THE EFFECT OF *ANETHUM GRAVEOLENS* UPON THE GROWTH OF *E. COLI*

Gabriela ISOPENCU[1], Mariana FERDEŞ[2]

Prezenta lucrare urmareşte evidenţierea efectului antibacterian al principiilor active din mărar (Anethum graveolens) sub formă de ulei esenţial şi pulbere rezultată din plante proaspete de mărar uscate şi macinate. Studiul a fost realizat asupra unei tulpini de Eschericia Coli (K12-MG1655). Studiul s-a efectuat în cultura lichidă, măsurându-se densitatea optică rezultată din creşterea culturii microbiene prin metode spectrofotometrice. Se constată o acţiune antimicrobiană evidentă din partea ambelor materiale utilizate, de aceea pentru a reduce consumul de solvenţi necesari pentru a produce uleiurile esenţiale, se pot utiliză şi plantele uscate, deoarece se constată că şi acestea prezintă activitate antibacteriană pronunţată.

This paper aims to evidence the antibacterial effect of active principles of dill (Anethum graveolens), as essential oil form and as powder of fresh plant of dill, resulting from drying and grounding. The study was conducted on a strain of Eschericia coli (K12-MG1655). The study was performed in liquid culture. It was measured the optical density of the liquid resulted from the microbial culture growth by spectrophotometric methods. It was evidenced an antimicrobial activity of both materials used and therefore we propose, with purpose to reduce solvent consumption needed to produce essential oils, the using of dried herbs, because it appears that they also show a pronounced antibacterial activity.

Keywords: dill, essential oil fresh plant, antibacterial activity

1. Introduction

Essential oil (EO) from plants includes a wide range of plant species, mainly used in the preparation of perfumes, cosmetics, beverages, medicinal foods, disinfectants, insecticides, fungicides, smoking, chewing, tobacco and condiments. These oils are found in various parts (seeds, leaves, fruits barks & roots) of aromatic plants. They can be obtained using various methods (function of destination of use) and refined. EO's contains a large percent of the volatile oils existing in different parts of the plant and other substances extracted during the fabrication process.

[1] Lect., Dept. of Chemical and Biochemical Engineering, University POLITEHNICA of Bucharest, Romania, e-mail: g_isopencu@chim.upb.ro, 021 402 3969

[2] Lect., Dept. of Chemical and Biochemical Engineering, University POLITEHNICA of Bucharest, Romania

The *Anethum graveolens* as dill or sow, how is called in subcontinent, is used as flavoring and preservative agent. Its medicinal uses are as an antispasmodic, carminative, diuretic, stimulant and ameliorate stomach problems.

Delaquis *et al*. (2002) separated heterogeneous mixture of compounds from essential oil of dill by fractional distillation and analyzed by gas chromatography mass spectrophotometery [1].

Constituents of dill include volatile oils, fatty oil, moisture (8.39%), proteins (15.68%), carbohydrates (36%), fibre (14.80%), ash (9.8%) and mineral elements such as calcium, potassium, magnesium, phosphorous, sodium, vitamin A and niacin. Fruits of dill contain 1 - 4% essential oil comprising of major compounds: carvone (30 - 60%), limonene (33%), β-phellandrene (20.61%), including pinene, diterpene, dihydrocarvone, cineole, myrcene, paramyrcene, dillapiole, isomyristicin, myristicin, myristin, apiol and dillapiol (Ishikawa et al., 2002; Raghavan, 2006). Other constituents of dill essential oil are furanocoumarin, 5-(4" - hydroxy - 3"methyl - 2"- butenyloxy)-6, 7 - furocoumarin, oxypeucedanin, oxypeucedanin hydrate and falcarindiol [1], [2].

Anethum graveolens oil contained saturated fatty acids namely: capric (decanoic), lauric (dodecanoic), myristic (tetradecanoic), palmitic (hexadecanoic) and stearic acids (octadecanoic), while unsaturated fatty acids were oleaic, linoleic, linolenic and arachidoic acids. The differences in geometry between various types of unsaturated fatty acids, as well as between saturated and unsaturated fatty acids, play an important role in biological processes and in the construction of biological structures, such as cell membranes. The toxic activity of fatty acids increases in order: oleaic < linoleic < linolenic acid.

It is important that free carboxylic group is necessary for bactericidal activity because ester formation generally decreases this activity of fatty acids (Wyss *et al.*, 1945 by [4]). The reduction of the carboxylic group to an aldehyde or alcohol or, changing to amine or amide group increases bacteriostatic effects [4].

Regarding the antioxidant activity, the antioxidant values reported in μM Trolox /100 g (TE) from DPPH (2,2-diphenyl-1-picyrlhydrazyl) radical scavenging activity of crude seed extracts from plants, *Anethum graveolens* L. *Apiaceae,* dill, is established at 7,828 by Joy R. Borchardt [5].

EO and extracts of dill have been reported to possess various degrees of antimicrobial activity and this property may be probably due to the presence of furanocoumarin in dill [2]. D-limonene and D-carvone, have exhibited strong antifungal activity against *Aspergillus niger*, *Saccharomyces cerevisiae* and *Candida albicans* [2], [3].

Gram-negative bacteria were shown to be generally more resistant than gram-positive ones to the antagonistic effects of essential oils because of the lipo-polysaccharide presents in the outer membrane. It restricts the diffusion of

hydrophobic compounds through its lipo-polysaccharide covering, but this was not always true [6], [7].
All studies to date, regarding the antimicrobial activity of dill oil, were performed on microorganism cultures grown on solid medium. This paper aims to analyze the antimicrobial activity of EO, obtained from dill, and also of plant of dill, dried and transformed in powder dissolved in growth medium, on a liquid culture of *Escherichia Coli.*

2. Material and methods

The bacterium used in the present study is *Escherichia Coli,* K12-MG1655, from Microbiology Laboratory of Politehnica University microorganism collection, Faculty of Applied Chemistry and Material Science. The antimicrobial activity was studied for essential oil of dill.

The EO used contained no synthetic chemicals or unnatural components. The essential oil was stored in brown bottles covered with aluminum foil at 4°C protected from light and air. Two different concentration of the essential oil were used in the experiments in the growth media: 10 μL/L and 20 μL/L respectively.

Fresh plants materials were washed under running tap water, air dried and than homogenized to fine powder and stored in airtight bottles at 4°C. For the experiments it was used a quantity of 1g/L and 2 g/L respectively.

The growth media is a classical **LB** (*Luria Bertani*) liquid medium obtained by classical formula: Triptone, 10 g/L; Yeast extract, 5g/L; NaCl, 5g/L (Merk Chemicals) and tap water, needed for the mineral salts contribution.

Experiments have been accomplished by the following procedure:

- the culture medium was prepared and sterilized;
- the essential oil or powder of fresh plant respectively, was introduced into the environment;
- the culture media obtained, was inoculated with *E. Coli* strain.

Three samples were stored as witness for both cases: a sample contains *E. Coli* inoculated on culture medium without essential oil (**Control sample *E. Coli***). For the experiments involving the EO, two control samples containing culture medium and essential oil in two concentrations was used: (**Control sample OD 10** - samples containing dill essential oil in concentration of 10 μL/L and culture medium; **Control sample OD 20** - samples containing essential oil of dill in a concentration of 20 μL/L and culture medium). For the experiments involving the fresh dried plants, were also used two control samples containing culture medium and power of dill in two concentrations: (**Control sample FD 1** -

samples containing dill powder in concentration of 1 g/L and culture medium; **Control sample FD 2** –

+ samples containing essential oil of dill in a concentration of 2 g/L and culture medium.

Growth of microorganisms in liquid culture of *E. Coli* was followed by spectrophotometer measurements. The measurements are made at 600 nm wave length**.** The OD600 value corresponds with the cell density or cell number in a given *E. Coli* culture volume. OD stands for optical density. Different cell strains may have different cell numbers at a given OD600 value, but OD 600 = 1 usually means there are about 1 x 10^9 cells per ml culture.

The containers were kept at a temperature of 39°C in a shaker under continuous stirring. Samples were taken every hour and analyzed in terms of absorbance achieved.

In order to track differences in absorbance induced by the presence of essential oil it was appropriate to use the two witnesses (culture medium with essential oil in two concentrations) due to the method of analysis. For determining the real absorbance, caused only by growing of the bacteria in the culture medium, was taken into account the level of absorbance induced by this oil, in the growth medium. Thus, the absorbance of the essential oil in the growth medium was measured and, after that the absorbance of the essential oil and bacteria in the growth medium were measured. The growth of the bacteria was visually observed by increasing turbidity of the medium.

3. Results and discussions

a. Experimental study follows the action of EO of dill on microbial growth of *E. Coli*. Values of absorbance for the liquid medium with bacterial inoculum and for control samples were obtained. Absorbance variation over time is shown in Fig. 1.

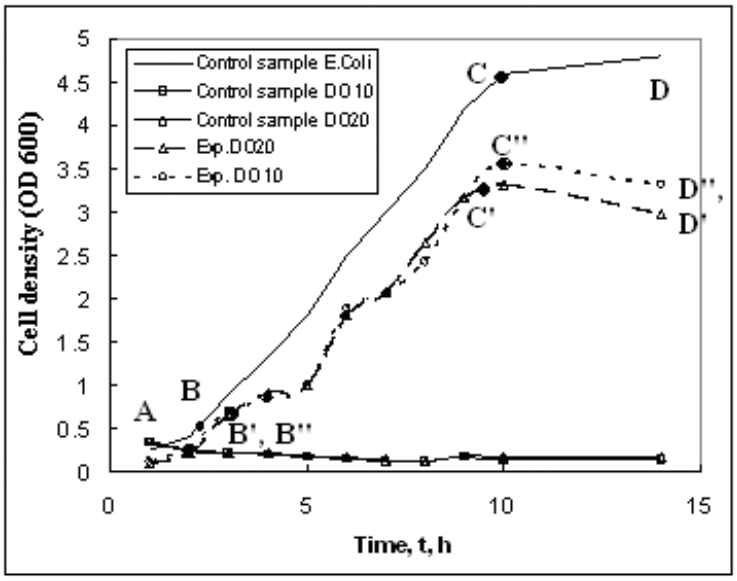

Fig. 1. The variation of the absorbance in time for the *E. Coli* growth in liquid medium with essential oil of dill.

The differences between those two curves of absorbance, corresponding to the exponential zone of growth, show the effects of the EO on the capacity of microorganisms to grow in such conditions. It can be seen that the values of concentration doesn't influence the developing capacity of the microbial culture, only affect the quantity of individuals, expressed through the turbidity degree.

The effect of the EO is evident for the steady zone and for the microorganism inactivation, because in the presence of the oil, the steady zone disappear (at different moments for different concentration of EO), and the microorganisms are inactivated more rapidly, the absorbance tending to the values of the control samples of the oil.

b. The antimicrobial effect of the processed fresh plants by drying, on the *E. Coli* is presented in Fig. 2.

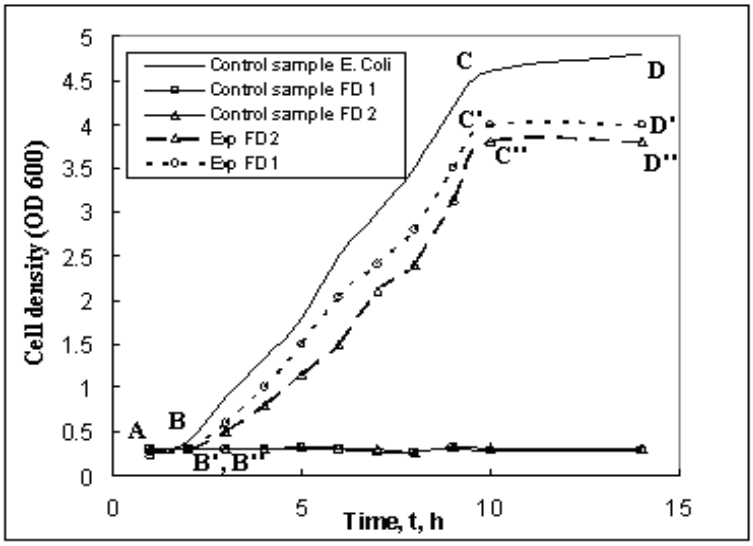

Fig. 2. The variation of the absorbance in time for the *E. Coli* growth in liquid medium with powder of fresh plant of dill.

The inhibition is present in the experiments when in growth medium of *E. Coli* was used powder of dill plant, but not so obvious than when is used EO of dill. The lag zone is smaller, fact which shows that the bacteria easy be accommodates to the growth medium. The exponential zone of growth, in the first period is no more different than that the **Control sample *E. Coli***, but in time, the releasing of the active substances in the culture medium, acts on the microorganism growth.

An empirical equation was determined for the exponential zone of growth, to evidence the kinetics of cell growth express like optical density:

$$\frac{d\,A}{dt} = const \cdot A^{x} \qquad (1)$$

The values for the constant and for the exponent determined from the experimental data were presented in table 1:

Table 1

The parameters of the kinetic equation of E. Coli growth in liquid medium with EO and powder of dill add

Nr. crt.	Probe code	const.	x
1	Control sample E. Coli	0.282	0.531
2	Exp. OD 10	0.162	0.367
3	Exp. OD 20	0.152	0.342
4	Exp. FD 1	0.233	0.341
5	Exp. FD 2	0.208	0.290

The value obtained shows the slightly influence of the quantity of EO used upon the cell density parameter, respectively on the cell growth. The influence of the added active substance is more obvious when it is used powder of fresh dill in culture medium.

4. Conclusions

The effect of the *Anethum graveolens* active substances upon the *E. Coli* growth was evidenced. The active substances come from two different sources: essential oil of dill and powder of dried fresh plant. It was expected that the EO action to be higher than the activity of the powder of fresh plant of dill. The experiments show that the differences are not significant. That is a real motive to use plant or aqueous extract of the active principles of aromatic plants instead of the EO processed through chemical processes.

Acknowledgments:

The work was financially supported by the project POSDRU/89/1.5/S/52432 from 1.04.2010 - Institutional organization of a postdoctoral school of national interest "Applied biotechnology with impact in the Romanian economy"; the project was co funded by the EU Social Fund in the framework of the Sectorial Operational Program 2007-2013 for Human Resources Development.

R E F E R E N C E S

[1]. *P.J. Delaquis, K. Stanich, B. Girard, G. Mazza*, Antimicrobial activity of individual and mixed fractions of dill, cilanro, coriander and eucalyptus essential oils, International Journal of Food & Microbilogy, **vol 74**, 2002, pp.101-109

[2]. *M. Stravri, S. Gibbons*, The antimycobacterial constituents of dill (Anethum graveolens), Phytoterapy Research, **vol. 19**, 2005, pp. 938-941

[3]. *G.J. Kaur, D.S. Arora*, 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, 2010, pp. 087-094

[4]. *N. Badar, M. Arshad, U. Farooq*, Characteristicsof *Anethum graveolens* (Umbelliferae) seed oil: extraction, composition and antimicrobial activity, International Journal of Agriculture &Biology, **vol. 10,** no. 3, 2008, pp.329-323

[5]. *J.R. Borchardt, D.L.Wyse, C.C. Sheaffer, Kendra L. Kauppi, R.G. Fulcher, Nancy J. Ehlke, D.D. Biesboer, R.F. Bey,* Antioxidant and antimicrobial activity of seed from plants of the Mississipi river basin, Journal of Medicinal Plant Research, **vol. 2**, no. 4, 2008, pp. 081-093

[6]. *P.O. Angienda, D.M. Onyango, D.J. Hill,* Potential application of plant essential oils at sub-lethal concentrations under extrinsic conditions that enhance their antimicrobial

effectiveness against pathogenic bacteria, African Journal of Microbiology Research, **vol. 4**, no.16, 2010, pp. 1678-1684

[7]. *Sonali Jana, G.S. Shekhawat,* Phytochemical analysis and antibacterial screening of *in vivo and in vitro* extracts of Indian medicinal herb: *Anethum graveolens*, Research Jourbal of Medicinal Plant, **vol 4**, no.4, 2010, pp. 206-212.