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Chemical composition and antimicrobial activity of the essential oils of selected Apiaceous fruits

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ABSTRACT

Antimicrobial properties of plants essential oils are continuously investigated to use them as potential drug candidates to overcome the problem of microbial drug resistance. The aim of this research is to study the antimicrobial effects of the essential oils of ten Apiaceous fruits [*Pimpinella anisum* L. (anise), *Carum carvi* L. (caraway), *Apium graveolens* L. (celery), *Coriandrum sativum* L. (coriander), *Cuminum cyminum* L. (cumin), *Anethum graveolens* L. (dill), *Foeniculum vulgare* L. (fennel), *Petroselinum crispum* L. (pasley), *Daucus carota* L. var. *sativus* (yellow carrot) and *Daucus carota* L. var. *boissieri (red carrot)*].

Results of agar-well diffusion method revealed that the maximum inhibition zones were obtained with cumin, coriander and caraway oils against the standard bacterial strains *Escherichia coli, Bordetella bronchiseptica* followed by *Staphylococcus aureus*.

Results of viable count time-kill method revealed that coriander oil had the highest antimicrobial activity with more than 99.99% killing of the exposed cells of the standard *E. coli* and *Bordetella bronchiseptica* standard strains. GC/MS was carried out to identify the chemical composition of the most active oils. The percentage of identified compounds by GC/MS was 92.5%, 99.43% and 98.66% for cumin, coriander and caraway oils, respectively. Monoterpenes were the most abundant components in the three oils.

1. Introduction

It is crucial nowadays to search for other sources of promising antimicrobial agents and strategies for the treatment of serious gram negative and gram positive infections due to the emergence of multidrug resistance in common pathogens. Toxicity and carcinogenicity of synthetic additives have led scientists in food industry to search for alternatives specially naturally occurring antimicrobial agents [1]. The medicinal effects of many common spices and herbs indicate the presence of antioxidant and antimicrobial constituents in their tissues [2]. Essential oils are complex mixtures comprising many single compounds. Each of these constituents contributes to the beneficial or adverse effects of these oils. Therefore, many researches aim at understanding their chemical composition to allow practical application [3]. Many oils have proven to be promising antimicrobial agents [4]. Plants from family Apiaceae are commonly used as food, flavoring agents and for medical purposes and are also known as nutraceutical plants. Based on many studies, it seems that several species in this family are good sources for phytochemicals with potent antimicrobial and anti-inflammatory properties [5]. Many members of this family have culinary uses and are very common in Egypt. In our work, we selected the most common Apiaceous members used traditionally in Egypt. The work was on the essential oil fraction since this family is popular for its richness in the essential oil content; that is mentioned traditionally to have several medicinal values. The essential oil of these Apiaceous fruits is also used in the food industry. These essential oils have been reported to have several medicinal effects. Anise oil is used as digestive, carminative and antispasmodic [6]. Caraway oil has been reported to have antimicrobial, antioxidant and cytotoxic activities [7–9]. Celery oil has antibacterial and antifungal effects [10]. Coriander oil has antimicrobial and insecticidal effects [11,12]. Cumin oil has antimicrobial, antifungal and hypoglycemic effects

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[13,14]. Dill oil has antispasmodic effects [15]. Fennel oil has been reported to have antifungal, antioxidant, and hypoglycemic effects [16,17]. Parsley oil has anti-inflammatory and immunomodulatory effects [18]. Red and yellow carrot oils have antioxidant and anti-inflammatory activities [19]. In this paper we investigate the antimicrobial activity of essential oils of the fruits of *Pimpinella anisum* L. (anise), *Carum carvi* L. (caraway), *Apium graveolens* L. (celery), *Coriandrum sativum* L. (coriander), *Cuminum cyminum* L. (cumin), *Anethum graveolens* L. (dill), *Foeniculum vulgare* L. (fennel), *Petroselinum crispum* L. (pasley), *Daucus carota* L. var. *sativus* (yellow carrot) and *Daucus carota* L. var. *boissieri (red carrot)* cultivated in Egypt by two different methods; agar-well diffusion method and viable-count method. Moreover, to identify the chemical composition of the most potent oils, GC/MS was carried out. Further studies on these oils using other antimicrobial assays and application of a suitable pharmaceutical dosage form is needed to find other sources of antimicrobial drugs other than synthetic ones to overcome the problem of multidrug resistance.

2. Material and methods

2.1. Plant material

The Apiaceous fruits were collected from a cultivated field at El-Sharkeyya governorate, Nile delta of Egypt in 2010. Plants were identified and authenticated according to Boulos [20].

2.1.1. Essential oils isolation

One hundred grams of whole fruits from each studied species were submitted to hydro-distillation for 4 h using a Clevenger apparatus according to the procedure described in the Egyptian pharmacopeia (2005) [21]. Oils were dried over anhydrous sodium sulfate and stored in dark vials at 4 °C until analysis. Yields, expressed as 100 g of dried weight, were stated in mean ± standard deviation of three replicates.

2.2. Essential oil analysis

2.2.1. Gas chromatography/flame ionization detection (GC/FID)

The GC analyses were carried out on a Focus GC ® (Thermo fisher scientific®, Milan, Italy) equipped with TR5-MS fused bonded column (30 m × 0.25 mm x 0.25 μm) (Thermo fisher scientific®, Florida, USA) and FID detector; carrier gas was nitrogen (1.5 ml/min); the operating conditions were: initial temperature 40 °C, 1 min isothermal followed by linear temperature increase till 230 °C at a rate of 4 °C/min. 230 °C, then 5 min isothermal. Detector and injector temperatures were 300 and 220 °C, respectively. The split ratio was 1: 20. Chrom-card® chromatography data system ver. 2.3.3 (Thermo Electron Corp. ®, Florida, USA) was used for recording and integrating of the chromatograms. Average areas under the peaks of three independent chromatographic runs were used for calculation the percentage composition of each component.

2.2.2. Gas chromatography/mass spectrometry (GC/MS)

The analyses were carried out on Focus GC ® (Thermo fisher scientific®, Milan, Italy) equipped with the same column and conditions mentioned in the GC/FID. The capillary column was directly coupled to a quadrupole mass spectrometer Polaris Q, (Thermo Electron Corp. ®, Milan, Italy). The injector temperature was 220 °C. Helium carrier gas flow rate was 1.5 ml/min. All the mass spectra were recorded with the following condition: filament emission current, 100 mA; electron energy, 70 eV; ion source, 250 °C; diluted samples were injected with split mode (split ratio, 1: 15). Compounds were identified by comparison of their spectral data and retention indices with Wiley Registry of Mass Spectral Data 8th edition, NIST Mass Spectral Library (December 2005), our own laboratory database and the literature [22].

2.3. Sources of microbial cultures

The antimicrobial activity of the essential oils was evaluated using laboratory reference strains (American Type Culture Collection "ATCC" for bacteria and Candida albicans, obtained from Laboratory of Microbiology, Pharco pharmaceuticals company, Egypt: Gram-positive bacteria: *Staphylococcus aureus* (ATCC 6538), *Micrococcus luteus* (ATCC 9341). Gram negative bacteria: *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027), *Bordetella bronchiseptica* (ATCC 4617), fungal microorganisms: *Candida albicans* (ATCC 10231). Microbial inocula of bacterial and yeast cultures were prepared as suspensions in Roux bottles using Trypticase soy agar (TSA) and Sabouraud dextrose agar (SDA) media according to USP procedure.

2.4. Antimicrobial screening

2.4.1. Preparation of the essential oils emulsion

Ten percent w/w essential oils emulsion was prepared using Tween 80 as emulsifying agent. The resultant emulsion was sterilized by filtration through 0.45 μm hydrophilic membrane filter and stored at 4 °C in well closed sterile container.

2.4.2. Determination of antimicrobial activity by the agar-well diffusion method

The antibacterial activity was performed with the agar diffusion method [23]. Fifty ml-portions of the melted sterile TSA, SDA maintained at 50 °C, were inoculated, each with 100 μl of properly diluted inoculum and mixed well. The inoculated medium was poured into sterile petri dish (15 cm) and allowed to solidify. Wells, each 6 mm, were cut through the agar using sterile cork porer and the agar removed leaving empty wells which was filled with 10% w/w oil emulsion. Maintain the plates at room temperature for about 2 h and then incubate the plates at 30-35 °C for 24 and 48 h in case of bacteria and yeast respectively. The resultant inhibition zones were measured and the average values are taken.

2.4.3. Determinations of the minimum inhibitory concentration (MIC)

Aliquots of each essential oil emulsion were properly diluted with sterile water, inoculated with the overnight culture of the tested organism diluted with water, vortexed and incubated at 37 °C. At specified time period, the inoculated systems were vortexed and aliquots were decimally diluted with sterile saline and the numbers of viable cells were determined by transferring 40 μl portion of each dilution onto the surface of over dried TSA plates. These plates were incubated at 37 °C for 48 h, the numbers of developed colonies were counted and the average number of cells calculated as cfu/ml. Controls lacking tested essential oils were included in the test.

2.4.4. Standard drugs

Fluconazole (Sigma) was used as positive controls for the fungi and chloramphenicol (Sigma) was the positive control for bacteria.

3. Results

3.1. Essential oils isolation

Hydrodistillation of the selected Apiaceous fruits gave essential oil yield as follows:

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