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Evaluation of the antimicrobial and cytotoxic activity of an aqueous extract of *Anethum graveolens* L. seeds

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Abstract

Anethum graveolens L. (dill) is a popular aromatic herb frequently used as a spice but also for its healing properties which are associated with antioxidant, anti-inflammatory, antibacterial and antiviral actions exerted by the synergistic action of classes of bioactive compounds present in the composition. The main purpose of this study was to evaluate the antibacterial action and cytotoxic potential of a crude aqueous extract of dill seeds. The antibacterial potential determined by the disc diffusion method was observed against E coli. Regarding the cytotoxic activity, evaluated by the MTT method, a reduction of the number of living cells was observed when using the highest concentrations on the SCC-4 tumor line, while at the same healthy line concentrations, PGK was not affected. In-depth studies related to the pharmacological activity of the extract must be carried out in order to be able to decipher the mechanisms of antibacterial or antitumor action.

Keywords: dill, seed extract, antibacterial, cytotoxic, squamous cell carcinoma

1.Introduction

Complementary and alternative medicine (CAM) has been in the public's attention for thousands of years, whether specialized practitioners are involved or consumers, while benefiting from clinical recognition [1,2]. Herbs are mainly used in CAM, due to their smell, aroma but especially due to the beneficial biological properties they exert both for maintaining health and for recovery in various disease processes [3]. It is well known that different drugs currently used are derived from medicinal plants.

Anethum graveolens L. (popularly known as dill), is a member of the Umbelliferae family, and has been used since ancient times (by the Greeks and Romans) as a spice or medicine.

Dill oil contains mainly paraffin hydrocarbon, d-carvone and d-limonene and residues of eugenol, anethole, α-felandren, umbelliferone, triterpenes, flavonic compounds, coumarins [4-6] and in seeds, leaves and roots have been identified numerous compounds belonging to the following classes: tannins, terpenoids, steroidal saponins, flavonoids, cardiac glycosides and anthraquinones [7,8]. The pharmacological properties of dill are related to its antioxidant, anti-inflammatory, antimicrobial and antiviral potential, being a good candidate for maintaining the health of the tissues of the oral cavity and respiratory system [9]. Different studies reported that dill extracts, both aqueous and hydroalcoholic have shown antidiabetic activity [10,11].

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Problems that affect the oral cavity are common and their preventive approach through the use of safe natural resources is desirable.

The main aim of this study was to obtain, characterize and evaluate from the point of view of antimicrobial (using both gram-negative and gram-positive bacteria) and cytotoxic (using a healthy monolayer cell line and a tumor monolayer cell line) activity of an aqueous extract of dill seeds.

2. Materials and methods

2.1. Extract preparation

The plant material was provided by specialists from the Faculty of Agriculture Timisoara. 10 g of dried dill seeds were extracted for 20 minutes with boiling water (MiliQ system Milli-Q® Integral Water Purification System) under stirring, after which the mixture was cooled to room temperature, filtered and subsequently lyophilized. The lyophilized sample, *Anethum graveolens* extract (AGE) was kept in the refrigerator until further determinations.

2.2. Determination of individual polyphenols by LC-MS

The main polyphenols from lyophilized samples were determined by LC-MS using the Shimadzu chromatograph. The system was equipped with two detectors (SPD-10A UV, LC-MS 2010) and an EC 150/2 NUCLEODUR C18 Gravity SB chromatographic column (150 x 2mm x 5 um). The chromatographic conditions applied were: (a) mobile phase A consisting of acidified water (pH = 3), mobile phase B consisting of acidified acetonitrile (pH = 3), (b) gradient program: 0-20 min. 5% B, 20-50 min 40% B, 50-60 min, 95% B, (c) flow rate 0.2 mL / min, (d) temperature 20 °C, (e) monitoring wavelength 280 and 320 nm. The calibration curves were performed in the range of 20-50 ug / mL and the data were expressed in mg/g dry material (d.m.). Standard polyphenolic compounds: Gallic Acid, Protocatechuic Acid, Caffeic Acid, Epicatechin, Coumaric Acid, Ferulic Acid, Rutin, Rosmarinic Acid, Resveratrol, Quercitin, and Kaempherol were aquired from Sigma-Aldrich.

2.3.Determination of antimicrobial activity

In vitro testing of dill extract was assessed against *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), *S. pyogenes* (ATCC 19615), and *S. flexneri* (ATCC 12022) by using the Disk diffusion (DD) method, according to the Standard Rules for Antimicrobial Susceptibility Testing using Impregnated Disks. Evaluations were performed in specific plates, filter papers impregnated with a water used as negative control (NC), microcomprimates with Gentamicin disks (10 mg, E110712, BioMaxima, Poland) for the antimicrobian activity as positive controls (PC), and filter papers impregnated with a known quantity of dill extract. A 10^{-3} dilution of the fresh *S. aureus*, *E. coli*, *P. aeruginosa*, *S. pyogenes*, and *S. flexneri* culture were used to perform the assays, an inoculum equivalent to a 0.5 McFarland standard. The Petri plates so seeded and the respective specimens with the extract were incubated at 37 °C in case of the antimicrobial testing, for 24-48 hours. Tests were performed in duplicate.

2.4. Determination of cytotoxic activity

The cells utilized in the current study were primary gingival keratinocytes (PGK - ATCC® PCS-200-014™) and tongue squamous cell carcinoma (SCC-4 - ATCC® CRL-1624™) purchased from ATCC (American Type cell Collection) as frozen samples and kept in liquid N_2 until the beginning of the experiments. All the specific reagents utilized for cell cultures were obtained from Sigma Aldrich. In brief, cells were seeded onto a 96-well plate in specific media (Dermal Cell Basal Medium supplemented with the keratinocyte Growth Kit for PGK and DMEM:F12 Medium supplemented with fetal bovine serum – 10 % for SCC-4) treated with a mixture of antibiotics and preserved in optimum conditions (humidified atmosphere with 5% CO_2, 37 °C) until a confluence of over 85% is obtained. The cells were stimulated with different concentrations of the dill extract – 10, 15, 25, 50, 75, 100 and 500 µL, respectively and incubated for 24h. Cell viability was assessed by MTT test according to the protocol described in the literature [12].

3.Results and discussions

LC-MS analysis of the Dill aqueous extracts individual polyphenols has shown the presence of rutin and resveratrol in significant quantities along with a series of phenolic acids in different proportions of which ferulic acid proved to be predominant, as can be seen from the table.

Table 1. Individual polyphenolic quantification regarding an aqueous extract from dill seeds

No.	Compound	Retention time (min)	Ion (M/Z)	Conc. mg/ g d.m.
1	*Gallic Acid*	4.6	169	0.11
2	*Protocatechuic Acid*	10.9	153	0.14
3	*Caffeic Acid*	21.4	179	0.23
4	*Epicatechin*	22.6	289	-
5	*Coumaric Acid*	24.2	163	0.61
6	*Ferulic Acid*	24.9	193	4.57
7	*Rutin*	25.5	609	5.06
8	*Rosmarinic Acid*	28.9	359	0.12
9	*Resveratrol*	31.6	227	3.82
10	Quercitin	32.2	301	0.09
11	*Kaempherol*	34.7	285	0.47

Table 2. Antimicrobial activity of dill extract expressed by inhibition zones measured in millimetres

Sample	*S. aureus*	*S. flexneri*	*E. coli*	*P. aeruginosa*	*S. pyogenes*
AGE (1:1)	13/13 mm	7/6 mm	17/17 mm	-	7/7 mm
AGE (1:2)	13/11 mm	-	17/15 mm	-	-
AGE (1:5)	9/7 mm	8/7 mm	15/15 mm	-	6/6 mm
AGE (1:10)	7/7 mm	-	13/11 mm	-	-
Gentamicin (PC)	17/15 mm	13/13 mm	13/13mm	15/15mm	21/21 mm
Water (NC)	-		-	-	-

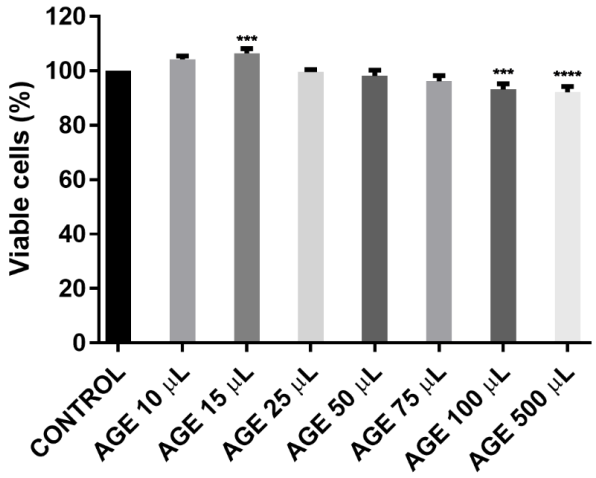

Figure 1. Percentage of PGK viable cells (primary gingival keratinocytes) evaluated *in vitro* after treatment with dill seeds aqueous extract (AGE; 10, 15, 25, 50, 75, 100, and 500 µL) by utilizing the MTT assay. Statistical differences were established by one-way ANOVA analysis, followed by Tukey's post-test (***p < 0.001 and ****p < 0.0001.

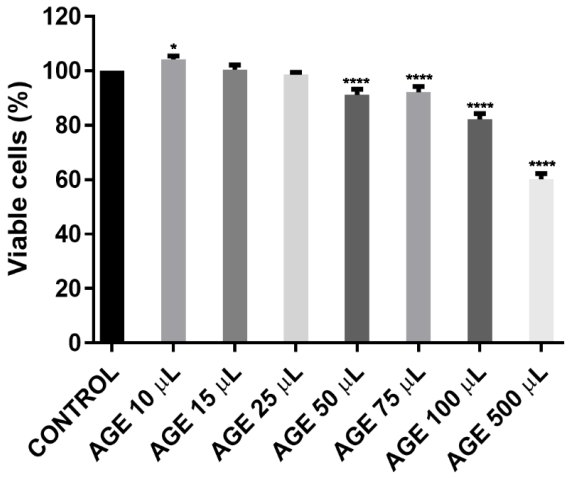

Figure 2. Percentage of SCC-4 viable cells (tongue squamous cell carcinoma) evaluated *in vitro* after treatment with dill seeds aqueous extract (AGE; 10, 15, 25, 50, 75, 100, and 500 µL) by utilizing the MTT assay. Statistical differences were established by one-way ANOVA analysis, followed by Tukey's post-test (*p < 0.1 and ****p < 0.0001).

In the case of carcinoma cells, the effect on viability was much more pronounced especially at the highest concentrations: ~82% for 100 µL and ~60% 500 µL (figure 2).

Studies conducted to evaluate the antiproliferative activity exerted by dill extract highlight the action against cancer cells K-562 (chronic myelogenous leukemia) and NCI-H460 (carcinoma; large cell lung cancer) [18].

At the same time, positive results were highlighted regarding the reduction of viability of breast and liver cancer cells [19,20].

4. Conclusions

The present study showed that *Anethum graveolens* L. seed aqueous extract is rich in phytocompounds which according to the data are responsible for the pharmacological properties exerted. The antimicrobial activity was increased against *E. coli* and for the rest of the strains studied (*S. aureus*, *S. flexneri*, *P. aeruginosa*, and *S. pyogenes*) it is not significant. In addition, the extract has been shown to be harmless for primary keratinocytes but toxic fo tumor cells, especially at the highest concentration tested. Further in-depth studies are needed to establish the mechanisms responsible for the exercise of pharmacological properties.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human or animal subjects (if exist) respect the specific regulation and standards.

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