OPEN ACCESS JOURNAL

BIOINTERFACE RESEARCH IN APPLIED CHEMISTRY

ORIGINAL ARTICLE

www.BiointerfaceResearch.com

ISSN 2069-5837

Volume 2, Issue 2, 2012, 300-305

Received: 10.03.2012 / Accepted: 10.04.2012 / Published on-line:15.04.2012

Phytochemical composition of the fennel fruits essential oil and its influence on prokariotic cells growth and pathogenic features

Crina Saviuc [1], Ioana Marinaş[1], Alexandru Mihai Grumezescu[2]*, Coralia Bleotu[3], Carmen Chifiriuc[1], Dan Mihaiescu[2], Veronica Lazar[1]

ABSTRACT

Medicinal plants, repository of phytochemicals with putative biological activity are nowadays subjected for screening in order to assess on a scientific basis the traditional medicine claims. Moreover the antimicrobial terapy shifted from a microbicidal to an antipathogenic concept and synergistic effects in plant extracts mixtures seems to be an apropriate solution for the control of microbial resistance emerging issue. Essential oil was obtained by microwave assisted hydrodistilation method in a Neo Clevenger type apparatus and subjected to GC-MS for the phytochemical analysis. The antimicrobial spectrum was established by the liquid medium microdilution assay, while the putative antipathogenic effect by studying the essential oil effect on biofilm development (microtiter assay using violet crystal staining and flow cytometry). The essential oil prooved to be rich in in anethol. All tested microbial strains were susceptible to the *F. vulgare* essential oil which exhibited anti-adherence properties at subinhibitory concentrations and antibiofilm activity on the preformed experimental fungal biofilms.

Keywords: *fennel fruits, prokariotic cells, essential oils, Foeniculum vulgare*

1. INTRODUCTION

Foeniculum vulgare Mill, commonly known as fennel, is a small genus of annual, biennial or perennial herbs. It is widely cultivated throughout the temperate and tropical regions of the world for its aromatic fruits, which are used as culinary spices. Fennel and its preparations are used to cure various disorders, and also act as a carminative, digestive and diuretic agent. Fennel increases elasticity of connective tissues and act as an anti-aging agent. There are some commercial pharmaceuticals with formula based on fennel essential oil. The antimicrobial properties of the essential oil have been also recognized [1]. The antibiotic era in clinical medicine, launched more than 70 years ago with the introduction of sulfonamides and the stunning success of penicillin which led to a drastic increase in survival from bacterial infections, clearly changed the world. But nowadays, the discovery of new antibacterial strategies represents a real scientific challenge, due to antimicrobial resistance in sessile and planktonic bacteria. Returning to natural products screening seems to be an appropriate solution [2]. A biofilm is a sessile microbial community composed of

[1] University of Bucharest, Faculty of Biology, Microbiology Immunology Department, Romania
*Corresponding author e-mail address: ***grumezescu@yahoo.com***
[2] University Politehnica of Bucharest, Faculty of Applied Chemistry and Materials Science, Bucharest, Romania
[3] S Nicolau Institute of Virology, Bucharest, Romania

cells irreversibly attached to a substratum, to an interface or between them, embedded in a matrix of extracellular polymeric substances produced by themselves and which present a modified phenotype, concerning the growth and gene transcription rate. Between the biofilm'cells a cell-density-dependent mechanism (quorum sensing) for controlling protein expression, including the production of the virulence factors is mediated by small molecules, making this cells different from their counterpart - the free or floating cells, i.e. more resistant to all kind of stress conditions, including the antimicrobial substances [3].The use of herbs as complementary and alternative medicine has faced an important increase in the last 20–25 years. According to World Health Organization (WHO) traditional medicine is used by 65–80% of the world's population for their primary health care needs [4]. Medicinal plants are rich repositories of natural products with pharmacological activity, complex mixtures of compounds as essential oils acting synergically for the achievement of a specific effect. The phenomenon of additive or synergistic effects is often crucial to bioactivity in plant extracts and in some cases, the activity is lost in purified fractions. Development of bacterial resistance to synergistic drug combinations, such as those found in plants, may be slower than for single drug therapies [5]. In this respect, the GC-MS analysis of the essential oil from *Feniculum vulgare* seeds was settled, in order to carry out the phytochemical assessment for the main compounds, with putative antimicrobial and antipathogenic agents.

2. EXPERIMENTAL SECTION

2.1. Essential oil extraction and phytochemical assessment by GC-MS analysis. Dried plant material represented by 150g seeds of *F. vulgare* purchased from a local supplier were subjected to essential oil extraction in a Neo Clevenger type apparatus in microwave assisted conditions, for 1h [6,7]. The obtained essential oil was subsequently dried on Na_2SO_4 anhidrous and stored in dark botles at 4°C untill further analysis. For GC-MS phytochemical assessment the essential oil was diluted 1:1000 with CH_2Cl_2 and injected. Gas chromatographic analysis was performed using an Agilent 6890 Series GC System. Detection was carried out with a 5973 mass-selective single quadrupole detector (Agilent technologies). Operation control and data process were carried out by Agilent Technologies ChemStation software (Santa Clara, CA, USA). The mass spectrometer was calibrated before use with perfluorotributylamine as a calibration standard. The working conditions were: H_2-carrier gas, flow: 1.2 ml/min, temperature program 50/300^{o}C with a ramp rate of 5^{o}C/min; the temperature of the injector and of the detector was 250^{o}C, and a DB5-MS (30m; 0.25 mm id; 0.25 μm) column.

2.2. Microbial strains. Eight microbial strains, Gram-positive and Gram-negative bacterial strains and a fungal strain, have been used in the study, either ATTC reference or recent clinical isolates, identified by using a Vitek II automatic system (table 1).

Table 1: Gram-positive and Gram-negative bacterial strains and a fungal strain, have been used in the study

N^o	Microbial strains
1	*Enterococcus. faecalis* ATCC 29212
2	*Staphylococcus aureus* ATCC 25923
3	*Bacillus subtilis* ATCC 6633
4	*Pseudomonas aeruginosa* ATCC 27853
5	*P. aeruginosa* 508
6	*Escherichia coli* ATCC 25922
7	*Klebsiella pneumoniae* 802
8	*Candida albicans* ATCC 10231

2.3. Antimicrobial activity screening and minimum inhibitory concentration (MIC) of the essential oil. The antimicrobial activity screening of the essential oil obtained from *F. vulgare* fruits was achieved by serial binary microdilution technique in liquid medium (broth) in 96-well plates. Microbial suspensions obtained from 18h cultures grown on solid medium, were adjusted to an optical density corresponding to the nephelometric standard McFarland 0.5. The concentrations obtained for the serial dilutions of the stock solution (1:1 v / v essential oil: dimethylsulfoxide) covered the 1.53-50µL/mL range. Subsequently, the plates were incubated for 24 h, and MIC was established macroscopically as the lowest concentration which inhibited the microbial growth. We have used a positive (microbial culture) and negative control (culture medium), as well as a vapor phase effect control.

2.3.1. Antipathogenic activity of the essential oil assessment. The influence on the microbial adherence capacity on the inert substratum and the ability to eradicate preformed biofilms of the *C. albicans* ATCC 10231 were studied.

2.3.2. Influence on the microbial adherence capacity on the inert substratum. The 96 multi-wells plates used for the antimicrobial screening protocol were subsequently subjected to violet crystal staining technique in order to assess spectrophotometrically the adhered biomass. Briefly, the method consists in removing microbial culture, fixing the adhered microorganisms by cold methanol for 5 minutes, staining with 0.1% violet crystal solution for 15 min, resuspension of the adhered biomass in 33% acetic acid solution and spectrophotometric reading at 490 nm. The amount of the biomass adhered correlates directly proportional to the absorbance values.

2.3.3. Assessment of the influence of the essential oil on preformed biofilms by flow cytometry. Flow cytometry analysis was carried out on preformed biofilms of *C. albicans* ATCC 10231 on glass coupons. The coupons were incubated in six-well plates (Sabouraud broth) with an inoculum adjusted at McFahrland 0.5 standard, obtained from a 24 h culture. Slides contaminated with adhered microbial cells were washed to remove sedimented cells and immersed in fresh culture medium. The experiment was conducted in temporal dynamic, samples represented by preformed biofilms of 48 and 72h respectively, being subjected to essential oil treatment for 24h. Treatment stock solution concentration essential oil: DMSO (1:2 v / v) used for the experimental biofilms eradication was 105µL/mL (2xMIC) and the incubation time was 24 hours. Treated samples were placed in recipients with sterile saline, sonicated for 30s, vortexed and the obtained suspension was stained using the propidium iodide, which specifically binds to the nucleic acids in cells with impaired coatings. Fluorochrome concentration was 5 µg/mL and the staining protocol was applied at room temperature for 10 min before data acquisition, which was performed using a FACS Calibur cytometer. CellQuest Pro software was used for statistical analysis.

3. RESULTS SECTION

The essential oil extraction yield was 0.8% (v/w) normalized to 100g of dried plant material, the results being in accordance with the literature, 0.31-14.7% extraction yields being mentioned by other authors, depending of the subjected extraction organ of the plant. The qualitative and semi-quantitative essential oil compositions for the microwave assisted hydrodistillation conditions are presented in Table 1. These data are in accordance with the main *F. vulgare* var. *vulgare* anethole chemotype [8,9,10].

Table 1: *Foeniculum vulgare* essential oil composition

Peak	R.T.	%	compounds
3	4.893	0.275	á-Myrcene
4	5.195	0.218	à-Phellandrene
5	5.721	0.192	o-Cymene
6	5.822	8.099	á-Terpinyl acetate
8	6.662	0.351	ç-Terpinene
9	7.445	1.698	Fenchone
11	10.625	80.557	Anethol
14	13.032	0.702	Estragole
15	14.969	3.986	Eugenol
19	16.525	1.277	Caryophyllene
23	19.312	0.275	Eugenol acetate

The screening and MIC assay revealed a broad spectrum of antimicrobial activity for the essential oil against the tested microorganisms, the most sensitive strains being *E. faecalis* ATCC 29212 and *E. coli* ATCC 25922. Kaur and Arora [11] reported a broad spectrum of antimicrobial activity for the *F. vulgarae* fruits essential oil and organic extracts, as well as Gulfraz et al. [1]. They confirm a strain specific activity in a dose dependent manner, the Gram-negative bacteria proving to be generally more resistant than the Gram-positives. Patra et al., [12] also showed a broad fungitoxic spectrum, inhibiting the mycelial growth of the nail-infective fungi, i.e. *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. ustus*, *Candida albicans*, *Epidermophyton floccosum*, *Microporum audouinii*, *M. canis*, *M. gypseum*, *M. nanum*, *Rhizopus nigricans*, *Trichophyton tonsurans* and *T. violaceum*. Moreover, they showed that the *F. vulgare* fruits essential oil did not exhibit any adverse effects on mammalian skin and nails up to 5% concentration. In our study comparative concentrations proved to have inhibitory effect against the tested strains, as shown in figure 1. The vapor phase effect did not affect the assay and the solvent had no inhibitory effect at the tested concentrations against the microbial strains.

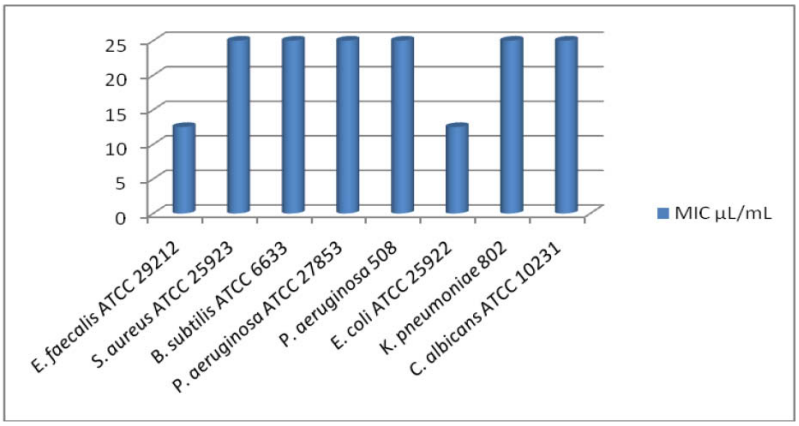

Figure 1: MIC values of the essential oil against the tested microbial strains

Concerning the anti-adherence activity a significant decrease of the 490 nm absorbance values was obtained at MIC/2 concentrations for the *S. aureus* ATCC 25923, *B. subtilis* ATCC 6633, *Ps. aeruginosa* ATCC 27853, and *C. albicans* ATCC 10231 (figure 2).

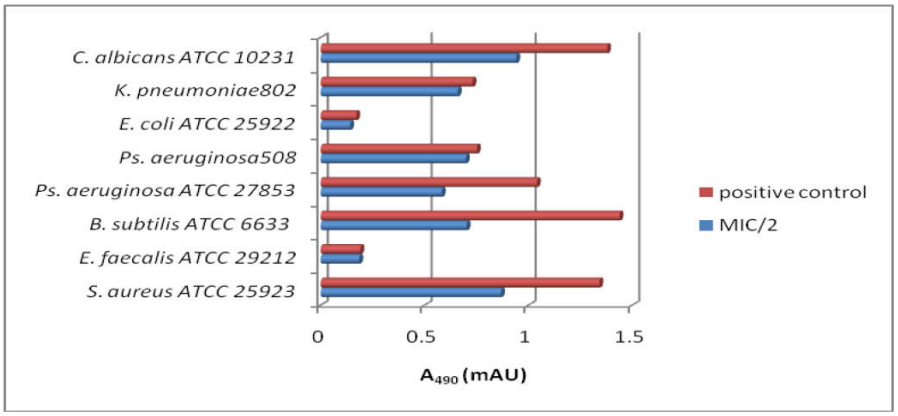

Figure 2: Absorbance values (mAU) for adhered biomass in the presence of MIC/2 concentrations

The evidence of the essential oil antipathogenic effect was reinforced by the microbicidal effect exhibited on the preformed experimental biofilm of the *C. albicans* ATCC 10231 strain of 48 and 72h (figure 3).

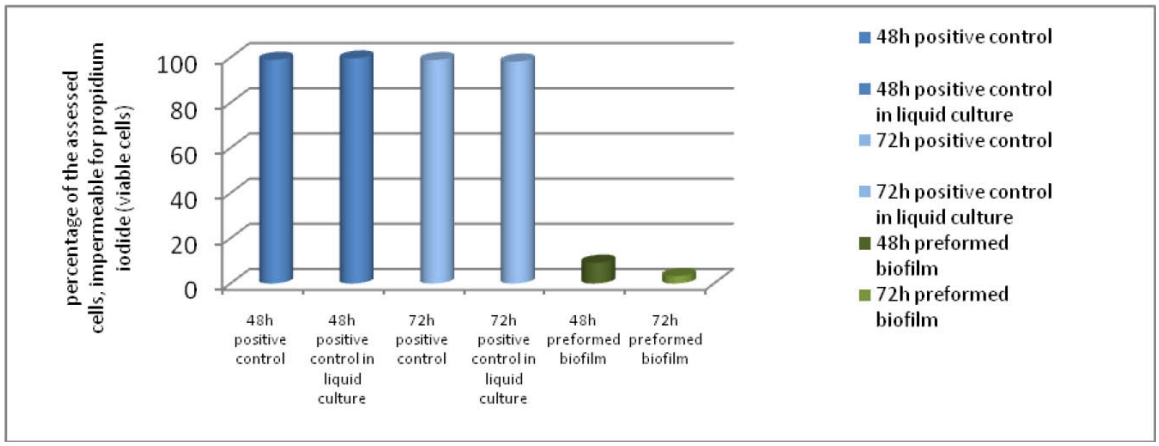

Figure 3: Percentage of viable cells m\measured by flow cytometry (10000 gated events /cells) performed for the positive controls and samples, represented by preformed experimental 48 and 72h biofilms developed on the inert substratum

4. CONCLUSIONS

The *F. vulgare* fruits essential oil extraction yield was 0.8%, with a phytochemical composition rich in anethol. The essential oil exhibited a broad spectrum of antimicrobial activity against Gram positive and Gram negative bacteria and fungal strains, with comparative MIC values, as well as anti-adherence and antibiofilm properties at specific concentrations.

5. ACKNOWLEDGEMENT

The results presented in this work were supported by the Human Resources 135/2010 grant (Contract no. 76/29.07.2010).

6. REFERENCES

[1] Gulfraz M., Mehmood S., Minhas N., Jabeen N., Kausar R., Jabeen K., Arshad G, Composition and antimicrobial properties of essential oil of Foeniculum vulgare, *African Journal of Biotechnology*, 7,24, 4364–4368, **2008**.

[2] Marinaş I., Grumezescu A.M., Saviuc C., Chifiriuc C., Mihaiescu D., Lazar V., Rosmarinus officinalis essential oil as antibiotic potentiator against *Staphylococcus aureus*, *Biointerface Research in Applied Chemistry*, 2, 1, 271-276, **2012**.
[3] Lazar V., Quorum sensing in biofilms e How to destroy the bacterial citadels or their cohesion/power?, Anaerobe, 17, 280-285, **2011**.
[4] Gurinder J.K., Daljit S.A., Antibacterial and phytochemical screening of Anethum graveolens, Foeniculum vulgare and Trachyspermum ammi, *BMC Complement Altern Med*, 9, 30, **2009**.
[5] Quave C.L., Plano L. R.W., Pantuso T., Bennett B. C., Effects of extracts from Italian medicinal plants on planktonic growth, biofilm formation and adherence of methicillin-resistant *Staphylococcus aureus, J Ethnopharmacol*, 118, 3, 418–428, **2008**.
[6] Saviuc C., Grumezescu A.M., Oprea E., Radulescu V., Dascalu L., Chifiriuc M.C., Bucur M., Banu O., Lazar V., Antifungal activity of some vegetal extracts on Candida biofilms developed on inert substratum, *Biointerface Research in Applied Chemistry*, 1, 1, 015-023, **2011**.
[7] Saviuc C., Grumezescu A.M., Holban A., Bleotu C., Chifiriuc C., Balaure P., Lazar V., Phenotypical studies of raw and nanosystem embedded Eugenia carryophyllata buds essential oil antibacterial activity on Pseudomonas aeruginosa and Staphylococcus aureus strains, *Biointerface Research in Applied Chemistry*, 1, 3, 111-118, **2011**.
[8] Piccaglia R, Marotti M., Characterization of some Italian types of wild fennel (Foeniculum vulgare Mill.), *J Agric Food Chem*, 49, 239–44, **2001**.
[9] Kruger H., Hammer K., Chemotypes of fennel (Foeniculum vulgare Mill.), *J Essential Oil Res*, 11, 79-82, **1999**.
[10]Tognolini M., Ballabeni V., Bertoni S., Bruni R., Impicciatore M., Barocelli E., Protective effect of Foeniculum vulgare essential oil and anethole in an experimental model of thrombosis, *Pharmacological Research*, 56, 254–260, **2007**.
[11] Kaur G. J., Arora D. S., Antibacterial and phytochemical screening of Anethum graveolens, Foeniculum vulgare and Trachyspermum ammi, *BMC Complement Altern Med*., 9, 30, **2009**.
[12] Patra M., Shahi S.K., Midgely G., Dikshit A., Utilization of essential oil as natural antifungal against nail-infective fungi, *Flavour Fragr. J.*, 17, 91-94, **2002**.