Chemical Composition and Antibacterial activity of *Origanum vulgare* Essential Oil Grown in Kerman, Iran

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ABSTRACT

Disease caused due to the consumption of contaminated foods has a wide economic and public health impact worldwide. Finding the natural and non-toxic preservatives have been encouraged by the scientists to investigate among the medicinal plants. Some essential oils derived from medicinal plants possess antibacterial properties and have been screened as potential sources of novel antimicrobial agents. The aim of this study was to evaluate chemical composition and antibacterial effects of *Origanum vulgare* essential oil. The test bacteria were *Staphylococcus aureus* (PTCC 1431), *Bacillus cereus* (PTCC 1015), *Salmonella enterica* (PTCC 1709) and *Listeria monocytogenes* (ATCC 7644). The essential oil showed higher antimicrobial activity against *L. monocytogenes* in comparison with other bacteria. Minimum bactericidal concentration of the oil for *S. aureus, B. cereus, L. monocytogenes* and *S. enterica* were 3.1%, 6.25%, 6.25% and 0.3% respectively. The chemical analyses showed that *Origanum vulgare* oil contained Thymol (32.7%), γ-terpinene (12.1%), cis-terpinene (5.9%) and p-cymene (5.2%) as the main components. Even if *in vitro* bioassays are only the first steps towards the use of essential oils in practical applications, these substances represent a potential alternative to chemical antimicrobial in food industry.

Keywords: Antibacterial activity, Chemical composition, *Origanum vulgare*, Essential oil

INTRODUCTION

Medicinal plants are as sources of new antimicrobial agents which are used in traditional medicine [1]. Essential oils which are available in aromatic plants are one of the important compounds having different therapeutic characteristics including antimicrobial effects [2]. Besides antimicrobial properties, essential oils have shown to exhibit antiviral [3], antimycotic [4], antitoxigenic [5], antiparasitic [6], and insecticidal [7] activities that are probably related to the function of these compounds in plants [8] . Increasing microbial resistance against antibiotics leads to trying to search new antimicrobial agents[9]. *Origanum vulgare* is a common species of *Origanum*, a genus of the Lamiaceae or Labiatae [10]. In Iran, the family Lamiaceae is represented by 46 genera, and 410 species/subspecies, of which 74 species have been mentioned as medicinal plants in the ancient Iranian medicinal literature [11]. A total of 38 *Origanum* species are documented in the world. *Origanum vulgare* L. is the only species of the *Origanum* genus growing wild in Iran. *Origanum vulgare* is commonly spread all over the country, particularly Gilan, Mazandaran ,West Azarbaijan and Kerman provinces [12]. It is perennial plant and sometimes called Oregano or wild marjoram. It can grow to 80 centimeters in height. Leaves are dark green, oval and opposite. Flowers are white, pink or purple, formed in erect spikes. *Origanum vulgare* has been used as a culinary and medicinal

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herb for thousands of years[13]. It has a beneficial effect upon the digestive and respiratory systems[14]. The leaves and flowering stems are strongly antiseptic, antispasmodic, carminative, cholagogue, diaphoretic, emmenagogue, expectorant, stimulant, stomachic and mildly tonic [15,16]. The present investigation was focused on chemical composition and antibacterial potential of essential oil of Oregano (*Origanum vulgare*) against 4 bacterial strains and the characterization of its minimum bactericidal concentration.

MATERIALS AND METHODS

Bacterial strains

Standard strains of bacteria including *Listeria monocytogenes* (ATCC 7644), *Staphylococcus aureus* (PTCC 1431), *Salmonella enterica* (PTCC 1709) and *Bacillus cereus* (PTCC 1015) were used for investigation of antibacterial activity of *Origanum vulgare* essential oil.

Preparation of Essential oil

Origanum vulgare was collected in June 2014 from plants growing wild in Hezar Mountain, Kerman province, Iran. The sample was crushed manually. The extraction was performed in a Clevenger apparatus for 180 min. After hydro-distillation runs, water was removed by anhydrous sodium sulfate and the essential oil obtained was stored at 4ºC and protected against light to avoid alteration in its composition.

Antibacterial activity

The antibacterial assay of the essential oil was performed by broth dilution method [17]. The bacterial suspensions were adjusted with sterile normal saline (0.9%) to a concentration of 1×10^8 CFU/ml equal 0.5 MC Farland solution. 100µl of each inoculum was inoculated to diluted the essential oil and incubated for 24,48,72,96 and 120 hour at 37˚C in shaker incubator (Labnet Company, USA) with 150 rpm. Following incubation period times, from each sample was cultured on plates with sterile Mueller Hinton agar medium (Merck Company) and incubated in 37˚C for 24 hours and minimum bactericidal concentration of the oil was determined [18].

GC and GC-MS analyses

A Hewlett-Packard 6890 gas chromatograph with HP-5MS column (30m×0.25mm i.d., 0.25µm film thickness) equipped with a mass detector (Hewlett-Packard model 5973 HP) was applied for the essential oil analyses. Helium (at 1 ml/min) was used as a carrier gas. The initial oven temperature was 40°C and was then raised at a rate of 3°C /min to 250˚C.The injection temperature was 250°C and the oil sample (100 µl) was injected with a split ratio of 1:90. The mass spectra were obtained by electron ionization at 70 eV. The retention indices (RI) of the compounds were calculated using a homologous series of n-alkanes injected in conditions equal to the samples. Identification of major compounds was accomplished by comparing their retention times with those of authentic standards, and by comparison their mass spectra with those from the Wiley library. Compositions are then expressed as percent of normalized peak areas [19,20].

RESULTS AND DISCUSSION

The essential oil showed antibacterial activity against all evaluated bacterial strains. *L. monocytogenes* (ATCC 7644) was the most susceptible bacteria in comparison with other bacteria. Minimum bactericidal concentration of the oil for *Staphylococcus aureus* (PTCC 1431)*, Bacillus cereus* (PTCC 1015)*, Listeria monocytogenes* (ATCC 7644) and *Salmonella enterica* (PTCC 1709) were 3.1%, 6.25%, 6.25% and 0.3% respectively. Twelve components identified in the oil of *Origanum vulgare* were Thymol (32.7%), γ- Terpinene (12.1%), Cis-Terpineol(5.0%), P-Cymene (5.2%), α-thujene (2.8%), α-Terpinolene (2.4%), E-Caryophyllene (2.3%), Sabinene (1.7%), β-Eudesmol (1.4%), Borneol (1.2%) , β-myrcene (1.1%) and α-Pinene (0.7%).

Lamiaceae or Labiatae family with a wide distribution all over the world are one of the main sources of aromatic and medicinal plants [11]. Several studies have been conducted regarding *in vitro* antimicrobial properties of plant essential oils and extracts. In present study, it was investigated the use of Oregano essential oil as antimicrobial agent against 4 bacteria strains. All bacteria were sensitive to the oil and *Listeria monocytogenes* (ATCC 7644) was the most susceptible bacteria and Thymol, γ-Terpinene, Cis-terpinene and p-Cymene were main components of the oil. Thymol has antimicrobial activity because of its phenolic structure [21]. Thymol is also used as a preservative, and as active antiseptic ingredient in some toothpastes when used to reduce plaque and gingivitis [22]. In another study, antibacterial activity of the essential oil of *Origanum vulgare* L. on multi-resistant bacteria isolated from biological materials was done and their results showed that four samples of *Escherichia coli*, *Enterococcus faecalis* and methicillin resistant *Staphylococcus aureus*, three samples of *Acinetobacter baumannii* and a sample, *Klebsiella pneumoniae* were inhibited by the essential oil at the concentration 0.125%, 0.125% and 0.5% respectively [15]. In similar investigation, the antimicrobial activity of Oregano essential oil in a food system was evaluated and results showed that the addition of Oregano essential oil to sausage could be a promising route as bacteriostatic effect and analysis of the oil identified Terpinen-4-ol and γ-terpinene were the major components [2]. Pirigharnaei and co-workers showed that the Oregano essential oil is composed of Carvacrol and Thymol as major components, followed by γ-terpinene, p-cymene, Linalool, terpinen-4-ol and sabinene hydrate [23] identified Linalool as the main components of *Origanum vulgare*.

CONCLUSION

The result of this investigation showed that *Origanum vulgare* oil contains antibacterial components and can be used as antibacterial agent for treatment of human pathogens, including those that cause enteric infections and food poisoning.

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