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Review

Zataria multiflora Boiss. (Shirazi thyme)—An ancient condiment with modern pharmaceutical uses

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ABSTRACT

Ethnopharmacological relevance: Zataria multiflora Boiss. (ZM) is a thyme-like plant belonging to the Lamiaceae family that grows wild only in Iran, Pakistan and Afghanistan. This plant with the vernacular name of Avishan-e-Shirazi (Shirazi thyme) in Iran is a valuable medicinal and condimental plant. It has several traditional uses as an antiseptic, carminative, stimulant, diaphoretic, diuretic, anesthetic, antispasmodic and analgesic.

Aim of the study: This paper reviews the ethnopharmacology, pharmacology, toxicology, modern pharmaceutical uses and phytochemistry of *Zataria multiflora*, and highlights the gaps in our knowledge deserving further research.

Materials and methods: All relevant databases were searched for the terms "Zataria", "Zataria multiflora", "Shirazi thyme" and "Iranian thyme" without limitation up to 24th October 2012. Information on *Zataria multiflora* was collected via electronic search using Pubmed, Scopus, Web of Science and SID (for articles in Persian language), and local books on ethnopharmacology.

Results: ZM has played an important role in Iranian traditional medicine. In light of the modern pharmacological and clinical investigations, ZM is a valuable medicinal and condimental plant that has anti-microbial, antioxidative, anti-inflammatory, spasmolytic and anti-nociceptive properties. The oil of ZM contains high percentages of oxygenated monoterpenes, in particular thymol and carvacrol, and exhibits excellent anti-microbial properties.

Conclusions: Overall, antimicrobial property appears to be the most interesting studied biological effect of ZM. The lack of a comprehensive phytochemical analysis of ZM is an important limitation that can be noted regarding most of the previous studies.

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1. Introduction

Zataria multiflora Boiss. (ZM) (synonyms: *Zataria bracteata* Boiss.; *Zataria multiflora* var. *elatior* Boiss) is a thyme-like plant belonging to the Lamiaceae family that geographically grows wild only in central and southern Iran, Pakistan and Afghanistan (Hosseinzadeh et al., 2000). It has chemical and pharmacological similarities to *Thymus vulgaris*, the well-known and widely investigated medicinal plant. For this reason, it is also called Avishan-e-Shirazi (Avishan meaning thyme in the Persian language and Shiraz being the name of a city in southern Iran). The genus name of the plant is derived from the Arabic word "Zaatar" which is a generic name of some Middle Eastern herbs including thyme, oregano and savory. ZM can be recognized by the orbicular, densely gland-dotted, ovate leaves and the dense white, hairy, round buds on the leaf axils. It is an aromatic shrub that reaches 60–90 cm in height. Mature branches are woody and leafless whereas young branches are white with dense glandular, spreading, pilose indumentums. Leaves ($5–10 \times 5–10\ mm^2$) are orbicular ovate to orbicular. Flowering stems are usually unbranched, sometimes having short lateral branches. Flowers are white, sub-sessile, very small and often male sterile (Simbar et al., 2008). ZM (aerial parts) is not only a popular condimental plant but is also used in traditional folk remedies for its antiseptic, analgesic, carminative, anthelmintic and antidiarrheal properties (Iranian Herbal Pharmacopoeia, 2002). Modern pharmacological studies show that ZM possesses wide ranging biological properties including antinociceptive, antimicrobial, spasmolytic and anti-inflammatory effects. Currently, some pharmaceutical forms of this plant, such as syrups, oral drops, soft capsules and vaginal creams are sold as treatments for various diseases (Table 1). In addition to modern medicinal uses, ZM is still used in folk remedies.

To outline the extensive uses of ZM in healthcare and medicine and to provide a probable scope for future research, several pharmacological and clinical studies on this plant and its active components are described in this review. In addition, this review critically evaluates the issues related to current pharmacological studies of ZM. All relevant databases were searched for the terms "Zataria", "Zataria multiflora", "Shirazi thyme" and "Iranian thyme" without limitation up to 24th October 2012.

2. Phytochemistry

2.1. Chemical composition of essential oil

Quantitatively, the most abundant components in hydrodistilled ZM essential oils are oxygenated monoterpenes (approximately 70%) followed (in order) by monoterpene hydrocarbons, sesquiterpene hydrocarbons and oxygenated sesquiterpenes (Mohagheghzadeh et al., 2000; Saei Dehkordi et al., 2010). Gupta et al. reported that the essential oil of the Indian ecotype consists of 69% phenols containing mainly carvacrol. In another survey, *p*-cymene was reported as the main constituent of the non-phenolic portion of the oil (Gupta and Gupta, 1972). Additionally, two studies from Pakistan identified carvacrol as the main constituent of the oil (Malik et al., 1987; Ahmad et al., 1999). To date, a large number of studies have focused on ZM essential oil: some reported carvacrol as the main compound, but others reported thymol, the isomer of carvacrol, as the main compound. Saei Dehkordi et al. (2010) collected ZM from five different areas of Iran and analyzed its oils. According to the GC–MS data, the main oil constituents remained similar between plants from different geographical regions, but their relative quantities differed among plants from different regions. Thymol was the most abundant compound among all constituents in all samples (Saei Dehkordi et al., 2010). Additionally, Saleem et al. showed that thymol is the main constituent of the fresh plant (73.21%), while carvacrol is the primary constituent in the dried plant (62.87%) (Saleem et al., 2004). Some studies on the essential oils of this plant are shown in Tables 2. It is clear that geographical variation, cultivar differences, stage of plant growth, preparation process and other factors may influence oil composition both quantitatively and qualitatively.

According to the data presented in Table 2, the essential oils of ZM contain significant amounts of thymol and carvacrol, which are well-known anti-microbial and anti-fungal agents (Shafiee and Javidnia, 1997; Baser, 2008). *p*-Cymene is the other main component in the ZM oils. Zatariol, zataroside A (glycoside and non-volatile), zataroside B (glycoside and non-volatile), multiflotriol and multiflorol have been reported as new derivatives of *p*-cymene isolated from ZM (Ali et al., 1999a, 1999b, 2000). Linalool, caryophyllene, γ-terpinene and borneol are some of the other main components in the oils (Fig. 1).

Table 1
Pharmaceutical forms of ZM on Iranian market.

Product name (trade name)	Dosage form	Active ingredient(s)	Application
Broncho T.D.	Syrup	Essential oil containing 1.0–1.5 mg thymol in 120 mL of syrup.	Cough, bronchitis and laryngitis
Tussivin	Oral drop	ZM and *Foeniculum vulgare* oils containing at least 2.2–3.5 mg thymol and 5.5–8.5 mg anethol in each mL of drop.	Cough
Gastrolit	Oral drop	ZM oil containing at least 4.4–7.0 mg thymol in each mL of drop.	Irritable bowel syndrome, stomachache, flatulence and reflux
Leucorex	Vaginal cream	ZM oil containing 0.027–0.033% thymol	Candidiasis, trichomoniasis and infection by *Gardnerella vaginalis*
Bronchobarij	Syrup	ZM and *Althaea officinalis* extracts and containing 2.55–3.06 mg thymol and 24.22–29.32 mg amino acid in each 5.0 mL of syrup	Cough, bronchitis, laryngitis and pertussis
Gastrolit	Soft capsule	ZM oil containing at least 5.5–6.5 mg thymol in each soft capsule	Irritable bowel syndrome

Table 2
The main components of different ecotypes of *Z. multiflora* essential oil.

Main components					Origins of plant	References
Carvacrol (57.40%)	Thymol (15.60%)	Caryophylene (8.30%)	*p*-Cymene (7.10%)	α-Phellandrene (2.40%)	Pakistan	Malik et al. (1987)
Thymol (48.4%)	Carvacrol (12.6%)	*p*-Cymene (13.5%)	Linalool (5.2%)	γ-Terpinene (3.9%)	Iran	Shafiee and Javidnia (1997)
Carvacrol (62.74%)	Methyl carvacrol (6.5%)	Carvacrol acetate (4.4%)	*p*-Cymene (7.1%)	γ-Terpinene (5.6%)	Pakistan	Ahmad et al. (1999)
Thymol (44.6%)	γ-Terpinene (21.5%)	*p*-Cymene (13.7%)	Carvacrol (2.35%)	β-Caryophyllene (2.20%)	Iran, Tehran	Ebrahimzadeh et al. (2003)
Thymol (52.4%)	γ-Terpinene (17.1%)	*p*-Cymene (13.2%)	Carvacrol (6.1%)	α-Terpinenyl acetate (5.4%)	Iran, Shiraz	Sadeghzadeh et al. (2005)
Carvacrol (71.12%)	γ-Terpinene (7.34%)	α-Pinene (4.26%)	Eucalyptol (3.37%)	Globulol (2.32%)	Iran, Shiraz	Basti et al. (2007a)
Thymol (37.59%)	Carvacrol (33.65%)	*p*-Cymene (7.72%)	γ-Terpinene (3.88%)	β-caryophyllene (2.06%)	Iran, Firoozabad	Sharififar et al. (2007)
Carvacrol (37%)	*p*-Cymene (15%)	Dodecane (8.9%)	γ-Terpinene (6.5%)	Carvacrol methyl ether (5.2%)	Iran, Fars province	Fakour et al. (2007)
Thymol (39.65%)	Carvacrol (36.21%)	*p*-Cymene (10.62%)	Carvacrol methyl ether (1.77%)	Linalool (1.76%)	Iran, Tehran	Sharif Roohani et al. (2007)
Thymol (33.6%)	Carvacrol (17.0%)	*p*-Cymene (11.7%)	Linalool (5.0%)	Caryophyllene (2.9%)	Iran, Tehran	Abkenar et al. (2008)
Carvacrol (26.08%)	*p*-Cymene (20.34%)	Thymol (17.23%)	Linalool (10.09%)	β-Caryophyllene (4.27%)	Iran, Karaj	Shahsavari et al. (2008)
Carvacrol (61.29%)	Thymol (25.18%)	Linalool (1.96%)	*p*-Cymene (1.90%)	β-Caryophyllene (1.82%)	Iran, Shiraz	Khosravi et al. (2009)
Thymol (38.7%)	Carvacrol (15.3%)	*p*-Cymene (10.2%)	γ-Terpinene (9.8%)	α-Pinene (4.6%)	Iran, Kashan	Mahboubi and Ghazian Bidgoli (2010)
Thymol (47.46%)	*p*-Cymene (13.16%)	Carvacrol (9.64%)	Linalool (7.92%)	γ-Terpinene (2.72%)	Iran, Hajiabad	Saei Dehkordi et al. (2010)
Thymol (46.61%)	Carvacrol (17.26%)	*p*-Cymene (11.51%)	γ-Terpinene (4.01%)	β-Caryophyllene (2.91%)	Iran, Farashband	Saei Dehkordi et al. (2010)
Thymol (40.94%)	Carvacrol (22.39%)	*p*-Cymene (7.73%)	γ-Terpinene (5.43%)	β-Caryophyllene (3.95%)	Iran, Yazd	Saei Dehkordi et al. (2010)
Thymol (64.87%)	γ-Terpinene (9.11%)	*p*-Cymene (5.63%)	Carvacrol (4.65%)	β-Caryophyllene (3.41%)	Iran, Najafabad	Saei Dehkordi et al. (2010)
Thymol (27.05%)	*p*-Cymene (9.49%)	Borneol (7.1%)	*cis*-Sabinene hydrate (6.12%)	Linalool (5.63%)	Iran, Poldokhtar	Saei Dehkordi et al. (2010)

2.2. Other compounds

ZM also contains other compounds belonging to different classes of natural products, including alkanes such as *n*-nonacosane (C29), *n*-hentriacontane (C31), *n*-dotriacontane (C32), *n*-tritriacontane (C33) and *n*-pentatriacontane (C35); fatty acids such as behenic acid (C22), lignoceric acid (C24), cerotic acid (C26) and montanic acid (C28) (Gupta and Gupta, 1972); phytosterols such as β-sitosterol and stigmasterol; triterpenes such as betulin, betulinic acid and oleanolic acid (Ali et al., 1999b, 2000; Rajaei and Khajehali, 2009); and hydroxycinnamic acids such as rosmarinic acid (Mohagheghzadeh et al., 2004) (Fig. 2). Moreover, flavonoids such as apigenin, luteolin and 6-hydroxyluteolin are also among the phytochemicals reported from ZM (Ali et al., 2000). This plant also contains small amounts of tannins, resins and saponins while lacking alkaloids (Jaffary et al., 2000).

3. Pharmacology (Table 3)

3.1. Traditional uses

ZM is extensively used as a flavor ingredient in a wide variety of foods. It has a long history of medicinal uses in its native regions. This plant has several traditional uses (via infusion, decoction or vapor) including as a carminative, stimulant, diaphoretic, diuretic (Gupta and Gupta, 1972), antiseptic, vermifuge, anesthetic, anti-spasmodic, anthelmintic, antidiarrheal and analgesic (Iranian Herbal Pharmacopoeia, 2002). Additionally, ZM has been commonly used as an antiseptic and anti-tussive agent for the management of respiratory tract disorders (Aynehchi, 1991). Other medicinal uses of ZM include treatment of some GI disorders, such as bloating, dyspepsia and irritable bowel syndrome (Amin, 1991), fever, premature labor pain, rupture, bone and joint pain, headache, migraine, gastrodynia, diarrhea, vomiting and the common cold (Naghibi et al., 2005).

3.2. Anti-bacterial properties

Plant essential oils are good sources of oxygenated monoterpenes, in particular thymol and carvacrol, with significant anti-microbial properties. Due to the differences in the test methods, bacterial strains and plant source, making a direct comparison of the findings from different studies is difficult. Moreover, it is well documented that genetic as well as environmental conditions could affect the yield and composition of the essential oils and thus their anti-microbial properties (Mansour et al., 2010). It has been accepted that the anti-microbial activity of most essential oils is related to their phenolic monoterpenes (Saei Dehkordi et al., 2010). ZM essential oil, with a very high percentage of thymol and carvacrol, possesses significant anti-microbial activity (Shafiee et al., 1999). Electron micrographs showed that these compounds, which are lipophilic in nature, act on the cell membrane and cause substantial morphological damage, resulting in a change in permeability and the release of cellular contents (Moosavy et al., 2008). *p*-Cymene is another common component of the oil; however, contradictory data have been reported regarding the anti-microbial role of *p*-cymene. Some studies have shown an antagonistic interaction between phenolic monoterpenes, thymol and carvacrol, and *p*-cymene. In contrast, other studies showed that *p*-cymene is an ineffective anti-microbial agent individually, and its combination with carvacrol led to a synergistic activity that resulted in destabilization of the microbial membrane (Saei Dehkordi et al., 2010). ZM leaf oil is stable at high temperatures and over a wide range of pH values. In addition, proteolytic treatment (through addition of protease) of the oil does not affect its anti-bacterial activity. Hence, it is plausible that non-proteinic compounds are responsible for the observed anti-bacterial properties of this plant (Mansour et al., 2010). The extracts of this plant showed a weaker anti-bacterial activity compared to the essential oil. Anti-bacterial properties of polar extracts could also be attributed to the presence of several types of compounds, such as flavonoids and more polar thermo-labile and/or thermo-stable phenolics. Moreover, the presence of rosmarinic acid in the plant

Table 3
Summary of pharmacological and clinical studies of *Zataria multiflora*.

Reported activity	Type of preparation	Dose	Route of administration	Study model	Control treatment	Main finding(s)	References
Anti-nociceptive	Aqueous and ethanolic extracts	0.1, 0.2, 0.8 and 1.4 g/kg (hot-plate test) 0.04, 0.08 and 0.16 g/kg (writhing test)	i.p.	Albino mice	Positive control: morphine Negative control: naloxone	Aqueous and ethanolic extracts showed antinociceptive activity in both hot-plate and writhing tests	Hosseinzadeh et al. (2000)
Anti-inflammatory	Aqueous and ethanolic extracts	0.2, 0.8 and 1.4 g/kg (acetic acid-induced vascular permeability) 0.2, 0.8 and 1.4 g/kg (xylene-induced ear edema test and cotton pellet test)	i.p.	Albino mice	Positive control: dexamethasone and diclofenac Negative control: naloxone	Aqueous and ethanolic extracts showed inhibitory activities against both acute (assessed through acetic acid-induced vascular permeability and xylene-induced ear edema) and chronic (assessed through cotton pellet test)	Hosseinzadeh et al. (2000)
Anti-nociceptive	Hydroalcoholic extract; essential oil of aerial parts	500 mg/kg (hydroalcoholic extract) and 0.3 ml/kg (essential oil)	i.p.	Male Wistar rats	Positive control: morphine Negative control: normal saline	Hydroalcoholic extract and essential oil of ZM showed anti-inflammatory activity in writhing, tail flick and formalin tests	Jaffary et al. (2004)
Anti-nociceptive	Fractionated extracts of the aerial parts	0.1, 0.2 and 0.8 g/kg	i.p.	Male albino mice	Positive control: morphine and diclofenac Negative control: normal saline	Hydroalcoholic extract of ZM showed anti-inflammatory activity in both hot-plate and writhing tests.	Ramezani et al. (2004)
Anti-nociceptive (against dysmenorrheal pain)	Essential oil at 1% and 2% concentrations	25 drops q.i.d.	Oral	Female subjects with primary dysmenorrhea	Placebo controlled	Both tested concentrations of ZM oil could significantly lessen dysmenorrheal pain as assessed by visual analog scale and multidimensional system scales.	Iravani (2009)
Anti-nociceptive (against dysmenorrheal pain)	Leaf infusion (obtained from 15 g of leaf powder)	One glass of infusion	Oral	Female subjects with primary dysmenorrhea	Positive control: mefenamic acid (250 mg)	ZM infusion could significantly lessen dysmenorrheal pain as assessed by visual analog scale. Antidysmenorrheal effects were comparable to mefenamic acid.	Rouzbahani et al. (2005)
Anti-leishmaniasis	Hydroalcoholic extract	Topical application of extract b.i.d.	Topical	Female Balb/c mice	Positive control: glucantime Negative control: 70% alcohol or no treatment	ZM extract effectively reduced ulcer size.	Hejazi et al. (2009)
Anti-inflammatory	Total extract, flavonoid and the essential oil	total extract (900 mg/kg oral, 500 mg/kg i.p.), flavonoid (900 mg/kg oral and i.p)	Oral and i.p.	Male Wistar rats	Positive control: indomethacin	Total extract, flavonoid fraction and essential oil of ZM showed significant preventive effect against carrageenan-induced rat paw edema.	Jaffary et al. (2000)
Antioxidant	Methanolic extract	50, 100 and 200 mg/kg/d	Intragastric intubation	Male Wistar rats	Negative control: Normal saline Positive control: α-tocopherol	The extract at doses of 50 and 100, but not 200 mg/kg/d, showed antioxidant activity in the DPPH test, reduced thiobarbituric acid reactive species and increased total antioxidant power.	Babaie et al. (2007)
Anti-diabetic	Essential oil	50 μL/kg body weight of 100 μL/mL essential oil	Oral	Streptozocin-induced diabetic rats	Negative control: vehicle	ZM oil protected against diabetic damage and reduced serum levels of glucose, alanine aminotransferase, aspartate aminotransferase while increasing those of total protein and insulin.	Kavoosi (2011)
Immunostimulatory activity	Essential oil	0.2 g/ Kg, 6 times with 6 days of interval	Oral and subcutaneous injection	Rabbits	Negative control: Normal saline	Subcutaneous but not oral administration of ZM oil stimulated lymphocyte transformation in response to *Candida albicans* and Con-A mitogen, and boosted the phagocytosis of *Candida albicans* by neutrophils.	Khosravi et al. (2007)
Immunostimulatory activity	Essential oil	100 mg/kg	i.p.	Female Balb/c mice	Negative control: Distilled water	ZM oil stimulated innate immunity in terms of increasing phagocytosis and TNF-α secretion	Shokri et al. (2006)
Immunostimulatory activity	Essential oil	30, 60 and 120 ppm of ZM oil for a	Oral		–		Soltani et al. (2010)

Table 3 (*continued*)

Reported activity	Type of preparation	Dose	Route of administration	Study model	Control treatment	Main finding(s)	References
		period of 8 continous days		Common carp (*Cyprinus carpio*)		ZM oil enhanced the antibody production, total white blood cell count and serum bactericidal activity	
Anti-aphthous activity	Essential oil	0.2% oral mouthwash 3 times a day for 4 weeks	Oral rinse	Patients with recurrent aphthous stomatitis	Matched placebo mouthwash	ZM oil exerted beneficial effects in the treatment of recurrent aphthous stomatitis.	Mansoori et al. (2002)
Anti-aphthous activity	Hydroalcoholic leaf extract	10 drops of extract 5 times a day for a week.	Topical application	Patients with recurrent aphthous stomatitis	Positive control: *Myrthus communis* mouthwash	ZM extract improved the healing of aphthous lesions and exerted pain relieving effects.	Jafari et al. (2003)
Anti-ulcerogenic	Hydroalcoholic extract	200, 400, 800 and 1200 mg/kg	Oral	Male wistar rats	Negative control: vehicle Positive control: ranitidine and sucralfate	Significant reduction of duodenal ulceration induced by cysteamine HCl	Minaiyan et al. (2005)
Treatment of inflammatory bowel disease	Total methanol extract	400, 600 and 900 ppm	Oral	NMRI albino mice	Negative control: acetic acid Positive control: prednisolone	Mitigation of oxidative stress and improvement of macroscopic and microscopic parameters of colon	Nakhai et al. (2007)
Regulation of MDM2 gene expression	Essential oil	50 µl/kg/d	i.p.	Male Sprague-Dawly rats	Negative control: dimethyl sulfoxide	Down-regulation of MDM2 gene expression	Gohar et al. (2010)
Modulation of hematological and immunological parameters	Essential oil	30, 60 and 120 ppm (for oral treatment), and 7.5, 15 and 30 ppm (for bath treatment)	Oral or bath treatment	Common carp (*Cyprinus carpio*)	nd	Enhancement of respiratory burst activity of neutrophils+moderate effect on erythrocyte count and hematocrit	Sheikhzadeh, et al. (2011)
Hepatoprotective effects	Methanol extract	800 ppm/d for 7 days	Oral	Male Wistar rats	-	Prophylaxis with ZM extract effectively prevented halothane-induced hepatotoxicity and ameliorated serum levels of hepatic injury biomarkers	Sakhaee et al. (2011)

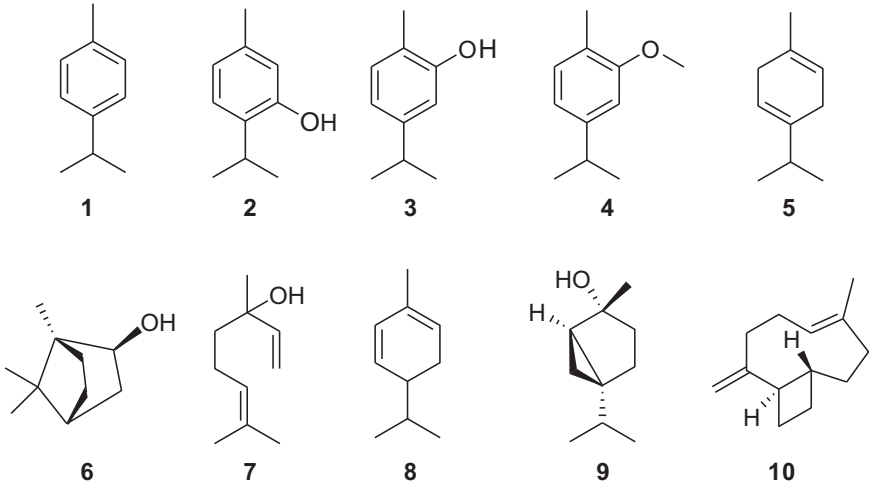

Fig. 1. Chemical structures of main volatile constituents of *Z. multiflora* essential oil; **1** (*p*-Cymene), **2** (Thymol), **3** (Carvacrol), **4** (Methyl carvacrol), **5** (γ-Terpinene), **6** (Borneol), **7** (Linalool), **8** (α-Phellandrene), **9** (*cis*-Sabinene hydrate) and **10** (β-Caryophyllene).

extract may be another contributing factor to the anti-bacterial activity of the plant polar fractions (Sharififar et al., 2007). The anti-bacterial effects of ZM was studied on different strains of bacteria (Table 4). In 2001, Rasouli et al. found that bactericidal concentrations of hydro-distilled essential oil of the plant on two prevalent pathogenic bacteria, *Staphylococcus aureus* and *Escherichia coli*, were less than that of ampicillin (Rasouli, 2001). ZM oil has also been found to be active against clinical isolates of extended spectrum β-lactamase-producing *Klebsiella pneumoniae* (Eftekhar et al., 2011). It has been shown that ZM extract can inhibit the release of the DNase enzyme and the production of enterotoxin in *Staphylococcus aureus* (Zaringhalam et al., 2007; Parsaeimehr et al., 2010), as well as the production of verotoxin *Escherichia coli* (Goudarzi et al., 2008). The essential oil of the plant could also serve as an efficient food preservative as it has been shown to inhibit the growth of several food-borne pathogens such as *Staphylococcus aureus*, *Escherichia coli*,

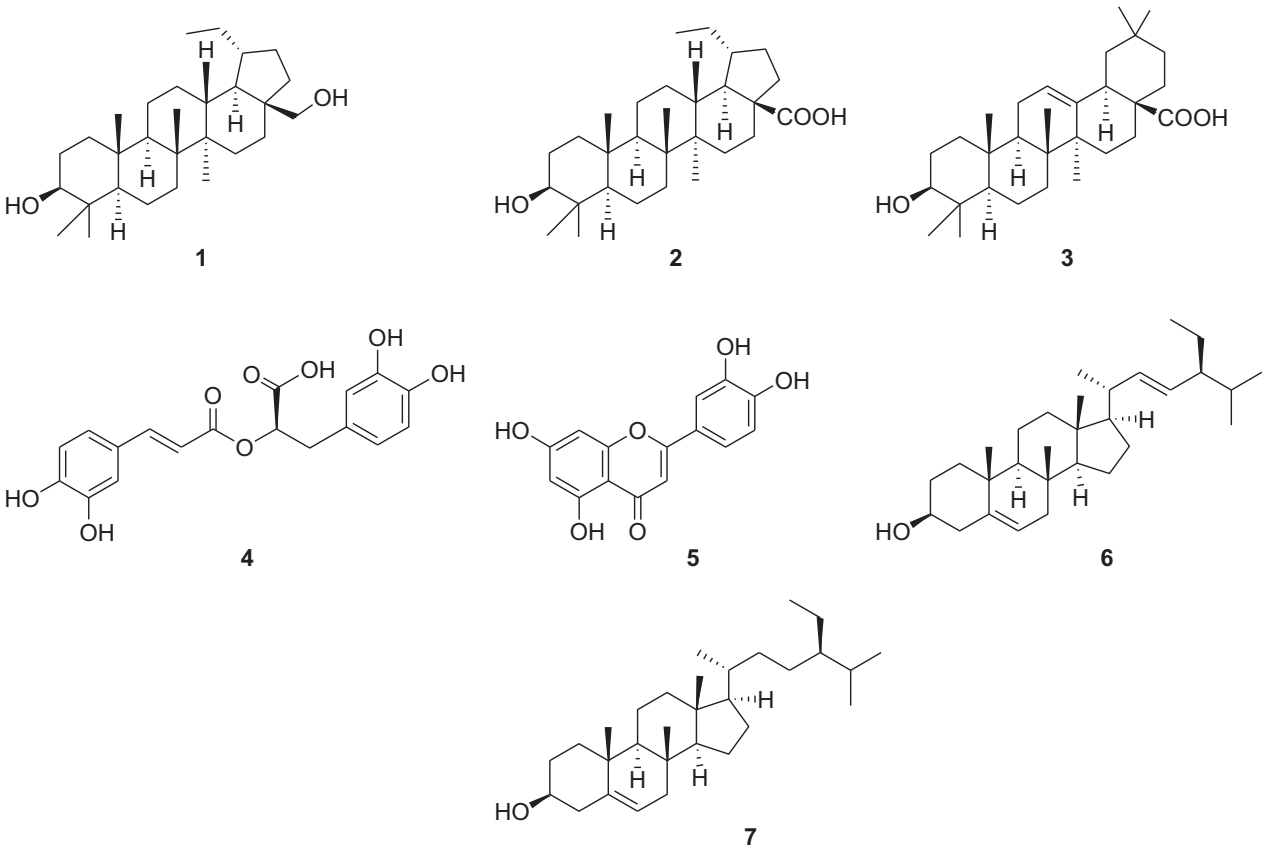

Fig. 2. Chemical structures of main non-volatile constituents of *Zataria multiflora*; **1** (Betulin), **2** (Betulinic acid), **3** (Oleanolic acid), **4** (Rosmarinic acid), **5** (Luteolin), **6** (Stigmasterol) and **7** (β-Sitosterol).

Salmonella typhimorium, *Bacillus cereus* and *Listeria monocytogenes* (Basti et al., 2007a, 2007b; Fazeli et al., 2007; Khanzadi et al., 2007; Abbasifar et al., 2008, 2009; Rahnama et al., 2009). Furthermore, the plant extract has been suggested to be effective as a mouthwash due to its significant effect against oral Streptococci (Oulia et al., 2004). The essential oil of ZM was effective in killing *Enterococcus faecalis* and therefore the cleansing of the root canal in dentistry (Ravanshad et al., 2007). Inhibitory effects of ethanol, methanol, chloroform and hexane extracts of ZM were also investigated against multiple drug resistant *Pseudomonas aeruginosa*. The results indicated that while all tested extracts were active, maximum anti-bacterial activity was observed from methanol extract. Additionally, the combination of extracts had variable synergistic/antagonistic effect (Rahman et al., 2008). Findings on the antibacterial properties of ZM are not limited to the aforementioned studies, and other reports exist confirming the inhibitory effects of the plant oil against different bacterial strains (Shakeri et al., 2011; Ghasemi et al., 2012; Zomorodian et al., 2011; Ekhtiarzadeh et al., 2012; Motaharinia et al., 2012). These results along with findings of other related studies have been summarized in Table 4.

3.3. Anti-fungal properties

Several studies have described the anti-fungal activity of ZM oil against *Candida* species (Abou fazeli et al., 2000; Eslami et al., 2004; Amanlou et al., 2006; Khosravi et al., 2009). Abou Fazeli et al. demonstrated the activity of the essential oil against *Candida albicans*. They also suggested ZM oil-containing vaginal suppositories as a successful replacement for current drugs for the treatment of vaginitis caused by *Candida albicans* (Abou fazeli et al., 2000). In a comparative study, a 7-d therapy with ZM as an intravaginal cream was more effective than clotrimazole vaginal cream in the treatment of *Candida* vaginitis (Eslami et al., 2004). An open-label, randomized and controlled study with two parallel treatment groups was conducted to evaluate the efficacy of a miconazole 2% gel compared with a ZM 0.1% gel applied four times daily for 2 weeks in the treatment of *Candida*-associated denture stomatitis. The results indicated that the ZM gel reduced the surface erythema of the palate more efficiently than miconazole gel but did not reduce the colony count on the denture surface as efficiently as miconazole (Amanlou et al., 2006). Another study compared the efficacies of the oil and itraconazole in clearing *Candida albicans* from the visceral organs of BALB/c mice suffering from disseminated candidiasis. The results demonstrated that intra-peritoneal administration of the oil had the highest efficacy in reducing *Candida albicans* (Khosravi et al., 2009). In another study, the minimum inhibitory activity of the ZM methanolic extracts against various dermatophytes was found to be approximately 0.5% (w/v). In the same study, the physicochemical properties and stability of creams containing different concentrations (1–3%) of ZM were evaluated and suggested as a successful replacement in the treatment of *Candida albicans*-induced vaginitis (Aghel et al., 2007). Yet another study examined the anti-fungal properties of the essential oil on growth inhibition of *Aspergillus parasiticus* and its aflatoxin production; the oil demonstrated powerful inhibitory properties on fungal growth and aflatoxin production (Fakour et al., 2007). Mahmoudabadi et al. studied the anti-*Candida* activity of three extracts of the aerial parts of ZM (aqueous, ethanolic and methanolic) against four *Candida* species (*Candida albicans*, *Candida tropicalis*, *Candida glabrata* and *Candida parapsilosis*). An aqueous extract showed no remarkable activity against the *Candida* species. In contrast,

Table 4
Summary of findings on the anti-bacterial effects of *Zataria multiflora*.

Organisms	Fraction	MIC (μg/mL)	DD (mm)	Positive control	MIC (μg/mL)	DD (mm)
Sharififar et al. (2007)						
Staphylococcus aureus	Essential oil	21	10 ± 1.0	Gentamicin	8	16 ± 1.5
Escherichia coli	Essential oil	42	9 ± 1.3	Gentamicin	16.5	10 ± 1.2
Klebsiella pneumoniae	Essential oil	30	16 ± 1.8	Gentamicin	12	9 ± 1.2
Staphylococcus epidermidis	Essential oil	5.5	14 ± 1.5	Gentamicin	4	18 ± 1.9
Enterococcus faecalis	Essential oil	38	18 ± 1.4	Gentamicin	6	11 ± 1.2
Bacillus subtilis	Essential oil	44	16 ± 1.8	Gentamicin	18	12 ± 1.3
Salmonella typhimorium	Essential oil	15	11 ± 1.8	Gentamicin	8.5	8 ± 1.1
Serratia marcescens	Essential oil	5.5	9 ± 1.3	Gentamicin	1.2	11 ± 1.1
Shigella flexneri	Essential oil	2.4	13 ± 1.4	Gentamicin	3	12 ± 1.2
Oulia et al. (2004)						
Streptococcus sanguis *Streptococcus salivarius* *Streptococcus mutans*	**Results:** The MIC values for *Streptococcus sanguinis*, *Streptococcus salivarius* and *Streptococcus mutans* were 15%, 20% and 10%. MBC values were 25%, 20% and > 25%, respectively.					
Sadeghzadeh et al. (2005)						
Salmonella paratyphi A *Salmonella paratyphi B*	**Results:** The essential oil at concentrations of 1%, 2.5% and 5% inhibited the growth of *Salmonella paratyphi A* with growth inhibition diameters of 0, 9.3 and 15.6 mm, respectively. The growth of *Salmonella paratyphi B* was also inhibited by 1%. 2.5% and 5% of the oil, with growth inhibition diameters of 8, 8.6 and 21.6 mm, respectively.					
Yazdi et al. (2008)						
Streptococcus pneumoniae	MIC = 163.88 μg/mL, DD = 52 ± 1 mm for the essential oil					
Moraxella catarrhalis	MIC = 81.94 μg/mL, DD = 55 ± 2 mm for the essential oil					
Haemophilus influenzae	MIC = 81.94 μg/mL, DD = 40 ± 1 mm for the essential oil					
Fazeli et al. (2007)						
Bacillus cereus	MIC = 0.4%, DD = 26 mm for the ethanolic extract.					
Proteus vulgaris	MIC = 0.4%, DD = 24 mm for the ethanolic extract.					
Zahraei salehi et al. (2005)						
Streptococcus agalactiae	MIC = 94 μg/mL, DD = 52 mm for the essential oil.					
Abbasgholizadeh et al. (2008)						
Proteus mirablis	MIC = 156 μg/mL for the essential oil.					
Khanzadi et al. (2007)						
Clostridium botulinum	The Log P% of growth of *Clostridium botulinum* was decreased by increasing the concentration of essential oil					
Oulia et al. (2009)						
Pseudomonas aeruginosa	MIC = 64 μg/mL and MBC = 128 μg/mL for the essential oil					
Rahnama et al. (2009)						
Listeria monocytogenes	MIC = 9.5 μg/mL for the essential oil. The inhibitory effect was enhanced in combination with nisin (an antibacterial peptide)					
Ekhtiarzadeh et al. (2012)						
Vibrio parahaemolyticus *Listeria monocytogenes*	**Results:** ZM oil had significant inhibitory effect on the growth of *Vibrio parahaemolyticus* and *Listeria monocytogenes* in salted fish fillet. The oil had considerable synergism with nicin					
Motaharinia et al. (2012)						
Brucella strains	MIC = 1237 μg/mL and MBC = 5902 μg/mL for the ethanol extract of ZM					
Purfard and Kavoosi (2012)						
Salmonella typhimorium	MIC = 2.65 μg/mL for essential oil					
Escherichia coli	MIC = 2.72 μg/mL for essential oil					
Klebsiella pneumoniae	MIC = 2.85 μg/mL for essential oil					
Staphylococcus aureus	MIC = 3.02 μg/mL for essential oil					
Staphylococcus epidermidis	MIC = 3.53 μg/mL for essential oil					
Bacillus subtilis	MIC = 3.65 μg/mL for essential oil					
Rahmani et al. (2012a)						
Listeria monocytogenes	**Result:** ZM oil dose-dependently and significantly prevented the growth of *L. monocytogenes* in the roast-chicken fillets					
Rahmani et al. (2012b)						
Escherichia coli O157:H7	**Result:** ZM oil inhibited the growth of *E. coli* O157:H7 in mechanical deboned meat					

a methanolic extract showed great inhibitory effects on *Candida* at 70.7 mg/L compared to an ethanolic extract 127 mg/L. In addition, the isolates of *Candida parapsilosis* were more susceptible to methanolic extract than other tested species (Mahmoudabadi et al., 2007). Moreover, the effect of the essential oil against growth, spore production and aflatoxin formation by *Aspergillus flavus* and its morphological alteration action were investigated. The results indicated that at an oil concentration of 150 ppm of the oil, the mycelial growth and aflatoxin accumulation were reduced by 90% and 99.4%, respectively (Gandomi et al., 2008, 2009). Furthermore, the anti-fungal activity of ZM extracts against *Aspergillus flavus* in a whey protein concentrate-based (WPC) coating on pistachio kernels was investigated. The results showed that WPC coating incorporated with 2500 ppm of extract on pistachio kernels completely inhibited *Aspergillus flavus* growth (Javanmard and Ramezan, 2009). In another study, the anti-fungal action of ZM essential oil was studied on five different dermatophyte and saprophyte species. It was found that the growth of the dermatophytes (*Trichophyton mentagrophytes*, *Trichophyton rubrum* and *Epidermophyton floccosum*) and saprophytes (*Aspergillus fumigatus* and *Aspergillus flavus*) were completely inhibited by an essential oil concentration of 8% and

10%, respectively (Effatpanah and Sabokbar, 2010). Other reports have demonstrated the antifungal activity of ZM against *Aspergillus* (Abdollahi et al., 2011), *Malassezia* (Naeini et al., 2011; Shokri et al., 2011), *Fusarium* (Shokri et al., 2011) and *Saprolegnia* (Khosravi et al., 2012) species.

3.4. Anti-protozoal properties

Abdollahy et al. (2004) studied the *in vitro* effects of hydrodistilled essential oils of ZM against *Tricomonas vaginalis* (TV) infection. The authors found that ZM oil was effective at the stars as well as after 2 and 4 h of inoculation. The alcoholic extract of the plant had also considerable dose-dependent effects in the treatment of leishmaniasis in mice, with a reported IC_{50} value of 7.4 μg/mL (Hejazi et al., 2009; Barati et al., 2010). Another recent report on the potent *in vitro* acaricidal activity of ZM oil by Pirali-Kheirabadi et al. showed that ZM oil possesses promising and dose-dependent toxic effects on the engorged stage of *Rhipicephalus* (*Boophilus*) *annulatus* (Pirali-Kheirabadi and Teixeira da Silva, 2011).

3.5. Spasmolytic properties

Gharib Naseri et al. found that ZM hydroalcoholic leaf extracts had inhibitory effects on isolated rat ileum contractions induced by known ileum stimulants (KCl, acetylcholine and $BaCl_2$) (Gharib Naseri, 2003). These results were consistent with those reported from the same plant against rat uterine contractions (Gharib Naseri et al., 2005). The ineffectiveness of propranolol and naloxone on the inhibitory effect of ZM indicated that adrenergic and opioids agonists were not present in the extract. In the same manner, it appeared that there were no anticholinergic substances in the extract. The authors speculated that ZM extract might induce the inhibitory effect through blockade of the voltage-dependent calcium channels and through releasing calcium from intracellular stores in rat ileum and uterus smooth muscles (Gharib Naseri, 2003; Gharib Naseri et al., 2005).

3.6. Anti-nociceptive properties

Hosseinzadeh et al. was the first group that studied the anti-nociceptive activity of the aqueous infusion and ethanolic maceration extracts of the aerial parts of ZM. Hot-plate and writhing tests were two applied models for the evaluation of anti-nociceptive activity in the study. Intraperitoneal injection of both extracts in mice showed significant and dose-dependent peripheral and central anti-nociceptive activity. Pretreatment with naloxone, an opioid antagonist inhibited the anti-nociceptive properties of the extracts. It was concluded that this activity may be mediated by opioid receptors, although other mechanisms of action such as inhibition of cyclooxygenase were also possible (Hosseinzadeh et al., 2000). The anti-nociceptive effects of ZM were further confirmed by other studies and attributed to the existence of flavonoids in the plant extract (Jaffary et al., 2004; Ramezani et al., 2004). The effect of this plant on decreasing pain of primary dysmenorrhea has been supported by two clinical studies. The findings indicated that the essential oil could be used as an effective herbal drug for primary dysmenorrheal, which appears to be due to its anti-prostaglandin and anti-spasmodic effects (Rouzbahani et al., 2005; Iravani, 2009).

3.7. Anti-inflammatory properties

The aqueous and ethanolic extracts from the aerial parts of ZM had protective activity against acute and, in particular, chronic inflammation (Hosseinzadeh et al., 2000). Jaffary et al. reported that all fractions of the plant, including total extract, flavonoid fraction and the essential oil, have significant preventive effect on carrageenan (CAR) induced rat paw edema while lacking any ulcerogenic effects on rat stomach, the latter being a typical side effect of indomethacin (a standard anti-inflammatory agent) (Jaffary et al., 2000). This anti-inflammatory effect of ZM was, at least in part, due to its flavonoids and the essential oil, but other active substances of the plant might also be involved. Following purification of active ingredients, further toxicological and *in vivo* investigations are needed to unravel the anti-inflammatory properties of this plant.

3.8. Antioxidative properties

Significant antioxidant activity of the methanolic extract of ZM has been measured by evaluating the inhibitory activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, total antioxidant power (TAP) and thiobarbituric acid reactive substances (TBARS) in the serum of treated rats and showed significant antioxidant activity (Babaie et al., 2007). Additionally, the essential oil of ZM possessed antioxidant activity as measured by the DPPH free radical scavenging and β-carotene bleaching assays (Sharififar et al., 2007; Shahsavari et al., 2008; Saei Dehkordi et al., 2010). In another study, ZM was reported to reduce the oxidative stress and genotoxicity induced by cyclophosphamide in mouse bone marrow cells (Hosseinimehr et al., 2010). Karimian et al. have also shown that ZM oil possess nitric oxide and malondialdehyde scavenging properties and thus could prevent nitrosative stress and lipid peroxidation (Karimian et al., 2011). In consistence, ZM oil has recently been reported to mitigate glucose-induced overproduction of nitric oxide and H_2O_2 along with NADH oxidase and nitric oxide synthase activities in cultured human monocytes. These effects were replicated by using thymol and carvacrol but not *p*-cymene, γ-terpinene and linalool (Kavoosi and Teixeira da Silva, 2012). The same effects for ZM oil, thymol and carvacrol were also found in another study on lipopolysaccharide-stimulated macrophages (Kavoosi et al., 2012). Such antioxidant effects of ZM oil have been confirmed *in vivo* using streptozocin-induced diabetic rats (Kavoosi, 2011).

3.9. Effects on the immune system

For the first time, Khosravi et al. showed that subcutaneous administration ZM essential oil stimulates a significant cellular immune response in rabbits. In the mentioned study, subcutaneous, but not oral, administration of ZM oil enhanced lymphocyte transformation against *Candida albicans* antigens and concanavalin A mitogens. Moreover, ZM oil had considerable effect on the phagocytosis of *Candida albicans* by neutrophils, which was again more prominent in the subcutaneous *vs.* oral route (Khosravi et al., 2007). Shokri et al. evaluated the effects of ZM essential oil on the function of innate immunity, including phagocytic activity and TNF-α production in an animal model. The results indicated a significant increase in TNF-α secretion in comparison with the control group after 4 and 7 days of treatment. Furthermore, the mean rate of phagocytosis (determined as respiratory burst) was stimulated by ZM oil up to 1.6 and 2.3 folds higher than that of a control group after 4 and 7 days of treatment, respectively (Shokri et al., 2006). A previous study proved that ZM essential oil had immunostimulatory effects on some immunological factors, such as antibody titers against *Aeromonas hydrophila*, total white blood cells and serum bactericidal activity, in fish. However, the latter study (Soltani et al., 2010) failed to detect any significant difference in serum

Table 5
Cytopathologic effect of *Zataria multiflora* extract and its essential oil on Vero, Hela, HEp II cell lines. All extracts were methanolic. Both ZM extract and oil were tested at 0.1875, 0.375, 0.75, 1.5, 3.0, 6.0, 12.0 and 24.0 ppm concentrations.

	Cytopathologic concentration of ZM extract (ppm)	Cytopathologic concentration of ZM essential oil (ppm)
HEp II	0.09375–0.75	0.046–0.1875
Hela	0.1875–0.375	0.093–0.375
Vero	0.1875–0.75	0.093–0.1875

lysozyme activity, total protein, globulin and albumin contents between ZM-treated and control group.

3.10. Efficacy of ZM for the treatment of RAS in clinical trials

Recurrent aphthous stomatitis (RAS) is a chronic inflammatory disease characterized by recurrent, painful single or multiple necrotizing ulcerations of the oral mucosal tissue. Despite substantial clinical attention, the causes of this disease are poorly understood (Mansoori et al., 2002). In 2002, a clinical study evaluated the efficacy of the topical use of 0.2% oral mouth wash consisting of ZM essential oil on RAS. In this randomized double-blind study, each of the 60 recruited RAS patients was assigned either an oral ZM oil-based rinse solution (three times a day for 4 weeks) or a placebo. The patients were monitored two times per week. Eighty-three percent of patients responded well to the essential oil formulation, whereas only 17% reported deterioration of their symptoms following use of the ZM mouth wash. In the placebo group, the rate of RAS improvement and deterioration were 13% and 87%, respectively. Overall, ZM oral rinse solution was significantly more effective compared to placebo in the treatment of patients with RAS (Mansoori et al., 2002). Jafari et al. compared the efficacy of ZM with *Myrtus communis* and *Anthemis nobilis* (Myrtle, mouth rinse) in 101 patients with minor aphthae. Findings of that study revealed that ZM extract was more effective than other interventions and acted faster in terms of pain relief and the extent of healing (Jafari et al., 2003). Hence, it was concluded that the ZM extract was an effective product for the management of minor aphthae.

3.11. Miscellaneous studies

ZM extract has been reported to be effective in protecting against duodenal ulceration, and at higher doses, its efficacy was comparable with those of the reference drugs, ranitidine and sucralfate. Although the mechanism of action for the mentioned effect has not been well clarified, local mucosal enhancement and cytoprotection have been suggested to be involved (Minaiyan et al., 2005). Research shows that ZM essential oil has an anesthetic effect on fish (Sharif Roohani et al., 2007). There is also evidence on the beneficial impact of ZM comparable with that of prednisolone, in an experimental model of inflammatory bowel disease (IBD). The antioxidant, anti-microbial and anti-inflammatory potentials of ZM could be regarded as plausible mechanisms by which this herb protects against experimentally induced IBD (Nakhai et al., 2007). Boskabady et al. showed a relatively potent stimulatory effect of the extract from ZM on β_2 adrenoceptors (Boskabady et al., 2009). Data from another study demonstrated that the essential oil of ZM acts to down-regulate MDM2 gene expression thus suggesting the availability of new tools for therapeutic intervention in the p53 pathway, a key genome-protective mechanism, for the purpose of cancer prevention and treatment (Gohar et al., 2010). Hosseinimehr and associates reported the radioprotective effects of hydroalcoholic ZM extract against γ-irradiation-induced genotoxicity in human lymphocytes. The authors attributed this effect to the free radical scavenging activity of ZM and proposed that such a protective activity might find applications in mitigating the side effects of γ-irradiation exposure in people (Hosseinimehr et al., 2011). There is also a report on the improvement of hematological parameters by ZM in fish (*Cyprinus carpio*) models. ZM oil enhances the respiratory burst activity of blood neutrophils and moderately increases red blood cell count and hematocrit levels (Sheikhzadeh et al., 2011). Another study has pointed to the hepatoprotective effects of ZM. Sakhaee et al. have reported that ZM confers significant protection against halothane-induced hepatotoxicity in rats by reversing the pathological changes in serum alanine aminotransferase, lactate dehydrogenase, alkaline phosphatase and total bilirubin concentrations (Sakhaee et al., 2011). Two previous studies have demonstrated the stimulatory activity of ZM oil and an aqueous-ethanolic extract on β-adrenoceptors (Boskabady and Tabanfar, 2011). This effect could be attributed, at least in part, to the carvacrol because the monoterpene has comparable agonistic activity (Boskabady et al., 2011).

4. Toxicity

The systemic toxicity and safety investigations of ZM are still inadequate. A prior study lists the LD_{50}'s of the aqueous (obtained through infusion of 100 g powdered plant in 1 L hot water) and ethanolic extracts (obtained through maceration of 200 g powdered plant in 500 mL 85% ethanol) of the aerial parts of ZM in albino mice to be 3.85 and 3.47 g/kg, respectively. Therefore, both extracts could be regarded as relatively toxic, based on the Loomis classification of toxicology (Loomis, 1968). Rahimifard et al. studied cytopathologic effects of ZM essential oil and extract on Vero, Hela and HEp II cell lines using an MTT assay. According to Rahimifard (Table 5), both the oil and extract had cytopathologic effects at specific concentrations (Rahimifard et al., 2008; Hedayati et al., 2009). Using an Ames *Salmonella* reversion assay, the essential oil from the aerial parts of the plant had no significant mutagenic effect in the *Salmonella typhimurium* strain TA100 with and without rat liver S9 fraction (Shoeibi et al., 2009). This result stands in contrast to the findings of Rahimifard et al. with the Ames assay using S9. Due to some drawbacks with the Ames test including false positive results, it was suggested that the mutagenicity of ZM should be evaluated using other methods. A yeast assay that screens for intra- and inter-chromosomal recombination in logarithmic phase cultures would be a good example of such a method (Schiestl et al., 1989).

As previously mentioned, there are some pharmaceutical forms of ZM on the market. The plant has been commonly prescribed by medical herbalists and physicians. Stimulation of mucosal membranes, dizziness, nausea, vomiting and dermatitis (Iranian Herbal Pharmacopoeia, 2002) are some common mild side effects of ZM and its products. Because of a lack of information concerning the safety of ZM in pregnancy, some pharmaceutical manufacturers recommend avoiding ZM administration to children less than 2 years of age and during pregnancy.

4.1. Acute toxicity, chronic toxicity and teratogenicity of thymol

Thymol is the main compound of ZM oil. According to European Chemicals Agency (ECHA), thymol has not shown chronic side effects or teratogenicity in previous studies, and can be considered as a safe compound. In a study, five male and five female rats per group were fed 67 and 667 mg/kg bw/d thymol in the diet for 19 weeks. The findings revealed no effect

on growth or hematology, and no macroscopic or microscopic change in the tissues (European Chemicals Agency).

Thymol was studied for teratogenicity in rats at doses 0 (solvent), 8, 40 and 200 mg/kg. The study showed no significance differences in numbers of offspring or live offspring at birth and viability index. In addition, there were no abnormal findings, clinical signs or necroscopy findings for neonates (European Chemicals Agency).

According to ECHA, LD_{50} of thymol in rat is 980 mg/kg (oral administration).

5. Conclusions and future perspectives

With a wide range of pharmacological and therapeutic properties, ZM has played an important role in Iranian traditional medicine. About 1000 years ago, Ibn Sina (Avicenna) applied ZM to cure stomachache and agitation, and to combat insect bites (Canon of Medicine). Gastrolite, a currently marketed formulation of ZM, is used for some gastrointestinal disorders such as stomachache, which is similar to the traditional application of the plant as described by Ibn Sina. This is a typical example of an ethnopharmacological relevance of a medicinal plant from past to present. However, researchers may like to investigate other pharmacological properties of ZM that were suggested in the past (*e.g.* agitation treatment) and find new therapeutic applications for this important plant.

In light of the modern pharmacological and clinical investigations, ZM is a valuable medicinal and condimental plant that has anti-microbial, antioxidative, anti-inflammatory, spasmolytic and anti-nociceptive properties. Overall, antimicrobial activity appears to be the most interesting studied biological effect of ZM. Nevertheless, no study has yet mechanistically investigated the impact of ZM on the growth of microbial strains. Anti-fungal properties of ZM oil are speculated to be due to its interaction with the plasma membrane and cell wall of hyphae, thereby leading to cellular deformity. Due to the lipophilic nature of ZM oil, its components could easily penetrate into the cell membrane and impair its integrity. At the next step, the infiltrated components could interact with the enzymes involved in cell wall synthesis (Gandomi et al., 2011). It appears that both volatile monoterpenes (*e.g.* thymol, carvacrol and *p*-cymene) and non-volatile compounds (*e.g.* flavonoids, tannins and saponins) could account for the anti-bacterial and anti-fungal properties of ZM. Moreover, further investigation is required to estimate the content of these phytochemicals in ZM and explore if the antimicrobial properties could be achieved through frequent consumption of plant in the daily diet.

Antimicrobial plants/spices/essential oils are used in food for two substantial reasons: (1) food preservation (to control natural spoilage processes) and (2) food safety (to inhibit the growth of pathogenic micro-organisms). Several studies showed that ZM oil could serve as an effective antimicrobial in the food industry (Fazeli et al., 2007; Abbasifar et al., 2008; Moosavy et al., 2008; Noori et al., 2008; Alipour-Eskandari et al., 2009; Gandomi et al., 2009; Ghasemi et al., 2012; Mashak and Moradi, 2012; Rahmani et al., 2012a, 2012b; Samadi et al., 2012). The edible and medicinal ZM has been successfully used alone or in combination with other preservatives to control the growth of pathogenic micro-organisms in some foods such as hamburger (Samadi et al., 2012), cheese (Abbasifar et al., 2008; Gandomi et al., 2009), barley soup (Alipour-Eskandari et al., 2009) and roast-chicken fillets (Rahmani et al., 2012a). Investigations showed that ZM essential oil can inhibit food-borne pathogenic micro-organisms such as *Staphylococcus aureus* (Abbasifar et al., 2008; Ghasemi et al., 2012), *Bacillus cereus* (Alipour-Eskandari et al., 2009; Mashak and Moradi, 2012), *Sallmonella typhimurium* (Moosavy et al., 2008), *Listeria monocytogenes* (Ghasemi et al., 2012) and *Aspergillus flavus* (Gandomi et al., 2009). The future will probably see a large number of food applications of ZM, especially the effectiveness of its essential oil, individually and in combination with other preservatives.

Another reported activity of ZM is its anti-inflammatory properties. However, the majority of previous studies have been performed on the plant essential oil. Given the distinct phytochemical differences between the composition of essential oil and extract, it is strongly recommended that future investigations evaluate the anti-inflammatory effects of standardized and fractionated ZM extracts. Because of the presence of several bioactive flavonoids in ZM and the prominent role of inflammation in the pathophysiology of a wide variety of human diseases, ZM should be investigated as an adjunctive therapy for cardiovascular, metabolic, dermatologic and neurodegenerative disorders. In addition, the observed chemopreventive and cytotoxic properties of flavonoids necessitate conducting future studies on the potential benefits of ZM as an adjuvant therapy for various types of cancers.

The lack of a comprehensive phytochemical analysis of ZM is an important limitation that can be noted regarding most of the previous studies. Phytochemical investigations of ZM have been frequently focused on the essential oil and flavonoids of the plant. It would be interesting to assess the presence and biological properties of other classes of secondary metabolites, such as sulfur compounds, betalains, hydroxycinnamic acids and stilbenes in ZM. Finally, future research should answer this question "Are the *in vitro* and *in vivo* findings describing antimicrobial, immunomodulatory, anti-inflammatory and antioxidant effects of ZM, applicable to clinical practice?" It should be noted, however, at ZM extract doses exceeding 300–400 mg/kg (Hosseinzadeh et al., 2000; Ramezani et al., 2004; Minaiyan et al., 2005), any pharmacological result may become evident.

Hosseinzadeh et al. (2000) showed that the anti-nociceptive activity of ZM may be mediated by opioid receptors. In contrast, Gharib Naseri et al. found that ZM extract doesn't contain any opioid agonist due to ineffectiveness of naloxone on the inhibitory effect of ZM. However, further investigations are necessary to confirm the mechanism(s) of anti-nociceptive and anti-spasmodic properties of ZM.

Because ZM is a widely-used dietary plant, there is negligible concern regarding any toxic or severe adverse effects following consumption at pharmacologically relevant doses.

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