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Chemical composition and antibiofilm activity of *Petroselinum crispum* and *Ocimum basilicum* essential oils against *Vibrio* spp. strains

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ABSTRACT

In this study, we evaluated the antibacterial activity of parsley and basilic essential oils tested against *Vibrio* strains and their abilities to inhibit and eradicate the mature biofilm using the XTT assay. *Petroselinum crispum* essential oil was characterized by 1,3,8-*p*-menthatriene (24.2%), β-phellandrene (22.8%), apiol (13.2%), myristicin (12.6%) and terpinolene (10.3%) as a major constituents. While, in the basilic oil, linalool (42.1%), (*E*)-methylcinnamate (16.9%) and 1–8 cineole (7.6%) were the main ones. These two essential oils exhibit high anti-*Vibrio* spp. activity with varying magnitudes. All microorganisms were strongly affected indicating an appreciable antimicrobial potential of basilic with a diameter of inhibition zones growth ranging from 8.67 to 23.33 mm and MIC and MBC values ranging from (0.023–0.047 mg/ml) and (>3->24 mg/ml), respectively. The two essential oils can inhibit and eradicate the mature biofilm formed on polystyrene surface even at low concentrations, with high magnitude for *Ocimum basilicum* essential oil. This study gives a better insight into the anti-*Vibrio* activity of parsley and basilc oils and the possibility of their use to prevent and eradicate contamination of sea products by these strains.

1. Introduction

Several spices and medicinal/aromatic plants have been tested against a large number of microorganisms including Gram+ and Gram-human pathogenic bacteria (*Staphylococcus aureus* MR, bacteria associate to skin infection, mycobacteria, *Cryptococcus neoformans*, caryogenic bacteria), fungi (dermatophytes, moulds, phyto-pathogenic fungi, yeasts) and viruses [1]. Interest in medicinal plants has burgeoned due to the increased efficiency of new plant-derived drugs and the growing interest in natural products. A larger number of these plants have shown beneficial therapeutic effects, including anti-oxidant, anti-inflammatory, anti-cancer, anti-microbial, and immuno-modulatory effects [2]. Foodborne illness caused by consumption of food contaminated with pathogenic bacteria like *S. aureus*, *Salmonella* sp., *Clostridium perfringens*, *Campylobacter* sp., *Listeria monocytogenes*, *Vibrio parahaemolyticus*, *Bacillus cereus* and entero-pathogenic *Escherichia coli* are a great problem in public health. These bacteria cause over 90% of all cases of food poisoning [3].

Member of the *Vibrionaceae* strains are a normal inhabitants in estuarine and marine environments and are frequently isolated from bathing area as free-living bacteria and attached to different biotic and abiotic surfaces [4–7]. Few reports dealing with the anti-*Vibrio* spp. activity of medicinal/aromatic plant extracts (essential oils and organic extracts) were reported. The efficacy of plants essential oil and organic extracts against foodborne pathogens, periodontopathic and cariogenic bacteria and fungal strains (*Candida* spp.) pre-formed biofilm was well documented [8–11]. Whereas, few data were available about the *Vibrio* spp. biofilm inhibition using plants derived molecules [12].

Many Lamiaceae species have long history of uses in culinary spices and folk medicine. In the Mediterranean region, parsley and basilic are typical seasonings. and Fresh basil leaves are consumed in larger quantities as ingredient in various dishes and food preparations [13]. *Petroselinum crispum* (Mill.) Nym. syn. *Pisum.. sativum* Hoffm. (family *Apiaceae*) is a medicinal and food plant commonly used to flavour the cuisine of China, Mexico, South America, India and South East Asia. Parsley is known for its aromatic leaves and roots, and is a raw material for resinoid, oleoresin and lipid production such as palmic, oleic, linoleic and petroselinic acids [14].

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This plant is also a good source of Ca, Fe, vitamin C and carotene [15]. The essential oil is present in all parts of the herb (root: up to 0.1% leaves: 0.05–0.3% seeds: 2–7%). Leaf oil have a flavour that resembles the fresh herb and can only be obtained in low yield, while the commercial oil derived from mature seeds (fruits) has a distinctly different flavour. It is used as a flavouring agent in food products (soaps and creams) or fragrance in perfumery and cosmetics. Antibacterial, diuretic and low antioxidant activities of parsley essential oil have been reported [16] and [17]. Myristicin from parsley oil is a potent cancer chemoprotective agent [18].

The *Ocimum* genus (Lamiaceae) is considered as one of the largest genera of the Lamiaceae family with more than 150 species. This genus comprises annual and perennial herbs and scrubs native to the tropical and sub-tropical regions of Asia, Africa and Central south America [19]. *Ocimum basilicum* L. (sweet basil), possess a large range of variability in their leaf colour (green or purple) and flower colour (white, red, purple). Sweet basil is considered a popular culinary herb originating in India, Africa and southern Asia and nowadays cultivated world-wide. In Mediterranean countries, it is extensively cultivated since both the fresh and dried leaves are widely used to enhance the flavour of foods such as salads, pizzas, sauces, pasta, meats, soups salad dressing, non alcoholic beverages, ice creams and confectionary it has also wide application in perfumery, as well as in dental and oral products [20] and [21]. Essentials oils extracted from fresh leaves and flowers can be used as aroma additives in food, pharmaceuticals, and cosmetics. Although the high chemical diversity in the composition of sweet basil essential oils, monoterpenes and phenylpropanoids are the main components. Many *Ocimum* species contain limonene, camphor, 1,8-cineole, linalool and geraniol as primarily monoterpene derivates, while other *O. basilicum* cultivars contain eugenol, methyleugenol, chavicol, estragole, methylcinnamate, in association with various concentrations of linalool [22]. The aerial parts of the plants are considered as a source of aroma compounds, and it possesses a range of biological activities being used as insect repellent, nematocidal, antimutagenic, antibacterial, antiviral, antifungal, antioxidant, antispasmodic, stomachic, anticonvulsant and hypotonic activities and carminative in native medicine [23]. Traditionally, basil has been used as a medicinal plant in the treatment of headaches, coughs, diarrhea, constipation, warts, worms, and kidney malfunction [24].

The aims of this work were (i) to study the chemical composition of Tunisian *P. crispum* and *O. basilicum* essential oils, (ii) to evaluate their antimicrobial effects against several pathogenic *Vibrio* spp. isolated from seawater and fish food and associated with human infection due to consumption of raw or undercooked sea products (iii) and to evaluate their ability to prevent and disrupt *Vibrio* spp. biofilm using the XTT technique.

2. Material and methods

2.1. Chemicals and mediums used

Mueller-Hinton agar, nutrient broth and Brain Heart Infusion broth (BHI) were purchased from Biolife (Monza, Italy). Glucose was purchased from (Scharlab S.L., Spain), NaCl was obtained from (CHEMI PHARMA, Tunisia). XTT [2, 3-bis (2-methyloxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide] was purchased from Sigma–Aldrich (GmbH, Sternheim, Germany) and the menadione was purchased from (MP Biomedicals, LLC, France).

2.2. Plant material and extraction of essential oil

P. crispum plants (aerial parts) were freshly collected in December 2010 from Soliman Tunisian locality (Nabeul) and *O. basilicum* plants were cultivated in a sandy soil in Mahdia until flowering stage. The two plants were identified according to the flora of Tunisia and 100 g of fresh material sample were subjected to hydrodistillation for 3 h with 500 ml distilled water using a Clevenger-type apparatus according to the *European Pharmacopoeia* [25]. The oil obtained from different hydrodistillation steps was collected and dried over anhydrous sodium sulphate and stored in sealed glass vials in a refrigerator at 4 °C prior to analysis.

2.2.1. GC–EIMS analysis

GC/EIMS analyses were performed with a Varian CP-3800 gas-chromatograph equipped with a HP-5 capillary column (30 × 0.25 mm coating thickness 0.25 μm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions: injector and transfer line temperatures at 220 and 240 °C, respectively oven temperature was programmed from 60 to 240 °C at 3 °C/min carrier gas helium at 1 ml/min injection of 0.2 ml (10% hexane solution) split ratio 1:30. Identification of the constituents was based on comparison of the retention times with those of authentic standards, comparing their Linear Retention Indices relative to the series of *n*-hydrocarbons, and by computer matching against commercial (NIST 98 and ADAMS 95) and home-made library mass spectra built up from pure substances and components of known essential oils and MS literature data. Moreover, the molecular weights of all the identified substances were confirmed by GC/CIMS, using methanol as CI ionising gas.

2.3. Antimicrobial activities

2.3.1. Microorganisms

The antibacterial effect of the *P. crispum* and *O. basilicum* essential oils were tested against 40 *Vibrio* spp. strains belonging to 17 different species and against *Aeromonas hydrophila* ATCC 7966^T. These microorganisms were isolated from diseased *Sparus aurata*, *Dicentrarchus labrax* and *Mytilus edulis* reared in aquaculture farms and medically important human pathogenic *Vibrio* type strains (ATCC and CECT) generally associated with seafoods consumption were also tested.

2.3.2. Disc-diffusion assay

Antimicrobial activity testing was done according to the protocol described by Snoussi et al. [26] for *Vibrio* strains. For the experiments, a loopful of the microorganisms working stocks were enriched on a tube containing 9 ml of Mueller–Hinton broth-1% NaCl then incubated at 37 °C for 18–24 h. The overnight cultures were used for the antimicrobial activity of the essential oils used in this study and the optical density was adjusted at 0.5 (OD_{520nm}). The inoculums were streaked onto MH-1% NaCl agar plates than the sterile filter discs (diameter 6 mm, Biolife Italy) were impregnated with 10 mg of essential oil (10 μl of parsley and basil essential oil weighted 4.5 and 8 mg, respectively).

Five antibiotics were used in this study as positive controls for *Vibrio* spp. strains. The antibiotic susceptibility was determined by using the Kirby–Bauer method and Mueller-Hinton agar plates supplemented with 1% NaCl [27]. After incubation at 37 °C for 18–24 h, the diameter of the inhibition zone was measured with 1 mm flat rule and the diameters were interpreted according to the Comité de la Société Française de l'Antibiogramme and followed the recommendations of the NCCLS 2002 guidelines [27]. The dishes were incubated at 37 °C for 18–24 h for microbial strains. The diameter of the zones of inhibition around each of the discs was taken as measure of the antimicrobial activity. Each experiment was carried out in triplicate and the mean diameter of the inhibition zone was recorded.