and solvent extraction (Guenther, 1972). The specific extraction method depends upon the plant materials to be distilled and the desired end product. The extract of dill showed no inhibitory activity to Gram negative and Gram positive bacteria, this may be due to the high volatility of the oil leading to the escape or evaporation of its major antibacterial constituents during boiling or insufficient release of oil during extraction. The lack of antibacterial activity in dill extract may also be due to absence or denaturation of some active components of the essential oil which are responsible for the bacteriostatic or bacteriocidal activities.

Lember kovics *etal.*, (2003) has recently shown that the composition of essential oils in aromatic plants is greatly affected by the method of extraction mainly the distribution of monoterpenes and azulene sesquiterpene. It has been reported that the antimicrobial properties can vary within the same plant species because the chemical composition and relative proportions of the individual constituents in the essential oils of the plants are influenced by genotype, growth stage, drying, climate, and geographical location (Rhyu, 1979 and Venskutonis, 1996).

A few studies have been reported the anticandidal effect of essential oil of dill seeds (Soylu, *etal.*, 2006 and Elgayyer, *etal.*, 2001). The dill extracts were consistently found to be effective on fungal growth by inhibition of sporangial production.

The ( $LD_{50}$ ) of dill extract was usually taken as one hundredth to one tenth to calculate the treatment dose. Such values provide a convenient way of comparing the potencies of drugs in experimental and clinical setting (Katzung, 2003). The study also showed that the ( $LD_{50}$ ) of dill extract was about 1486 mg/kg B.W and this considered that the extract is safe when injected subcutaneously. There were no previous toxicological studies that focused on the ( $LD_{50}$ ) of dill extract for comparison. The estimation of ( $LD_{50}$ ) may differ in its value among other studies which were achieved, This is due to the differences in dill sources and consequently differences in chemicals composition, the differences in lab . animals used, their species and number, the method of ( $LD_{50}$ ) calculation and other circumstances that related to the researchers.

Further studies will be done to investigate the best extraction method for obtaining the pure essential oils with determination of their antimicrobial activity against other pathogenic microorganism.

In conclusion , this study shows that the dill seeds oil can inhibit the growth of *C.albicans* and this oil can be used in same pharmaceutical preparation as anti candidal agents.

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