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ORIGINAL ARTICLE

Diverse biological effects of the essential oil from Iranian *Trachyspermum ammi*

Luca A. Vitali [a], Daniela Beghelli [b], Prosper C. Biapa Nya [c], Onelia Bistoni [d], Loredana Cappellacci [a], Silvia Damiano [a], Giulio Lupidi [a], Filippo Maggi [a,*], Giuseppe Orsomando [e], Fabrizio Papa [f], Dezemona Petrelli [b], Riccardo Petrelli [a], Luana Quassinti [a], Leonardo Sorci [e], Majid Majd Zadeh [g], Massimo Bramucci [a]

[a] *School of Pharmacy, University of Camerino, Camerino, Italy*
[b] *School of Bioscience and Veterinary Medicine, University of Camerino, Camerino, Italy*
[c] *Laboratory of Medicinal Plant Biochemistry, Food Science and Nutrition, Department of Biochemistry, Faculty of Sciences, University of Dschang, Dschang, Cameroon*
[d] *Rheumatology Unit, Department of Medicine, University of Perugia, Perugia, Italy*
[e] *Department of Clinical Sciences, Section of Biochemistry, Polytechnic University of Marche, Ancona, Italy*
[f] *School of Science and Technology, University of Camerino, Camerino, Italy*
[g] *School of Plant Biology, Sapienza University, Rome, Italy*

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Abstract *Trachyspermum ammi* (Apiaceae) is a plant with a good reputation in the traditional Persian and Ayurvedic medicine. The hydrodistilled essential oil from the fruits of *T. ammi*, known as 'ajwain oil', is used in countries such as Iraq, Iran, Afghanistan, Pakistan, and India in the preparation of curry, to flavour several foods, as preservative, and in perfumery. At therapeutic level, ajwain oil is employed in the treatment of gastrointestinal ailments, lack of appetite and bronchial problems. In the present work, the essential oil of *T. ammi* growing in Iran was analysed by GC–FID and GC–MS showing thymol (67.4%), *p*-cymene (17.9%) and γ-terpinene (11.3%) as the major constituents. Afterwards, we investigated the biological effects displayed by ajwain oil, namely the antimicrobial and antioxidant activity, the cytotoxicity on tumour cells, and the induction of lymphocyte proliferation. In addition, the inhibition on nicotinate mononucleotide adenylyltransferase (NadD), which is a promising new target for developing novel antibiotics, was evaluated. The antimicrobial effects of ajwain oil, measured by the agar disc diffusion method, were relevant, with inhibition zones higher than those of reference antibiotics, especially on

* Corresponding author. Tel.: +39 0737404506.
E-mail address: filippo.maggi@unicam.it (F. Maggi).
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Staphylococcus aureus and *Candida albicans* (34.7 and 54.3 mm, respectively). This effect was not due to the enzymatic inhibition on NadD. The ajwain oil exhibited a considerable dose-dependent inhibition on the ABTS radical cation, with an IC_{50} value of 22.4 μg/mL. MTT assay revealed that ajwain oil is particularly cytotoxic on colon carcinoma cells, with a IC_{50} value of 9.6 μg/mL. Finally, PBMC proliferation assay revealed some role for the ajwain oil within the network of interactions of the cells of the immune system.

1. Introduction

Herbal medicine represents one of the most important fields of traditional medicine all over the world. To promote the proper use of herbal medicine and to determine their potential as sources for new drugs, it is essential to study medicinal plants, which have folklore reputation in a more intensified way (Parekh and Chanda, 2007). Thousands of plant secondary metabolites have been identified and it is estimated that other thousands are yet to be discovered. Since secondary metabolites from natural sources have been elaborated within living systems, they are often perceived as showing more "drug – likeness and biological friendliness than totally synthetic molecules" (Koehn and Carter, 2005), making them good candidates for further drug development.

Trachyspermum ammi (L.) Sprague [syn. *Carum copticum* (L.) Benth. & Hook.f. ex C.B. Clarke], also known as ajwain (Bairwa et al., 2012), is a highly reputable plant as a source of constituents with promising bioactivity to be exploited at pharmaceutical level. *T. ammi* belongs to the Apiaceae family and is an annual herb up to 90 cm tall, native to arid and semi-arid regions of Egypt (Ashraf, 2002); it is also widely distributed and cultivated in Iraq, Iran, Afghanistan, Pakistan, and India. The plant is a highly valued medicinally seed spice. The fruits, having a bitter and pungent taste, are used to flavour and preserve foods, in perfumery for the manufacture of essential oil and in medicine (Pruthi, 1992). In the area of origin of the plant, the fruits are believed to exert aphrodisiac effects. They possess stimulant, antispasmodic and carminative properties, and are used traditionally in the treatment of flatulence, atonic dyspepsia, diarrhoea, abdominal tumours, abdominal pains, piles, bronchial problems, lack of appetite, asthma and amenorrhoea (Bairwa et al., 2012). The fruits contain 2–5% of a brown coloured essential oil, responsible for plant odour and taste, known as 'ajwain oil'. It is used in the treatment of gastrointestinal ailments, lack of appetite, and bronchial problems (Bairwa et al., 2012). The main component of this oil is thymol (35–60%), which is a strong germicide, antispasmodic and fungicide agent. The non-thymol fraction contains *p*-cymene, γ-terpinene, α-pinene, β-pinene, and other minor components (Zarshenas et al., 2014). However, sometimes γ-terpinene and *p*-cymene exceed the thymol content (Omer et al., 2014; Moein et al., 2015), and in other cases thymol and *p*-cymene are not among the predominant components (Singh et al., 2008).

The ajwain essential oil exhibited nematicidal (Park et al., 2007), scolicidal (Moazeni et al., 2012), antitermitic (Seo et al., 2009), antibacterial (Kumar et al., 2011; Paul et al., 2011; Moein et al., 2015), antifungal (Ashrafi Tamai et al., 2013; Moein et al., 2015; Kedia et al., 2015), and antioxidant (Chatterjee et al., 2013; Gandomi et al., 2014) effects. Interestingly, the oil showed appreciable spermicidal potential, which may be explored as an effective ingredient of male contraceptives (Paul and Kang, 2011, 2012). Ajwain oil also showed vapour toxicant and repellent effects against adults of *Anopheles stephensi* (Pandey et al., 2009), as well as larvicidal activity against *Aedes aegypti* (Seo et al., 2012), thus having promising applications in the management of malaria and yellow fever.

In the present work, we have evaluated the *in vitro* biological effects of ajwain oil, namely the antimicrobial and antioxidant activities, cytotoxicity on human tumour cells, and the induction of lymphocyte proliferation. These activities were evaluated by agar disc-diffusion, microdilution, DPPH, ABTS, FRAP, and MTT methods, and *in vitro* peripheral blood mononuclear cells (PBMC) proliferation assay, respectively. To complete the work we have evaluated the inhibitory effects of ajwain oil on nicotinate mononucleotide adenylyltransferase (NadD), which is a promising new target for developing novel antibiotics.

2. Materials and methods

2.1. Plant material

Fruits of *T. ammi* were collected at ripening from wild plants growing in Baluchistan (Iran) in the summer 2012. A voucher specimen was identified and deposited in the *Herbarium* of the University of Isfahan, Iran, under the code 125/21224/2012.

2.2. Hydrodistillation

The ripe fruits (78 g) were subjected to hydrodistillation in a Clevenger-type apparatus using 3 L of distilled water for 3 h until no more essential oil was obtained. The essential oil was stored in a sealed vial protected from light at – 20 °C before chemical analysis and biological assays. The oil yield (2.7%) was estimated on a dry weight basis.

2.3. GC–FID and GC–MS analyses

For GC separations, an Agilent 4890D instrument coupled to an ionization flame detector (FID) was used. Volatile components were separated on a HP-5 capillary column (5% phenylmethylpolysiloxane, 30 m, 0.32 mm i.d.; 0.25 mm film thickness; J and W Scientific, Folsom, CA, USA), with the following temperature programme: 5 min at 60 °C, subsequently 4 °C/min up to 220 °C, then 11 °C/min up to 280 °C, held for 15 min, for a total run of 65 min. Injector and transfer line