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Review

Melissa officinalis L. – A review of its traditional uses, phytochemistry and pharmacology

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ABSTRACT

Ethnopharmacological relevance: *Melissa officinalis* L. is a medicinal plant that has long been used in different ethno-medical systems especially in the European Traditional Medicine and the Iranian Traditional Medicine for the treatment of several diseases. It is also widely used as a vegetable and to add flavor to dishes

Aim of the review: This review aimed to provide a summary on the botanical characterization, traditional uses, phytochemistry, pharmacological activities, pharmacokinetics and toxicity of *M. officinalis*, and discusses research gaps and future opportunities for investigations on this plant.

Materials and methods: We extensively reviewed major unpublished old texts, and published and electronic literature on traditional medicines of different regions of the world to find traditional uses of *M. officinalis*. Electronic databases including Web of Science, PubMed, ScienceDirect, Google Scholar and Scopus were searched to find articles (published between 1956 and 2015) on pharmacology and phytochemistry of *M. officinalis*.

Results: Traditional uses of *M. officinalis* have been recorded mostly in European countries, Mediterranean region and Middle East countries. Phytochemical investigations revealed that this plant contains volatile compounds, triterpenoids, phenolic acids and flavonoids. Crude extracts and pure compounds isolated from *M. officinalis* exhibited numerous pharmacological effects, from which only anxiolytic, antiviral and antispasmodic activities of this plant as well as its effects on mood, cognition and memory have been shown in clinical trials. AChE inhibitory activity, stimulation of the acetylcholine and $GABA_A$ receptors, as well as inhibition of matrix metallo proteinase-2 are the main mechanisms proposed for the widely discussed neurological effects of this plant.

Conclusions: Modern pharmacological studies have now validated many traditional uses of *M. officinalis*. The data reviewed here revealed that *M. officinalis* is a potential source for the treatment of a wide range

Abbreviations: ABTS, 2,2′-Azinobis (3-ethylbenzothiazoline-6-sulphonate); AChE, Acetylcholinesterase; Ach, Acetylcholine Chloride; AD, Alzheimer's Disease; AGEs, Advanced Glycation End Products; ALP, Alkaline Phosphatase; ALDH, Aldehyde Dehydrogenases; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; AT1R, Angiotensin II Type 1 Receptor; AUC, Area Under the Curve; BAX, BCL2-Associated X Protein; BHA, Butylated Hydroxyanisole; CMAI, Cohen-Mansfield Agitation Inventory; CNS, Central Nervous System; CNV, Choroidal Neovascularization; COPD, Chronic Obstructive Pulmonary Disease; DISS, Defined Intensity Stressor Simulation; DPPH, 1,1-Diphenyl-2-picrylhydrazyl; ECG, Electrocardiogram; EC_{50}, Half Maximal Effective Concentration; EDHF, Endothelium-derived Hyperpolarizing Factor; EO, Essential Oil; EPM, Elevated Plus Maze; FGF-2, Fibroblast Growth Factor-2; FS, Forced Swimming; GABA-T, GABA Transaminase; GBM, Glioblastomamultiforme Cell Line; Glc, Glucose; GSH, Glutathione; HCV, Hepatitis C Virus; HEp-2, Human Epithelial Type 2 Cells; Hep G2, Hepatocellular Carcinoma; HIF1α, Hypoxia-inducible Factor 1-Alpha; HPLC-DAD, High Performance Liquid Chromatography with Diode-array Detector; HSV, Herpes Simplex Virus; HSV-1, Herpes Simplex Virus Type 1; HSV-2, Herpes Simplex Virus Type 2; 5-HT, 5-Hydroxytryptamine; HUVEC, Human Umbilical Vein Endothelial Cells; IC_{50}, Half Maximal Inhibitory Concentration; IL-1β, Interleukin-1 Beta; IL-6, Interleukin 6; ISO, Isoproterenol; ITM, Iranian Traditional Medicine; KA, Kainic Acid; LDH, Lactate Dehydrogenase; LDL, Low-Density Lipoprotein; LPO, Lipid Peroxidation; LPS, Lipopolysaccharide; MAO-A, Monoamine Oxidase A; MDA, Malondialdehyde; MDMA, 3,4-Methylenedioxymethamphetamine; MES, Maximal Electroshock Induced Seizures; MIC, Minimum Inhibitory Concentration; MMP-9, Matrix Metallopeptidase 9; MMS, Methyl Methanesulfonate; MCT, Monocarboxylic Acid Transporter; MRP1, Multidrug Resistance Associated Protein 1; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-Diphenyl Tetrazolium Bromide; ND, Neurodegenerative Diseases; NF- κB, Nuclear Factor-κB; NPI, Neuropsychiatric Inventory; NR, Neutral Red; PAS, Pittsburgh Agitation Scale; PCAF, P300/CBP-Associated Factor; PPAR, Peroxisome Proliferator-Activated Receptor; PTZ, Pentylenetetrazole-Induced Seizures; PLP2, porcine liver primary cells; RA, Rosmarinic Acid; RAW 264.7, Mouse Leukaemic Monocyte Macrophage Cell Line; ROS, Reactive Oxygen Species; α-SMA, α-Smooth Muscle Actin; SOD, Superoxide Dismutase; SREBP-1c, Sterol Regulatory Element-binding Protein-1c; TAG, Triacylglycerol; TBARS, Thiobarbituric Acid Reactive Substances; TEAC, Trolox Equivalent Antioxidant Capacity; TG, Triglyceride; TGF, Transforming Growth Factor; TNF-α, Tumor Necrosis Factor Alpha; TUNEL, Terminal Deoxynucleotidyl Transferase dUTP Nick End Labeling; VEGF, Vascular Endothelial Growth Factor; VF, Ventricular Fibrillation; VLDL, Very-Low-Density Lipoprotein; VPB, Ventricular Premature Peats; VT, Ventricular Tachycardia; ZI, Zone Inhibition

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of diseases especially anxiety and some other CNS disorders, though confirmatory trials are warranted to substantiate these effects in the clinical setting. Data regarding many aspects of this plant such as mechanisms of actions, pharmacokinetics, adverse effects of the extracts, potential interactions with standard-of-care medications and active compounds is still limited which call for additional studies particularly in humans.

Contents

1. Introduction

More than 20,000 plant species are used in various traditional medicines around the world, and are considered as potential reservoirs for discovery of new drugs (Amor et al., 2009). *Melissa officinalis* L., commonly known as lemon balm, is a well-known medicinal plant of Lamiaceae. For more than 2000 years, fragrant leaves of this plant have been widely used in cooking to add flavor to dishes. The plant has also been used for the treatment of mental and CNS diseases, cardiovascular and respiratory problems, various cancers, and as a memory enhancer, cardiac tonic, antidepressant, sleeping aid and antidote (Cogan, 1636; Osbaldeston, 2000; Evelyn, 1699; Dastmalchi et al., 2008; Jorjani., 1976; Ibn Sina, 1987). Medicinal properties of this plant were first introduced by Dioscorides (40–90 CE), the father of pharmacology, in *De Materia Medica.* Since then, medicinal properties of *M. officinalis* were mentioned in many other landmark medical books including *Canon of Medicine by Avicenna*, *Zakhireh Kharazmshahi* "The Treasure of Kharazmshah" by Jorjani and *Al-Hawi "The Continens" by Rhazes*. Since 1984, *M. officinalis* has been listed in "The Commission E Monographs" (Blumenthal et al., 2000). The plant is also listed in several pharmacopoeias including Iranian Herbal Pharmacopeia, British Herbal Pharmacopeia and European Pharmacopeia (British Herbal Pharmacopoeia, 1996; European Pharmacopoeia, 1999; Iranian Herbal Pharmacopoeia, 2002). Modern pharmacological studies demonstrate that *M. officinalis* has several biological activities including antioxidant, hypoglycemic, hypolipidemic, antimicrobial, anticancer, antidepressant, anxiolytic, antinociceptive, anti-inflammatory and spasmolytic properties (Lopez et al., 2009; Weidner et al., 2014; Zarei et al., 2014; Birdane et al., 2007; Mimica-Dukic et al., 2004; Queiroz et al., 2014; Taiwo et al., 2012; Sadraei et al., 2003). Additionally, phytochemical investigations have revealed the presence of volatile compounds, triterpenes, phenolic acids and flavonoids as the main active constituents of *M. officinalis* (Mencherini et al., 2007). Among the identified phytochemicals, hydroxycinnamic acids, commonly expressed as RA, are biomarkers of quality control according to WHO monographs on selected medicinal plants (WHO, 1994). The present paper aimed to review traditional uses of *M. officinalis* in different ethnomedical systems as well as botany, phytochemistry,

pharmacological activities, safety and clinical applications of *M. officinalis*. Critical evaluation of pharmacological studies in terms of their relation to ethnomedical use was also performed. This information might be useful in designing future studies in particular clinical trials, and in developing new pharmaceuticals containing *M. officinalis* or its active ingredients.

2. Botany

M. officinalis, also known as lemon balm, common balm or sweet balm, is a perennial lemon-scented herb belonging to the Lamiaceae (Awad et al., 2009). According to "The Plant List" *Melissa officinalis* L. is the only accepted name for the plant, with nine synonyms including "*Melissa officinalis* subsp. *altissima* (Sm.) Arcang., *Melissa officinalis* var. *altissima* (Sm.) K.Koch, *Melissa officinalis* var. *cordifolia* (Pers.) K.Koch, *Melissa officinalis* var. *foliosa* Briq., *Melissa officinalis* var. *graveolens* (Host) Nyman, *Melissa officinalis* var. *hirsuta* K. Koch, *Melissa officinalis* subsp. *officinalis, Melissa officinalis* var. *romana* (Mill.) Woodv. and *Melissa officinalis* var. *villosa* Benth.". There is also an infraspecific taxon of the species *Melissa officinalis* L.: *Melissa officinalis* subsp. *inodora* Bornm. (The Plant List, 2013).

Melissa officinalis grows to the height of 30–125 cm, with soft short hairs surrounding all parts. The stem is erect, branched, usually glabrous and quadrangular. Leaves are petiolate, ovate, to 6 cm long, 3 cm broad, the upper cuneate, the lower cordate at base, crenate-toothed, subglabrous, sometimes with glandular hairs or punctuate glands beneath (Komarov, 1977). Flowers are white or pale pink consisting of small clusters of 4–12 blossoms in the summer. It has two stamens and four lobed ovaries forming 1–4 nutlets. The seeds are very small about 1–1.5 mm long, with ovate dark brown or black color. Lemon balm can rapidly grow at a temperature range of 15–35 °C, and requires 500–600 mm precipitation well distributed throughout the growing season, otherwise it should be irrigated (Saeb and Gholamrezaee, 2012). *M. officinalis* has a hairy root system with many lateral roots, which makes the plant more adaptable to different environmental conditions. The upper parts of the plant die off at the start of winter but new shoots re-emerge from roots at the beginning of spring (Turhan, 2006). *M. officinalis* grows worldwide but its origin has not been well defined. However, the Eastern Mediterranean region, Western Asia and Southern Europe, Caucasus and Northern Iran are considered as areas of origin (de Sousa et al., 2004; Fernandes, 1973).

3. Traditional uses

Medicinal uses of *M. officinalis* date back to over 2000 years ago. In the European Traditional Medicine, *M. officinalis* was known as melissophyllon, baulme, melissa and balm. Dioscorides (40–90 CE), the father of pharmacology, addressed the plant in his *De Materia Medica* as follows: 'A decoction of the leaves is good for those touched by scorpions, or bitten by harvest spiders or dogs'. He also recommended the plant for the treatment of amenorrhea, dysentery, suffocation caused by mushrooms toxicity, intestinal ulcers, gripe, difficult breathing, scrofulous tumors and other swellings, arthralgia and toothache (Osbaldeston, 2000). In the Middle Ages, lemon balm was used to stop bleeding and to treat toothache, earache, morning sickness, crooked neck, and baldness (Saad and Said, 2011). An early prescription by Paracelsus (1493–1541) illustrated that the use of *M. officinalis* would be enlivening and effective in alleviating nervous system diseases (Scholey et al., 2014). It has also been noted that *M. officinalis* can increase lifespan and restore memory (Sun et al., 2013). Thomas Cogan (1545–1607) stated that daily consumption of *M. officinalis* tea by his students improves the understanding capacity and memory. He also recommended the distilled water obtained from a mixture of bugloss, borage and lemon balm for the treatment of melancholy. Evelyn (1620 –1706) in his book "*Acetaria, a discourse of Sallets*" – which is the first recorded book on salads – has mentioned this plant as '*Baulm, Melissa and Baum*" and described it with properties like "hot and dry", "cordial and exhilarating", memory enhancing, and anti-melancholic. The London Dispensary (1696) says "An essence of balm given in Canary wine every morning will renew youth, strengthen the brain, relieve languishing nature and prevent baldness' (Grieve, 1971). There is an old European prescription for the treatment of high blood pressure containing *M. officinalis*, *Tilia europaea* L., *Crataegus oxyacantha* L. and *Achillea millefolium* L. (Wood et al., 2015).

In the Danish folk medicine, *M. officinalis* is used for the treatment of sleeplessness caused by heart break, melancholy and sadness (Jäger et al., 2006). In Austria's folk medicine, lemon balm tea and external application of its essential oil (EO) is used to treat gastrointestinal, nervous, hepatic and biliary ailments (Vogl et al., 2013). In Croatia, *M. officinalis* is used for the treatment of sore throats and cough (Albala, 2011). Spanish physicians in the Islamic era used *M. officinalis* as an exhilarant, antidote, emmenagogue and pain killer (al-Ghassani, 1990). Oral administration of the plant leaves with natron has been practiced to treat intestinal ulcers, gripes and heart palpitation caused by consumption of toxic mushrooms. A multi-herbal lozenge containing *M. officinalis* has been used for the treatment of orthopnea. It has been recorded that application of a poultice comprising common salt and *M. officinalis* leaves can cure scrofula, swellings, ulcers, arthralgia and toothache (Ibn Beytar, 2001). Lebanese herbalists used *M. officinalis* leaves to treat migraines and stomach problems, and to enhance cardiac function and memory (Salah and Jäger, 2005). In the traditional Moroccan medicine, *M. officinalis* is used as a tranquilizing medicine, antispasmodic and heart tonic (Bounihi et al., 2013). In ancient Iran, *M. officinalis* was known as Wadrangboy and Wattrangboy which mean "Citron's aroma" referring to the citrus-like smell of the plant (Soltani, 2012). *M. officinalis* is also an important medicinal plant in the Iranian Traditional Medicine (ITM). In his *Canon of Medicine,* Avicenna (981–1037), the genius Iranian philosopher and physician, recommended this plant as a medication for all diseases caused by phlegm and black bile including depression, anxiety, obsession and psychosis (Ibn Sina, 1987). He also believed that the aroma of the plant is responsible for its potent exhilarant effects (Javadi and Emami, 2015). He used *M. officinalis* for purging excessive black bile out of the blood in the heart and to cure heart palpitation (Ibn sina, 1984). Jorjani (1042–1136), one of the most distinguished Iranian scientists, and other ITM physicians used *M. officinalis* for the treatment of various central nervous system diseases such as dementia, epilepsy, paralysis, stroke, tremor, migraine and vertigo (Ahwazi Arjani, 1877; Chashti, 1884; Jorjani, 1976). Moreover, this plant has been reported to be a cardiac and gastric tonic, memory enhancer, mild sedative, antidote (for toxic mushrooms) and wound disinfectant (Tonekaboni, 2007). Inhalation aromatherapy with this plant has been recommended for the treatment of nightmares (Aqili Khorasani, 1992). *M. officinalis* is also used in some eye diseases such as severe conjunctivitis and lack of eyesight caused by the opacity of the aqueous humour (Chashti, 1884). According to the main ITM pharmacopoeias, 40 g of the dry leaves, 80 g of the fresh leaves and 9 g of the dry seeds of the plant are applied in simple preparations. However, *M. officinalis* is mainly used in multi-component preparations to enhance its therapeutic effectiveness as a result of synergism between herbal products. The dosage of *M. officinalis* in these preparations varies widely. This plant is a crucial component of around 400 ITM medicinal preparations for the

treatment of cancer, syncope, heart palpitation, asthma, diabetes, various fevers, hiccups, joint inflammation and pain, halitosis, aphtha, rabies and gastrointestinal problems (Chashti, 1884; Aqili Khorasani, 1992; Jorjani, 1976). For example, according to "*Zakhire Kharazmshahi pharmacopeia*", a multifunctional decoction comprising *M. officinalis* along with several other plants including *Terminalia chebula* Willd. ex Flem., *Phyllanthus emblica* L., *Anchusa italica* Retz., *Lavandula stoechas* L., *Polypodium vulgare* L., *Ipomoea turpethum* (L.) R. Br., *Cuscuta epithymum* Mur. and raisin is beneficial in curing croup, swellings and cancers of the tongue and ears, piles and menorrhagia (Jorjani,1976). In Ayurveda, *M. officinalis* is believed to sharpen memory (Patel et al., 2014).

Interestingly, similarities exist in traditional applications of this plant in different cultures. The examples are treatment of mental disorders especially anxiety and depression, reliving heart illnesses and enhancing memory. As mentioned above, *M. officinalis* is usually used in compound herbal formulae in order to enhance its effectiveness. It is worth noting that only aerial parts of the plant were used traditionally and less attention was devoted to its roots. It is also noteworthy that *M. officinalis* preparations are mainly in the forms of infusion and tea in order to preserve and prevent decomposition of active ingredients especially EO components. Table 1 provides a summary of the ethnopharmacological uses of *M. officinalis* in different countries.

4. Phytochemistry

Phytochemical investigations on *M. officinalis* have revealed the presence of various phytochemicals including terpenes (monoterpenes, sesquiterpenes and triterpenes) and phenolic compounds (phenolic acids, flavonoids and tannins) (Allahverdiyev et al., 2004; Moradkhani et al., 2010). The main active constituents of *M. officinalis* are volatile compounds (e.g. geranial, neral, citronellal and geraniol), triterpenes (e.g. ursolic acid and oleanolic acid), and phenolics (e.g. *cis*-and *trans*-RA isomers, caffeic acid derivatives, luteolin, naringin and hesperidin) (Argyropoulos and Müller, 2014; Awad et al., 2009; Ibragić et al.). Table 2 summarizes the major phytochemicals that have been isolated and characterized from *M. officinalis.* Medicinal activities of *M. officinalis* are considered to be attributed mainly to its essential oil (EO) and phenolic compounds (Schnitzler et al., 2008). Chemical structures of secondary metabolites that have been isolated and characterized from *M. officinalis* are shown in Fig. 1.

4.1. Volatile compounds

EO from *M. officinalis* has commercial importance owing to its applications in the pharmaceutical and food industry. *M. officinalis* EO is used as an additive in foods, herbal teas, cosmetics, and also in ornaments. The production cost and price of the oil are very high, because of the low yield of EO extraction (Sari and Ceylan, 2002; Sousa et al., 2004). The EO is considered to be mainly responsible for the antibacterial and antifungal activities of the plant (Mimica-Dukic et al., 2004). EO is obtained from fresh or dried flowers, leaves, and branches of *M. officinalis*, and has a fresh lemon odor and a light yellow color. The citrus-like aroma of *M. officinalis* is due to the presence of citral isomers i. e. geranial and neral as well as lesser amounts of citronellal and geranyl acetate (Dawson et al., 1988). Generally, the EO content of *M. officinalis* ranges between 0.02% and 0.30%, which is low compared with other members of the Lamiaceae (Moradkhani et al., 2010). The composition of EO varies as a function of climate, but the majority of studies have shown that *M. officinalis* EO is dominated by the presence of oxygenated monoterpenes including citral isomers (geranial and neral) (1–2), citronellal (3) and geraniol (4) as the main components (Meftahizade et al., 2010; Mimica-Dukic et al., 2004). For instance, the EO obtained from the aerial parts of cultivated *M. officinalis* collected from Vojvodina (Serbia) was characterized by the presence of high concentrations of geranial (23.4%), neral (16.5%) and citronellal (13.7%) (Mimica-Dukic et al., 2004). Similarly, Carnat et al. (1998) identified the main components of the leaf oils of *M. officinalis* (of France origin) as citral (neral + geranial; 48%) and citronellal (40%). Abdellatif and Hassani (2015) evaluated the effect of different extraction techniques (traditional hydrodistillation, steam distillation, organic solvent extraction and microwave-assisted hydrodistillation) on the chemical composition of the EO of *M. officinalis* leaves collected from the north-eastern Algeria. All obtained EOs were predominated by two components i. e. neral (18.86–38.18%) and geranial (27.79–37.91%). Saeb and Gholamrezaee (2012) investigated the EOs of *M. officinalis* leaves harvested in three different stages (before, during and after the flowering stage) from Iran. Their results showed that the major components of EO are (decadienal (5), 29.38% and geraniol, 25.3%), (decadienal, 28.04% and geraniol, 24.97%) and (carvacrol (6), 37.62% and methyl citronellate (7), 32.34%), respectively. In another study, the EO of *M. officinalis* collected from Turkey was reported to be dominated by sesquiterpene hydrocarbons, mainly β-cubebene (15.41%) (8) and β-caryophyllene (14.24%) (9) (Allahverdiyev et al., 2004). Van den Berg et al. (1997) reported the main components of the EO obtained from cultivated *M. officinalis* subsp. *altissima* (which is a synonym for *M. officinalis*) leaves from the Greek origin to be germacrene D (34.79–51.50%), sabinene (0.91–14.68%), β-caryophyllene (7.27–12.66%) and β-pinene (0.53–8.03%). Analysis of the EO from the leaves of the plant from the New Zealand origin identified sesquiterpene hydrocarbons β-cubebene (39%) and terpinolene (9.6%) as the major constituents (Dawson et al., 1988). These compositional variations in the *M. officinalis* EO could stem from differences in climatic, seasonal and geographic conditions, harvesting time, and procedural details of the applied distillation technique (Shakeri et al., 2014).

4.2. Triterpenes

Triterpenes are one of the largest classes of plant natural products that are widely distributed in the plant kingdom. Hitherto, over 20,000 different triterpenes have been identified from plants (Thimmappa et al., 2014). The main triterpenes that have been isolated from *M. officinalis* are ursolic and oleanolic acids (Mencherini et al., 2007). These compounds have a wide spectrum of biological effects including antifungal, cytotoxic and hemolytic activities (Han et al., 2009). Bioassay-guided fractionation of the methanol extract of *M. officinalis* has led to the identification of the triterpenoids ursolic acid (10) and oleanolic acid (11) as the major compounds responsible for the inhibition of rat brain GABA transaminase (GABA-T) (Awad et al., 2009). Some triterpenes contain different numbers of sulfate groups bound with sugars or aglycones. Triterpenes containing sulfate groups attached to the sugar chain have higher biological activity, e. g. hemolytic and cytotoxic effects, compared to those attached to the aglycone group (Kim and Himaya, 2012; Park et al., 2014). A literature survey revealed that only triterpenes containing sulfate groups in the aglycone unit occur in *M. officinalis* (Tantry et al., 2014). Mencherini et al. (2007) isolated five new disulfated ursene or oleanene triterpenes (12–16) and a new ursene glycoside (17) from a polar extract (EtOH 50%) of the stems and leaves of *M. officinalis*. In another study, two new sulfated triterpenes namely 3,23-disulfate ester of 2α,3β−23,29-tetrahydroxyolean-12-ene-28-oic acid, 28-*O*-β-D-glucopyranoside (18), and 23-monosulfate ester of 2α,23-dihydroxyurs-12-ene-28-oic acid, 3-*O*-β-D-glucopyranoside (19), with antioxidant and antimicrobial activities were identified (Tantry et al., 2014).

Table 1
Ethnopharmacological uses of *M. officinalis* in different countries.

Country	Part used	Dosage form	Medicinal use/disease treated	Reference(s)
Austria	Leaves	Tea essential oil (external application)	Curing gastrointestinal, nervous, hepatic and biliary ailments	Vogl et al. (2013).
Bolivia	Aerial part	Infusion	Curing heart ailments	Macia et al. (2005)
Bosnia and Herzegovina	Leaves	Oral preparations	Curing insomnia, restlessness, arrhythmia, increased lactation, flatulence, depressions, morning sickness, diarrhea, migraine, rheumatism strengthening the body, internal purification, blood purification	Šarić-Kundalić et al. (2011)
Brazil	leaves	Infusion tea	Sedative for children, curing stomach disturbances, bad cold, cough, infection, fever	Di Stasi et al. (2002) Oliveira et al. (2012)
Brazil	leaves	Bath	Wound healing (external use)	
Brazil	Roots	Decoction	Curing Bad cold, cough	
Bulgaria	Leaves	Infusions	Sedative, hypotensive, spasmolytic	Ivanova et al. (2005)
Catalonia	leaves	Infusion	Sedative	Bonet et al. (1999)
Croatia		–	Curing sore throats and cough	Albala (2011)
Denmark	Aerial parts	–	Curing sleeplessness caused by heart break, melancholy and sadness	Jäger et al. (2006)
Ecuador	Stems, leaves	Infusion	Relaxant, curing insomnia	Tene et al. (2007)
Greece	aerial parts	Infusion, decoction	blood circulation and heart stimulant, curing hypertension, ear aches, bloating, dyspepsia, spasm, headache, depression, dizziness, migraine common cold, decreasing cholesterol and uric acid, brain stimulant, calmative	Hanlidou et al. (2004)
India	Leaves	–	promoting memory	Patel et al. (2014)
Iran	Leaves	Infusion (oral)	Curing depression, anxiety, psychosis, palpitation, obsession, dementia, epilepsy, stroke, tremor, paralysis, migraine and vertigo, syncope asthma, diabetes fevers, hiccups, joint inflammation and pain, cancer, halitosis, aphtha, rabies, gastrointestinal problems, piles, menorrhagia exhilarant, cardiac and gastric tonic, mild sedative, memory enhancer, antidote, disinfectant	Ibn sina (1984); Ahwazi Arjani (1877); Chashti (1884); Jorjani (1976); Tonekaboni (2007); Aqili Khorasani (1992); Jorjani (1976)
Iran	Leaves	Oil (inhalation)	Treatment of nightmares	Aqili Khorasani (1992)
Iran	Leaves	Infusion (external use)	Curing conjunctivitis and lack of eyesight	Chashti (1884)
Iraq	Leaves	Tea	Diuretic, analgesic, for headache, toothache, galactogenic	Al-douri et al. (2000)
Italy	Leaves	Poultice infusion	Wound healing	Vitalini et al. (2009)
			Curing abdominal pains	and Pieroni (2000)
Italy	Leaves	Compress of crushed fresh leaves	Healing insect bite	Pieroni et al. (2004)
Jordan	aerial parts	Infusion	sedative, carminative, antispasmodic, curing abdominal pain and digestive, gynecological disorders, arthritis	Al-Khalil (1995) and Afifi and Abu-Irmaileh (2000)
Kosovo	Areal parts	Infusion	Curing abdominal pains during pregnancy	Mustafa et al. (2012)

Kosovo		Decoction	Neuro-relaxant	
Lebanon			Strengthening the heart, curing migraine, stomach disorders enhancing memory	Salah and Jäger (2005).
Morocco	Aerial part	Infusion	Spasmolytic, depurative and tranquilizing, heart tonic, cholagogue	Merzouki et al. (2000). and Bounihi et al. (2013).
Palestine		–	Antimicrobial	Abu-Shanab et al. (2006).
Patagonia		–	Sedative	Estomba et al. (2006).
Peru	leaves	Infusion	Sedative and hypotensive	Hammond et al. (1998).
Poland		–	Nervous excitability, curing vegetative neurosis, tension, anxiety, motor agitation, menopause	Leśniewicz et al. (2006).
Portugal	Aerial part, stems and flowers, fresh or dried.	Infusion	Relaxation (insomnia, nervousness, spasms and nervous indigestion), Intestinal gases and pain, stimulation of digestion and bile, Curing fever, headache and influenza, stomach ache, gastritis; For the heart, memory stimulation	Neves et al. (2009).
Republic of Macedonia	leaves	Tea, oil	Curing heart problems, headache	Rexhepi et al. (2013).
Spain	leaves	–	exhilarant, antidote, emmenagogue pain killer curing intestinal ulcers, gripes, heart palpitation caused by consumption of toxic mushrooms, orthopnea	al-Ghassani (1990).
		Poultice	Curing scrofula, swellings, ulcers, arthralgia and toothache	Ibn Beytar (2001).
Turkey	Aerial parts	Infusion Decoction	Curing cancer, asthma, cardiovascular diseases stomach diseases, nephritis, forgetfulness, diabetes, cold, bronchitis, enteritis	Kultur (2007).
	leaves		antiseptic, antispasmodic	Karaman and Kocabas (2001).
		Infusion	memory-enhancing	Orhan and Aslan (2009).
Uzbekistan		–	Curing coughs, lung disease, and skin infections	Egamberdieva et al. (2013).

4.3. Phenolic compounds

4.3.1. Phenolic acids

Phenolic acids are a class of plant secondary metabolites containing at least one phenol ring that possesses one carboxylic acid group (Heleno et al., 2015). Phenolic acids are the main components of *M. officinalis* and are divided into two classes: derivatives of benzoic acid e. g. gallic acid, and derivatives of cinnamic acid e.g. caffeic acid (Dai and Mumper, 2010). These compounds are widely distributed throughout the plant kingdom and possess antioxidant effects owing to their hydrogen-donating or singlet oxygen-quenching activities (Proestos et al., 2005). The antioxidant activity of *M. officinalis* extracts is attributed to the presence of phenolic acids, mainly hydroxycinnamic acid derivatives such as rosmarinic acid (RA) (20) (Caniova and Brandsteterova, 2001). Owing to its four hydroxyl groups, RA's antioxidant activity may be even stronger than that of trolox or vitamin E (Ibragić et al. 2014). Acetylcholinesterase inhibitory guided fractionation of the *M. officinalis* extract revealed those fractions containing RA derivatives to be more potent compared with other fractions. Dastmalchi et al. (2009) found that the most potent anti-acetylcholiestease fraction of the plant extract (25.36 ± 1.63 μg physostigmine/milligram of dry weight of the extract) contains RA and two of its derivatives. Since *M. officinalis* L. is mainly consumed as an infusion, Barros et al. (2013) evaluated phenolic profiles of *M. officinalis* L. infusions. Ten caffeic acid derivatives including 3-(3,4-dihydroxyphenyl)-Lactic acid (21), caffeic acid A (22), lithospermic acid A (23), salvianolic acid F (24), salvianolic acid A (25), salvianolic acid C (26), salvianolic acid B (27), sagerinic acid (28) and Yunnaneic acid F (29) were identified using HPLC-DAD-ESI/MS. The phenolic content of *M. officinalis* varies in different regions. Samples collected from Bosnia and Herzegovina had a higher content of RA, chlorogenic (30) and gallic acid (31) compared with those collected from Turkey (Ibragić et al., 2014). In another study, qualitative and quantitative analysis of the *M. officinalis* extract using HPLC-DAD showed the presence of caffeic acid, *m*-coumaric acid (32) (the least frequent compound), and RA (major component) (Dastmalchi et al., 2008). Furthermore, two other RA derivatives have been isolated from the aerial parts of *M. officinalis*; melitric acids A (33) and B (34), which contain three caffeic acid units (Agata et al., 1993). Finally, Pereira et al. (2014) reported gallic acid, chlorogenic acid, caffeic acid and ellagic acid (**35**) from ethanol extract of *M. officinalis*.

4.3.2. Flavonoids

Flavonoids are a family of plant secondary metabolites that contain a benzopyran heterocycle linked to a benzene ring (Testai, 2015). Numerous biological and pharmacological activities have been reported for flavonoids including antioxidant, anti-inflammatory, antimicrobial, anticancer, anti-HIV, anticoagulant, immunomodulatory, antitubercular and anti-allergic activities (Cao et al., 2015). In Lamiaceae, flavonoids occur in every part of the plant but the richest content is usually found in the aerial parts (Ulubelen et al., 2005). Flavonoids that have been isolated from *M. officinalis* can be categorized into four subgroups namely flavones (nine compounds), flavanones (five compounds), flavonols (three compound) and flavanols (two compounds). Luteolin 3′-*O*-β-D-glucuronide (36) has been reported as the major flavone present in *M. officinalis* (Heitz et al., 2000). Mulkens and Kapetanidis (1986) isolated two flavone glycosides namely luteolin7-*O*-glucoside (37) and apigenin 7-*O*-glucoside (38), one flavonol glycoside (isoquercitrin) (39), and one flavonol (rhamnocitrin) (40) from the leaves of the plant. In another report, Patora and Klimek (2002) isolated and characterized six major flavones (apigenin and luteolin derivatives) including luteolin 7-*O*-β-D-glucopyranoside-3′-*O*-β-D-glucuronopyranoside (41), Apigenin (42), Luteolin 7-*O*-β-D-glucuronopyranoside (43), Luteolin (44), Apigenin 7-*O*-β-D-glucopyranoside (45) and Luteolin 3′-*O*-β-D-glucuronopyranoside (46) from the leaves of *M. officinalis* collected before the flowering time. Also, five flavanones including hesperidin (47), hesperetin (48), eriodictyol 7-*O*-glucoside (49), naringin (50) and naringenin (51) were isolated from 45% (V/V) ethanolic extract of *M. officinalis* (Dastmalchi et al., 2008). Two flavanols namely catechin (52) and epicatechin (53) and one flavonol glycoside namely rutin (54) were also identified in *M. officinalis* by Pereira et al. (2014).

4.4. Other compounds

The chemical composition of *M. officinalis* stalk has been evaluated by Ashori et al. (2011). They found that the main components of *M. officinalis* stalk include holocellulose, lignin, and extractives in various amounts. In addition, the plant contains a relatively high percentage of alpha-cellulose (32.7%) but a low percentage of lignin (25%). The hemicelluloses are mainly glucose and xylose.

5. Pharmacological reports

5.1. Antianxiety effect

There are several records on the traditional use of *M. officinalis* as a tranquilizing medicine. In recent decades, several studies have supported the anxiolytic effects of *M. officinalis*. In an *in vitro* study on rat brain, methanol extract of *M. officinalis* and its main component RA showed GABA-T inhibitory activity (Awad et al., 2009). In another *in vivo* research, oral administration of the hydroalcoholic and ethanolic extracts of the plant induced anxiolytic-like effects, possibly through GABA-T inhibition resulting in an increase in brain GABA levels (Ibarra et al., 2010; Taiwo et al., 2012). In addition, aqueous extract of a mixture of *M. officinalis* and *Passiflora caerulea* reduced plasma levels of corticosterone, the most important mediator associated with physiological stress in mice (Feliú-Hemmelmann et al., 2013). The relevance of evidence from the above-mentioned animal studies (with a dose range of 240–360 mg/kg) for clinical practice has been supported by the findings of two clinical trials as follows. In a randomized double-blind placebo-controlled crossover clinical trial, 18 healthy volunteers subjected to laboratory-induced stress – confirmed through completing a 20-minute version of the Defined Intensity Stressor Simulation (DISS) battery – received two single 300 and 600 mg doses (separated by 7 days) of a standardized methanolic extract of *M. officinalis*. The 600 mg dose of *M. officinalis* improved the negative mood effects of DISS, increased self-ratings of calmness and reduced self-ratings of alertness (Kennedy et al., 2004). Findings of a prospective open-label pilot study in stressed volunteers with mild-to-moderate anxiety disorders and sleep disturbances showed that oral administration of Cyracos® (a patented standardized extract of M. officinalis leaves containing more than 7% RA and 15% hydroxycinnamic acid derivatives) at a dose of 600 mg/day for 15 days significantly reduces anxiety manifestations by 18%, ameliorates anxiety-associated symptoms by 15%, and lowers insomnia by 42% (Cases et al., 2011). The above-mentioned data supports the traditional use of *M. officinalis* as an anti-anxiety and calming drug. However, still more evidence from randomized controlled trials is required to elucidate other possible mechanisms underlying these anxiolytic effects, and also to evaluate anti-anxiety effects of other active ingredients present in *M. officinalis*.

Table 2
Major phytochemicals isolated and characterized from *M. officinalis*.

No.	Classification	Chemical component	Part of plant	References
1	Oxygenated monoterpene	Geranial	Aerial parts	Meftahizade et al. (2010)
2	Oxygenated monoterpene	Neral	Aerial parts	Meftahizade et al. (2010)
3	Oxygenated monoterpene	Citronellal	Aerial parts	Mimica-Dukic et al. (2004)
4	Oxygenated monoterpene	Geraniol	Aerial parts	Saeb and Gholamrezaee (2012)
5	Oxygenated monoterpene	Decadienal	Leaves	Saeb and Gholamrezaee (2012)
6	Oxygenated monoterpene	Carvacrol	Leaves	Saeb and Gholamrezaee (2012)
7	Oxygenated monoterpene	Methyl citronellate	Leaves	Saeb and Gholamrezaee. (2012)
8	Sesquiterpene hydrocarbon	β-Cubebene	Aerial parts	Allahverdiyev et al. (2004)
9	Sesquiterpene hydrocarbon	β-Caryophyllene	Aerial parts	Allahverdiyev et al. (2004)
10	Triterpene	Ursolic acid	Aerial parts	Awad et al. (2009)
11	Triterpene	Oleanolic acid	Aerial parts	Awad et al. (2009)
12	Triterpene	3β,16β,23-Trihydroxy-13,28-epoxyurs-11-ene-3-*O*-β-D-glucopyranoside	Stems and leaves	Mencherini et al. (2007)
13	Triterpene	3,23-Disulfate ester of 3β,19α,23-trihydroxyurs-12-en-28-oic acid	Stems and leaves	Mencherini et al. (2007)
14	Triterpene	3,23-Disulfate ester of 2α,3β,19α,23-tetrahydroxyurs-12-en-28-oic acid	Stems and leaves	Mencherini et al. (2007)
15	Triterpene	3,23-Disulfate ester of 2α,3β,19α,23-tetrahydroxyurs-12-en-28-oic acid 28-*O*-β-D-glucopyranoside	stems and leaves	Mencherini et al. (2007)
16	Triterpene	3,23-Disulfate ester of 3β,23,29-trihydroxyolean-12-en-28-oic acid	Stems and leaves	Mencherini et al. (2007)
17	Triterpene	3,23-Disulfate ester of 2α,3β,23,29-tetrahydroxyolean-12-en-28-oic acid	Stems and leaves	Mencherini et al. (2007)
18	Triterpene	3,23-Disulfate ester of 2α,3β-23,29- tetrahydroxyolean-12-ene-28-oic acid, 28-*O*-β-D-glucopyranoside	Leaves	Tantry et al. (2014)
19	Triterpene	23-Monosulfate ester of 2α,23-dihydroxyurs-12-ene-28-oic acid, 3-*O*-β-D-glucopyranoside	Leaves	Tantry et al. (2014)
20	Phenolic acid	Rosmarinic acid	Tops	Caniova and Brandsteterova (2001)
21	Phenolic acid	3-(3,4-dihydroxyphenyl)-Lactic acid	Aerial parts	Barros et al. (2013)
22	Phenolic acid	Caffeic acid	Aerial parts	Barros et al. (2013)
23	Phenolic acid	Lithospermic acid A	Aerial parts	Barros et al. (2013)
24	Phenolic acid	Salvianolic acid F	Aerial parts	Barros et al. (2013)
25	Phenolic acid	Salvianolic acid A	Aerial parts	Barros et al. (2013)
26	Phenolic acid	Salvianolic acid C	Aerial parts	Barros et al. (2013)
27	Phenolic acid	Salvianolic acid B	Aerial parts	Barros et al. (2013)
28	Phenolic acid	Sagerinic acid	Aerial parts	Barros et al. (2013)
29	Phenolic acid	Yunnaneic acid F	Aerial parts	Barros et al. (2013)
30	Phenolic acid	Chlorogenic acid	Leaves	Ibragić et al. (2014)
31	Phenolic acid	Gallic acid	Leaves	Ibragić et al. (2014)
32	Phenolic acid	m-Coumaric acid	Leaves	Dastmalchi et al. (2008a)
33	Phenolic acid	Melitric acid A	Aerial parts	Agata et al. (1993)
34	Phenolic acid	Melitric acid B	Aerial parts	Agata et al. (1993)
35	Phenolic acid	Ellagic acid	Aerial parts	Pereira et al. (2014)
36	Flavone glycoside	Luteolin 3′-*O*-β-D-glucuronide	Leaves	Heitz et al. (2000)
37	Flavone glycoside	Luteolin7-*O*-glucoside	Leaves	Mulkens and Kapetanidis (1986)
38	Flavone glycoside	Apigenin7-*O*-glucoside	Leaves	Mulkens and Kapetanidis (1986)
39	Flavonol glycoside	Isoquercitrin	Leaves	Mulkens and Kapetanidis (1986)
40	Flavonol	Rhamnocitrin	Leaves	Mulkens and Kapetanidis (1986)
41	Flavone glycoside	luteolin 7-*O*-β-D-glucopyranoside-3′-*O*-β-D-glucuronopyranoside	Leaves	Patora and Klimek (2002)
42	Flavone	Apigenin	Leaves	Patora and Klimek (2002)
43	Flavone glycoside	Luteolin 7-*O*-β-D-glucuronopyranoside	Leaves	Patora and Klimek (2002)
44	Flavone	Luteolin	Leaves	Patora and Klimek (2002)
45	Flavone glycoside	Apigenin 7-*O*-β-D-glucopyranoside	Leaves	Patora and Klimek (2002)
46	Flavone glycoside	Luteolin 3′-*O*-β-D-glucuronopyranoside	Leaves	Patora and Klimek (2002)
47	Flavanone glycoside	Hesperidin	Leaves	Dastmalchi et al. (2008)
48	Flavanone	Hesperetin	Leaves	Dastmalchi et al. (2008)
49	Flavanone glycoside	Eriodictyol-7-*O*-glucoside	Leaves	Dastmalchi et al. (2008)
50	Flavanone glycoside	Naringin	Leaves	Dastmalchi et al. (2008)
51	Flavanone	Naringenin	Leaves	Dastmalchi et al. (2008)
52	Flavanol	Catechin	Aerial parts	Pereira et al. (2014)
53	Flavanol	epi-Catechin	Aerial parts	Pereira et al. (2014)
54	flavonol glycoside	Rutin	Aerial parts	Pereira et al. (2014)

5.2. Antidepressant effect

Traditional use of *M. officinalis* as an exhilarant and enlivening medication has been recorded in ancient medical books. In an *in vitro* study, aqueous and methanol extracts of *M. officinalis* could mildly inhibit monoamine oxidase (MAO)-A, and the latter extract was more potent in this inhibition (Lopez et al., 2009). Nevertheless, the IC_{50} values calculated for the methanol and aqueous extracts were 19.3 and 48.2 μg/mL, doses that are unlikely to be reproducible in clinical settings even with the highly bioavailable preparations. Ethanol extract of the plant also exerted antidepressant effects in the forced swimming (FS) test via enhancing norepinephrine neurotransmission (Emamghoreishi and Talebianpour, 2009; Taiwo et al., 2012). RA reduced the duration of immobility in FS test in mice via antidepressant mechanisms different from monoamine transport or MAO inhibition (Takeda et al., 2002a, 2002b). Therefore, studies should be performed to elucidate the main anti-depressant mechanism of *M. officinalis*, explore

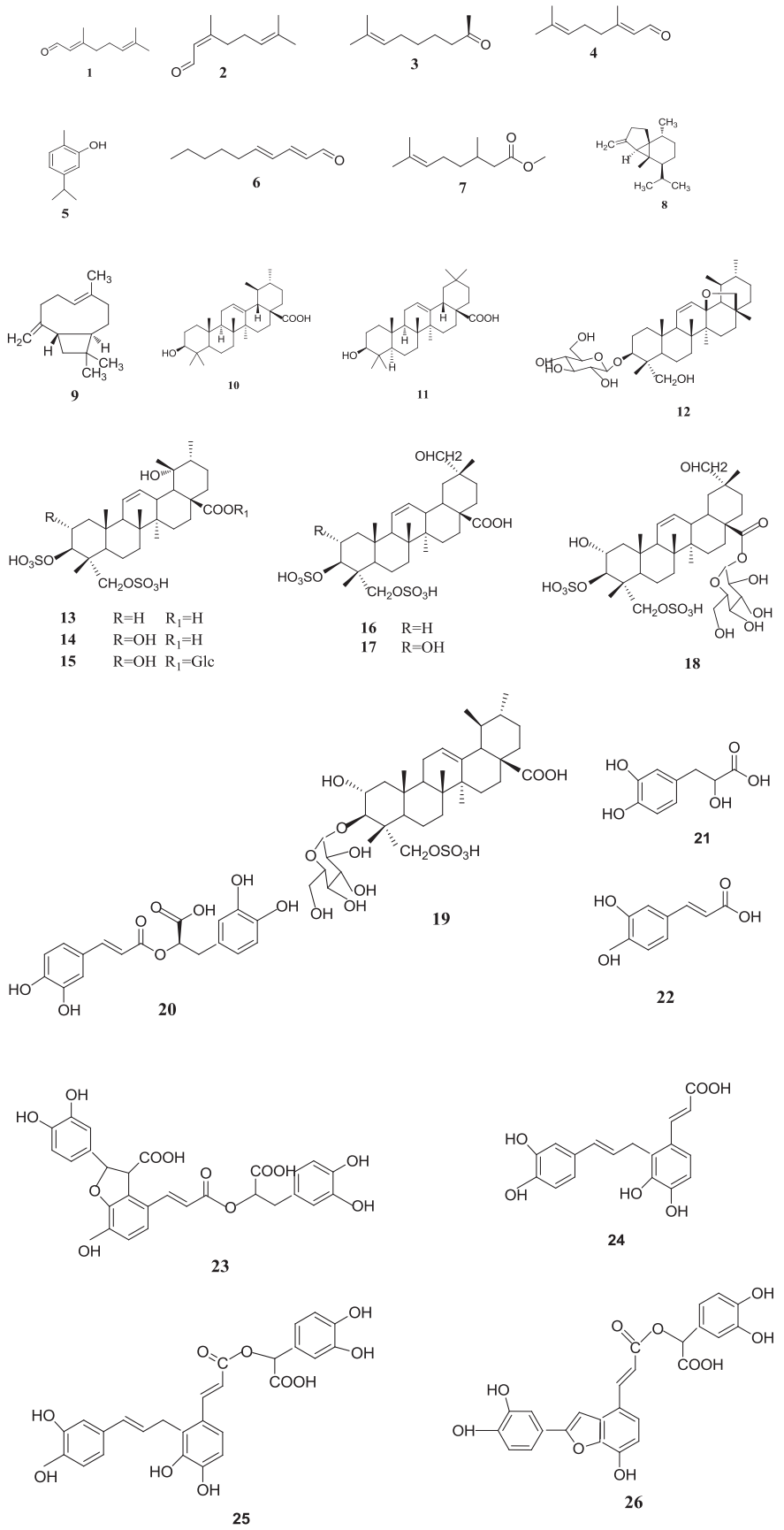
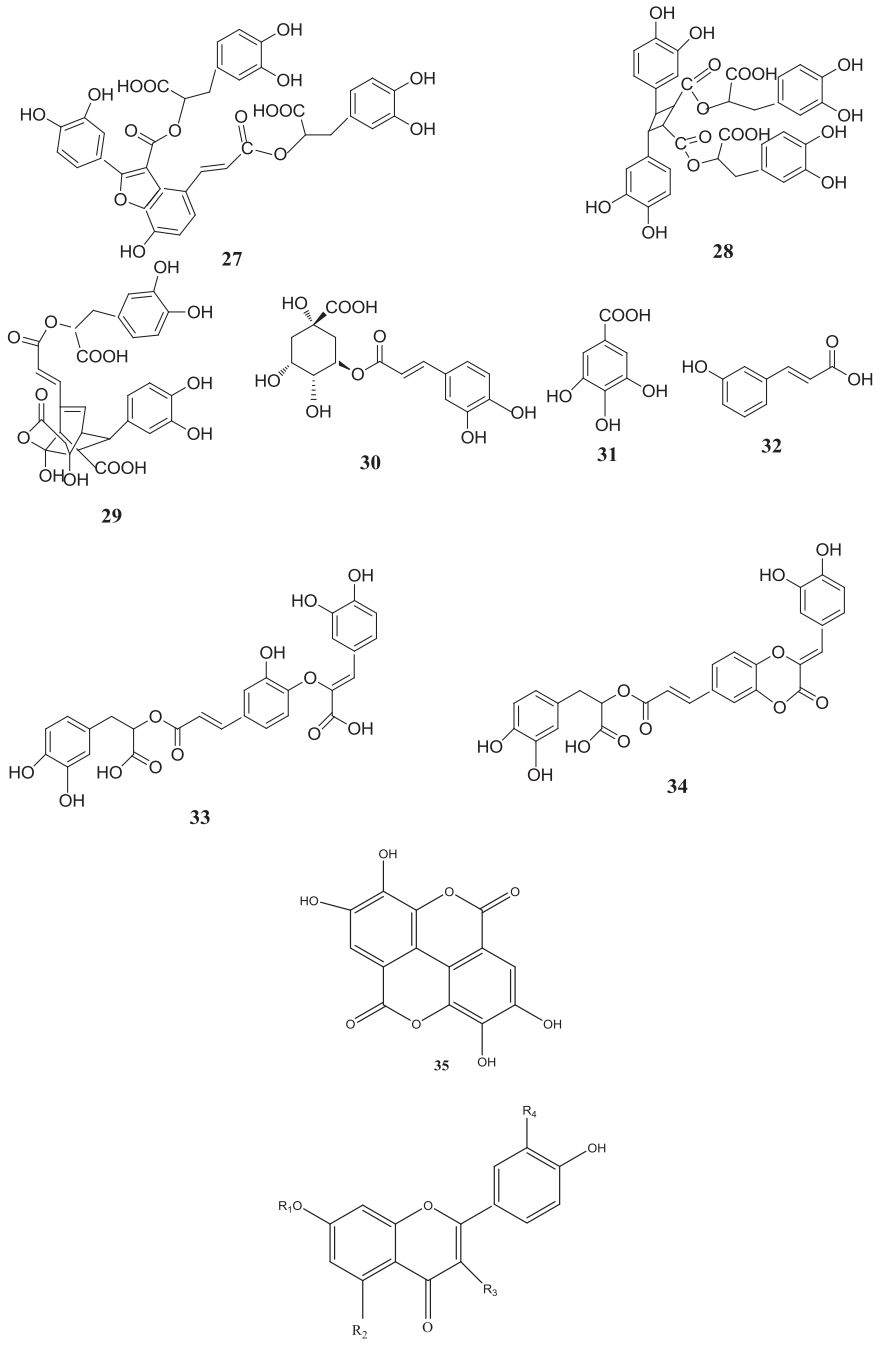
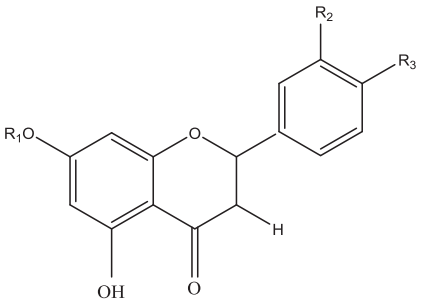


Fig. 1. Chemical structures of secondary metabolites isolated form *M. officinalis*.

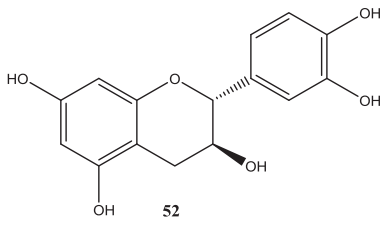


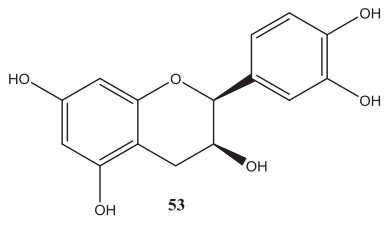
	R1	R2	R3	R4
36	H	OH	H	*O*-glucuronide
37	Glc	OH	H	OH
38	Glc	OH	H	H
39	H	OH	*O*-glc	OH
40	Me	OH	OH	H
41	Glucopyranoside	OH	H	*O*-glucuronopyranoside
42	H	OH	H	H
43	Glucuronopyranoside	OH	H	OH
44	H	OH	H	OH
45	Glucopyranoside	OH	H	OH
46	H	OH	H	*O*-glucuronopyranoside

Fig. 1. (*continued*)



	R1	R2	R3
47	Rutinoside	OH	OMe
48	H	OH	OMe
49	Glc	OH	OH
50	Neohesperidose	H	OH
51	H	H	OH





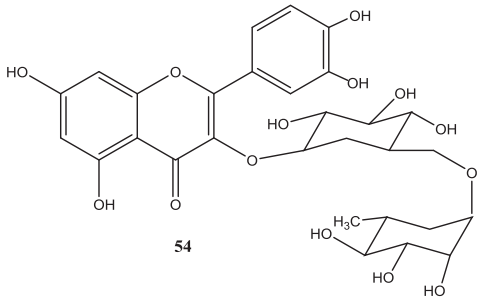


Fig. 1. (*continued*)

the MAO-A inhibitory activity of major polar compounds of *M. officinalis* other than RA, and investigate their possible synergistic effects with RA. Finally, although the above-mentioned animal studies approved the traditional use of *M. officinalis* as an antidepressant medication (at doses from 25 mg/kg to 300 mg/kg), no IC_{50} or EC_{50} value was reported to allow better assessment of the translational value of the effects, though translation of such doses is theoretically applicable in humans based on the principles of allometric scaling of experimental doses.

5.3. Neuroprotective effects

The use of *M. officinalis* in the treatment of several CNS diseases in different countries is supported by *in vitro* and *in vivo* studies in models of neuroprotection. Treatment of PC12 cells with the methanol extract of *M. officinalis* protects these cells against H_2O_2 toxicity in MTT and LDH assays (cell viability assays that are often used to determine cytotoxicity following exposure to toxic substances). Moreover, both aqueous and methanol extracts produced a significant reduction in the intracellular ROS formation, suggesting a significant neuroprotective effect (Lopez et al., 2009). Pretreatment of PC12 cells with the acidic fraction of *M. officinalis* ethanol extract containing polyphenols, flavonoids and terpenoids exerted a significant protective effect on Aβ-induced toxicity and oxidative stress, an effect that can be attributed to the antioxidant activity (Sepand et al., 2013). It has been reported that the *M. officinalis* extract can displace [3H]-(N)-nicotine from human brain cell membranes bearing acetylcholine receptors. IC_{50} values for the [3H] nicotine displacing activity of the ethanol extract of *M. officinalis* were lower than 100 μg/mL (Wake et al., 2000). A number of studies showed that nicotine has protective effects on Aβ-induced toxicity and pretreatment of neurons with nicotine attenuates Aβ-induced oxidative stress and apoptosis (Kihara et al., 1997; Liu et al., 2004). Accordingly, protective effect of *M. officinalis* extract on Aβ-induced toxicity can also be attributed to its stimulating effects on the nicotinic receptor. In addition, aqueous

extract of *M. officinalis* exerted neuroprotective effects against apoptosis induced by 3,4-methylenedioxymethamphetamine (MDMA or Ecstasy) in the primary neurons of hippocampal culture, these effects being possibly due to the free radical scavenging and MAO inhibition (Hassanzadeh et al., 2011). Pre- and post-treatment of human primary neuronal cells with RA has been shown to maintain neuronal integrity and attenuate the neurotoxicity induced by ciguatoxin (Braidy et al., 2014). Taken together, potent antioxidant and radical scavenging activities of the hydroalcoholic extract of *M. officinalis* and its main phenolic compounds RA, quercetin, gallic acid, caffeic acid, chlorogenic acid and rutin, could partially account for the neuroprotective effect of the plant. Moreover, *in vitro* cytotoxicity assays demonstrated the protective effects of *M. officinalis* EO on neuronal hypoxia-induced death. The EO of the plant has been reported to decrease caspase-3 activity and TUNEL-positive cells significantly. Furthermore, a decrease in malondialdehyde (MDA) and an increase in Trolox equivalent antioxidant capacity (TEAC) levels in the hippocampus of EO-treated ischemic animals were observed following treatment with a dose of 100 mg/kg. Nonetheless, EO induced neurotoxic effects in a primary neuronal culture system at a concentration of 0.1 mg/mL (Mahita et al., 2014). These results suggest a protective activity of *M. officinalis* EO against various CNS diseases associated with ischemic brain injury, and this protection could be mediated by the inhibition of hypoxia-inducible factor-1α (HIF-1α; a transcription factor that plays a critical role in hypoxia-induced gene expression), oxidative stress, and apoptosis (Bayat et al., 2012). Several studies have reported moderate antioxidant activity for oxygenated monoterpenes such as citral isomers and citronellal, which are abundant in *M. officinalis* EO (Ruberto et al., 2000; Mimica-Dukic et al., 2004; Misharina et al., 2008). These findings provide evidence for the traditional use of *M. officinalis* in the treatment of neurodegenerative diseases (ND) such as dementia, epilepsy, stroke and paralysis.

5.4. Effects on mood, cognition and memory

M. officinalis has been traditionally used for the treatment of dementia and amnesia, two disorders that are closely associated with the Alzheimer's disease (AD). The plant has also been recorded as a treatment for psychosis. Acetylcholinesterase (AChE) inhibitors increase the level and duration of action of acetylcholine in brain synapses. This cholinergic effect can alleviate AD and its cognitive symptoms (mostly in the form of memory and learning deficits) as well as cognitive impairments in patients with schizophrenia (Ellis, 2005; Cummings, 2000). Ethanol extract of *M. officinalis* can exert AChE inhibitory activity in a time- and dose-dependent manner (Ferreira et al., 2006; Dastmalchi et al., 2009) with a potency of 1.72 ± 0.16 μg equivalents of physostigmine/mg of the extract (physostigmine was used as the reference standard) (Dastmalchi et al., 2009). AChE inhibitory guided fractionation of the same extract (12 fractions (F1–F12) on a time based scheme) revealed that most fractions have inhibitory activities that are more potent than the crude extract. The main components of the most potent fraction (F8: 25.36 ± 1.63 μg physostigmine/mg) were *cis*- and *trans*-RA isomers and a RA derivative with a methyl ester or a methoxy group (Dastmalchi et al., 2009). Moreover, ethyl acetate fraction of the hydroalcoholic extract from *M. officinalis* was characterized by a high flavonoid content and antioxidant properties, and could significantly inhibit AChE in a concentration-dependent manner (Pereira et al., 2014). Aqueous and methanol extracts of the plant had no AChE inhibitory activity (Adsersen et al., 2006; Pereira et al., 2014) that can be explained by the antagonism between various constituents of the plant extract.

M. officinalis EO can also inhibit AChE in a dose-dependent manner (Ferreira et al., 2006). A few studies have reported the AChE inhibitory activity of citral (the major component of *M. officinalis* EO) and some other monoterpenes (Cunningham et al., 1995). Based on the above-mentioned findings, it is possible to establish that AChE inhibitory activity of *M. officinalis* is mainly attributed to RA and some of its derivatives, and the plant EO. Nevertheless, the presence of possible antagonism among the phytochemical constituents of the plant may be a possible explanation for the above-mentioned complicated results.

Gallic acid (an important constituent of *M. officinalis*) could inhibit matrix metalloproteinase-2 (MMP-2), an enzyme that has been suggested to be involved in AD, *in vitro*. Since ascorbic acid (as a potent antioxidant) had no effect on MMP-2 activity, the MMP-2 inhibitory effect of gallic acid seems to be related to mechanisms other than antioxidant activity (Pereira et al., 2014).

It has been shown that stimulation of acetylcholine receptors is another strategy for the treatment of AD (Kihara and Shimohama, 2004). An 80% ethanol extract of *M. officinalis* could displace [3H]-(N)-nicotine and [3H]-(N)-scopolamine (the ligand for muscarinic receptors) from human brain cell membranes bearing nicotinic or muscarinic acetylcholine receptors. This finding suggests that compounds with a higher solubility in 80% ethanol than water, can be responsible for the observed displacing effect (Wake et al., 2000).

It has been shown that dysfunction of the GABAergic system may contribute to cognitive impairment and AD in humans (Solas et al., 2015). Radiolabeling and electrophysiological evaluations have shown that *M. officinalis* EO can bind to $GABA_A$ receptors suggesting anti-agitation properties (Huang et al., 2008; Abuhamdah et al., 2008). *Trans*-ocimene, a monoterpene present in the *M. officinalis* EO, has been proposed to be responsible for this effect, acting in a concentration-dependent manner with an IC_{50} of 40 μM) (Mahita et al., 2014).

In vivo, ethanol extract of *M. officinalis* enhanced learning and memory of naive rats and improved scopolamine-induced learning deficit in a manner similar to the effect of other cholinesterase inhibitors. These effects were not dose-dependent, and doses above 200 mg/kg – which is itself high for clinical application – could neither enhance memory in naive rats nor reverse scopolamine-induced memory impairment; this being possibly due to over-stimulation of the nicotinic receptors, nicotinic receptor blockade and memory impairment. Also, inhibition of AChE activity was observed in both naive and scopolamine-induced memory-impaired rats (Soodi et al., 2014). The involvement of other mechanisms in the memory-enhancing activity of some constituents of the extract has also been reported. Luteolin improved scopolamine-induced impairment of passive avoidance response in rats and attenuated memory impairment induced by β-amyloid in water maze performance (Tsai et al., 2007, 2010). Ursolic acid improved age-related cognitive deficit through activation of antioxidant enzymes and reduction of lipid peroxidation (Lu et al., 2007). Clinical trials have confirmed the beneficial effects of *M. officinalis* in the treatment of some symptoms of AD and cognitive impairment. A 4-week double-blind placebo-controlled trial was conducted to determine the effects of massage aromatherapy with *M. officinalis* EO on the agitation behavior of people with severe dementia (the EO preparation was applied to patients' faces and arms twice a day by caregiving staff). The results showed that 60% of subjects in the treatment group and 14% of subjects in the placebo group experienced a 30% reduction of Cohen-Mansfield Agitation Inventory (CMAI) score. Overall improvements in agitation were 35% in patients receiving EO and 11% in those treated with placebo. The quality of life of patients in the EO group was also improved significantly (Ballard et al., 2002). Nevertheless, the results of another 12-week trial demonstrated no difference in agitation between the groups of patients with AD treated with *M. officinalis* EO, massage aromatherapy (with a 10%

w/w EO in a base lotion), donepezil and placebo (massage therapy with 10% w/w sunflower oil in a base lotion) (Burns et al., 2011). There were improvements in all 3 treatment groups, with an 18% improvement in the Pittsburgh Agitation Scale (PAS) and a 37% improvement in the Neuropsychiatric Inventory (NPI), over 12 weeks. However, involvement of elements of touch and social interaction by the carer in control group in the aforementioned study may explain the substantial improvements in this group.

Another clinical trial investigating the effects of *M. officinalis* on cognition and mood of healthy humans showed that acute administration of the plant's ethanol extract (600 mg) can modulate mood and cognitive performance (Kennedy et al., 2002). The results of an *in vitro* study conducted by the same authors suggested cholinergic receptor-binding properties in the human cerebral cortex tissue as a potential mechanism of action for the *M. officinalis* extract (Kennedy et al., 2003). Similarly, a16-week placebo-controlled trial on 42 patients with mild-to-moderate AD demonstrated reduction of agitation and improvement in cognitive and behavioral functions after administration of hydroalcoholic extract of *M. officinalis* (60 drops/day) standardized to contain 500 μg citral/mL (Akhondzadeh et al., 2003). These data suggest that the memory enhancing effects of *M. officinalis* can possibly be attributed to its AChE inhibitory activity, stimulation of the acetylcholine (nicotinic and muscarinic receptors) and $GABA_A$ receptors, as well as inhibition of MMP-2.

5.5. Cardiovascular effects

M. officinalis is a common traditional treatment for heart palpitation and is reputed to be a potent heart tonic and cardioprotective plant. Pharmacological studies mainly focus on anti-arrhythmic effects of *M. officinalis* extracts. In an *in vivo* study, ethanol extract of the plant reduced the occurrence of ventricular premature beats (VPB), ventricular fibrillation (VF) and ventricular tachycardia (VT) following $CaCl_2$-induced arrhythmias in rats (Akhondali et al., 2015). These antidysrhythmic and bradycardic effects have been suggested to be due to the β-adrenergic antagonistic activity of the plant (Somova et al., 2003, 2004). Aqueous extract of *M. officinalis* contains high concentrations of RA and other phenolics and can prolong QRS, QTc, JT and TpTe intervals of ECG in rats possibly via modulating the expression or conductance properties of sodium and potassium channels in the heart (Joukar and Asadipour, 2015). Administration of such doses is expected to be applicable in clinical practice (according to allometric scaling of animal dose to human) and carefully designed clinical trials are needed to verify if the above-mentioned effects on heart rhythm could be exerted in human. Although *M. officinalis* provokes cardiac rate reduction in isolated rat heart (Gazola et al., 2004), no significant heart rate reduction in human has been observed (Alijaniha et al., 2015). However, it reduced frequency of palpitation episodes in patients with benign palpitations (Alijaniha et al., 2015). Aqueous extract of *M. officinalis* also showed a mild protection against reperfusion-induced lethal ventricular arrhythmias in rats via muscarinic receptor stimulation (Joukar et al., 2014), but again at high doses (50–40 mg/kg/day) adminsitered via intraperitoneal route. These findings may indicate that anti-arrhythmic effects of *M. officinalis* possibly involves mechanisms related to β-adrenergic blockade and parasympathetic regulation along with slowing ventricular conductivity through blocking sodium and/or potassium currents.

Aqueous extract of *M. officinalis* also exhibited a concentration-dependent vasorelaxant activity in isolated rat thoracic aorta via stimulation of endothelial nitric oxide formation as well as possible involvement of prostacyclin and endothelium-derived hyperpolarizing factor (EDHF) pathways (Ersoy et al., 2008).

An investigation of the effects of pretreatment with *M. officinalis* aqueous extract on the resistance of the heart to myocardial injury induced by isoproterenol (ISO) was carried out in rats. The lower tested dose of the extract (50 mg/kg) increased the heart resistance to cardiac injury as evidenced by decreased MDA levels of the injured hearts. This protective effect was likely mediated by reducing the heart rate and improving the balance of the redox system. However, the higher tested dose of the extract (200 mg/kg) intensified the injury of ischemic heart (as evidenced by higher serum cardiac troponin I levels) possibly via increasing cardiac contractility and myocardial oxygen demand (Joukar et al., 2015a, 2015b). These observations are supported by a study reporting prevention of myocardial fibrotic changes and improvement in cardiac function in insulin resistant rats after treatment with RA (10 mg/kg). The fibrogenic process is accompanied by increased expression of fibrogenic genes including transforming growth factor (TGF)-β1, and differentiation of fibroblasts into myofibroblasts that generate excess extracellular matrix proteins and tissue inhibitors of MMP (TIMPs) causing hypertrophy. Furthermore, activation of angiotensin II type 1 receptor (AT1R) by angiotensin II (a potent profibrotic agent) enhances fibrosis by activating both TGF-β1 and MMPs (Leask et al., 2007). RA treatment significantly reduced the levels of superoxide anion, angiotensin II and collagen, the expression of TGF-β1, α-smooth muscle actin (α-SMA), MMPs and AT1R, and increased the expression of TIMPs (Karthik et al., 2012). Since fibrosis is thought to contribute to the development of heart failure and cardiac arrhythmias in patient with heart disease, RA as the main ingredient of *M. officinalis* might have a role in these effects. These results along with the scientific evidence on the antioxidant, hypolipidemic and antianxiety properties of *M. officinalis* may partly explain the traditional use of this plant for the treatment of heart diseases. However, further studies are needed to assess dose dependence of these effects.

5.6. Cytotoxic effects

M. officinalis has been widely used for the treatment of several types of cancer (Javadi et al., 2015). *In vitro*, *M. officinalis* EO and its major component, citral, induced apoptosis of GBM cell lines that expressed active MRP1. In the same study, citral induced production of ROS and inhibited MRP1 expression (Queiroz et al., 2014). Citral also reduced the viability of several human tumor cell lines and a mouse melanoma cell line (de Sousa et al., 2004). *M. officinalis* hydroalcoholic extract exerted antiproliferative effects on colon carcinoma cells and induced apoptosis via induction of intracellular ROS generation (Weidner et al., 2015). However, the reported IC_{50} values for the cytotoxic activity were >100 μg/mL, which is hardly to be replicated for therapeutic purposes in clinical practice. *M. officinalis* decoctions containing RA and lithospermic acid A showed growth inhibition activity against different human tumor cell lines (Carocho et al., 2015). A 50% ethanol extract of *M. officinalis* has been reported to have cytotoxic effects on human colon cancer cell lines according to MTT and NR tests that are cell viability assays often used to determine cytotoxicity following exposure to toxic substances (Encalada et al., 2011; Fotakis et al., 2006). Dichloromethane and *n*-hexane fractions of the plant extract showed strong inhibitory effects on both K562 and Jurkat cells in a dose-dependent manner. The dichloromethane fraction significantly induced apoptosis in leukemia cell lines via up-regulation of Fas and Bax mRNA expression and increasing the Bax/Bcl-2 ratio, indicating its capacity in activating both extrinsic and intrinsic pathways of apoptosis. Nevertheless, the *n*-hexane fraction of the plant did not significantly change the expression of apoptosis-related genes indicating that induction of apoptosis may not be the main cause for cell growth inhibition by this fraction of the plant (Ebrahimnezhad Darzi and Amirghofran, 2013). These

results suggest that apoptosis-inducing activities of the plant are possibly mediated by EO components as well as lipophilic constituents (present in the dichloromethane and *n*-hexane fractions) which can interact with the cell membrane and pass through it.

In vivo, treatment of mice with an ethanol extract of *M. officinalis* resulted in antigenotoxic and antimutagenic effects (de Carvalho et al., 2011). However, the effect was observed at a high dose of 500 mg/kg and through parenteral administration (i.p.), which is far from being feasible to be translated in human. The extract was more effective in reversing the genotoxic damage (comet assay) than mutagenic damage (micronucleus assay) induced by methyl methanesulfonate (MMS), an alkylating agent. As phenolic compounds have been shown to exert antioxidant properties, they could be considered as potential candidates for the mentioned activities (de Carvalho et al., 2011). RA can reduce the chromosome damage induced by doxorubicin and ethanol in the micronucleus assay in mice and V79 cells (Furtado et al., 2008, 2010; De Oliveira et al., 2012). Moreover, gallic acid acts as an antimutagenic agent via altering the activity of DNA repair enzymes and modulating the expression of these enzymes (Abdelwahed et al., 2007). Therefore, it is likely that phenolic acids, especially RA, which are abundant in polar extracts, account for antimutagenic and antigenotoxic effects of *M. officinalis.* In spite of the mentioned evidence on the cytotoxic properties of *M. officinalis* and its preparations, it must be noted that mentioned studies did not provide any information on the cytotoxic effects of the tested extract on normal cells, thus necessitating further evaluation of the selective cytotoxicity of the tested extracts.

5.7. Anti-inflammatory and anti-nociceptive effects

With reference to traditional records, *M. officinalis* has been used to treat several inflammatory diseases including asthma and joint inflammation. It is also used as a pain killer. Several pharmacological experiments revealed anti-inflammatory and anti-nociceptive effects of the plant. Pretreatment with aqueous extract of *M. officinalis* significantly reduced inflammagen-induced paw edema in rats and decreased the nociceptive response in mice (Birdane et al., 2007). Moreover, ethanol extracts from *M. officinalis* exerted dose-related antinociceptive effects in chemical models of nociception in mice through inhibition of the L-arginine-nitric oxide pathway and activation of cholinergic systems. RA content of the extract was found to be responsible for its antinociceptive properties (Guginski et al., 2009). While the reported IC_{50} values for the extract are relatively high (around 200 mg/kg), that of the RA (2.6 mg/kg) appears to be more applicable for clinical application. Long-term oral administration of *M. officinalis* EO (0.01–0.04 mg/day) exhibited a significant antinociceptive effect in an animal model of diabetic hyperalgesia (Hasanein and Riahi, 2015). The EO also showed significant reduction and inhibition of paw edema induced by carrageenan and experimental trauma in rats. However, the tested doses (200 and 400 mg/kg) appear to be high for translation to human, though no safety concern was reported at doses as high as 2000 mg/kg (Bounihi et al., 2013). The anti-inflammatory mechanism of EO seems to be related to its citral content which has been reported to inhibit TNF-α in RAW 264.7 cells stimulated by lipopolysaccharide (LPS), and suppress IL-6 and IL-1β in LPS-stimulated peritoneal macrophages of normal mice (Bounihi et al., 2013).

5.8. Hypoglycemic effects

M. officinalis has demonstrated obvious hypoglycemic effects *in vitro* and *in vivo* and has been traditionally used to treat diabetes. Application of *M. officinalis* ethanol extract (0.6 mg/mL) to human primary adipocytes caused specific PPAR gene expression in metabolically relevant target cells. However, replication of the same effect in clinical conditions needs further studies with lower doses that are more likely to be administered in human. *In vivo*, treatment of insulin-resistant obese mice for 6 weeks with *M. officinalis* ethanol extract (200 mg/kg) significantly reduced hyperglycemia and insulin resistance (Weidner et al., 2014). Moreover, EO of this plant restored normal plasma glucose levels and reduced the body weight of diabetic rats (Hasanein and Riahi, 2015) with doses of 0.02 and 0.04 mg/day that appear to be administratable in human; However, additional toxicological investigations should confirm the safety of EO. Ursolic acid, a natural pentacyclic triterpenoid carboxyl acid found in *M. officinalis*, has anti-diabetic activity and increases both insulin sensitivity and insulin secretion in high-fat fed streptozotocin-induced diabetic mice, resulting in the elevation of plasma and pancreatic insulin levels (Jang et al., 2009). Oleanolic acid, another triterpenoid constituent of *M. officinalis*, has potent antidiabetic effects. It improves insulin response, preserves functionality and survival of β-cells, and protects against diabetic complications through several mechanisms such as enhancement of the expression of antioxidant enzymes and phase II response genes, blocking NF-κB, and suppressing the polyol pathway, AGEs production, and hyperlipidemia (Castellano et al., 2013). Therefore, lipophilic triterpenoids along with the EO of the plant could be considered as potential anti-diabetic agents.

5.9. Hypolipidemic effects

According to ITM, *M. officinalis* is capable of expelling bad matters from the blood. Moreover, its extensive use as a cardiovascular remedy may be secondary to its hypolipidemic effects. Ethanol extract of *M. officinalis*, intraperitoneally administered to hypercholesterolemic rats (25–75 mg/kg), decreased liver enzymes (alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST)) levels in serum with an effect comparable to that of atorvastatin (Zarei et al., 2014). Moreover, treatment of insulin-resistant obese mice with ethanol extract (200 mg/kg/day) significantly reduced plasma triacylglycerol (TAG), LDL/VLDL cholesterol and non-esterified fatty acids (Weidner et al., 2014). Oleanolic and ursolic acids have been demonstrated to decrease serum levels of LDL-cholesterol and triglycerides and could thus explain, at least in part, the observed lipid-lowering effects of *M. officinalis* extract (Somova et al., 2003). Oral administration of the aqueous extract of the plant to hyperlipidemic rats also reduced serum total lipids, cholesterol and liver enzyme levels, and enhanced lipid peroxidation (LPO) and glutathione (GSH) levels in the liver tissue, indicating hypolipidemic and hepatoprotective activities of the plant (Bolkent et al., 2005).

Oral administration of *M. officinalis* EO significantly reduced plasma triglyceride (TG) concentrations in mice. Cellular TG and cholesterol concentrations were also significantly decreased in a dose- and time-dependent manner in HepG2 cells following treatment with *M. officinalis* EO. The mechanisms of the hypotriglyceridemic effects can be a reduction in the translocation of sterol regulatory element-binding protein-1c (SREBP-1c) and its responsive genes involved in fatty acid synthesis via decreased P300/CBP-associated factor (PCAF) histone acetylase activity, resulting in reduction of hepatic fatty acid synthesis (Jun et al., 2012). In spite of the interesting results, doses of *M. officinalis* extract applied in the above-mentioned studies are far from replication in clinical studies. This necessitates further investigations with lower doses that could be translated to human use.

5.10. Antioxidant

It is evident that oxidative stress plays an important role in the pathogenesis of many diseases including neurodegenerative diseases, cardiovascular diseases, diabetes, and various types of cancer (Melo et al., 2011; Elnakish et al., 2013; Sosa et al., 2013; Rains et al., 2011). Free radicals, particularly superoxide and non-radicals, such as hydrogen peroxide, can be generated in large quantities that may overcome endogenous protective antioxidants, such as reduced GSH and superoxide dismutase (SOD) (Slemmer et al., 2008), and this condition results in oxidative stress.

Several *in vitro* and *in vivo* studies have indicated antioxidant activity for EO and extracts of *M. officinalis* (Bayat et al., 2012; Canadanovic-Brunet et al., 2008; Carocho et al., 2015; Ferreira et al., 2006; Lopez et al., 2009; Luno et al., 2014; Mimica-Dukic et al., 2004; Zeraatpishe et al., 2011). Aqueous ethanol extract of *M. officinalis* was tested for its *in vitro* antioxidant activity using iron (III) reduction, iron (II) chelation, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azinobis (3-ethylbenzothiazoline-6-sulphonate) (ABTS), superoxide anion and nitric oxide free-radical scavenging, and inhibition of β-carotene–linoleic acid bleaching assays (Dastmalchi et al., 2008). The antioxidant activity of the extract (90.43 ± 1.55 μg/mL) was statistically superior to those of gallic and caffeic acids ($44.29 \pm 1.92\%$ and $44.59 \pm 7.10\%$ respectively) and was statistically comparable with that of quercetin ($98.46 \pm 0.89\%$) and butylated hydroxyanisole (BHA) ($96.08 \pm 1.58\%$) (Dastmalchi et al., 2008). The presence of high levels of phenolic substances may be attributed to the antioxidant properties of the extract. RA has a DPPH scavenging activity with an EC_{50} value of 26.03 μg/mL (Erkan et al., 2008). Also, caffeic acid, at the concentration of 10 μg/mL, showed 68.2% inhibition of lipid peroxidation of linoleic acid emulsion, and was reported to be effective in DPPH scavenging, superoxide anion radical scavenging, and reduction and chelation of ferrous ions (EC_{50} values lower than 5 μg/mL) (Gülçin, 2006). Furthermore, in another study, quercetin, gallic acid, caffeic acid, chlorogenic acid and rutin exhibited high antioxidant activities against DPPH and thiobarbituric acid reactive substances (TBARS) (Pereira et al., 2014). On the other hand, the high scavenging capacity of EO from *M. officinalis* (IC_{50} value of 7.58 μg/mL in DPPH assay) has been attributed to the presence of monoterpene aldehydes and ketones (citrals, citronellal, isomenthone, and menthone) and the mixture of mono- and sesquiterpene hydrocarbons (Mimica-Dukic et al., 2004).

In 2011, a single-arm clinical trial was conducted to evaluate the capacity of *M. officinalis* infusion in improving oxidative stress in radiology staff. The results showed a significant improvement in plasma levels of SOD, catalase, and GSH peroxidase as well as reduction of plasma DNA damage, lipid peroxidation and myeloperoxidase activity (Zeraatpishe et al., 2011).

These data indicate that *M. officinalis* and its phenolic compounds exert antioxidant activity through free-radical scavenging, inhibition of lipid peroxidation, and increasing endogenous antioxidant enzymes. Therefore, the therapeutic effects of *M. officinalis* in the prevention and treatment of oxidative stress-related diseases such as neurodegenerative and cardiovascular illnesses might be attributed to its antioxidant activity (Bayat et al., 2012).

5.11. Antimicrobial effects

In vitro, *M. officinalis* EO exerted notable antimicrobial effects on Gram-negative pathogenic bacteria, such as *Pseudomonas aeruginosa, Salmonella enteritidis, Salmonella typhi, Escherichia coli,* and *Shigella* strains, particularly on the multiresistant strains. The highest activity of the EO was observed on *E. coli* and the multiresistant strain of *Shigella sonei* (Mimica-Dukic et al., 2004). In addition, the EO exhibited antibacterial activity against food-borne pathogens and spoilage bacteria with MIC values ranging from 72.0 to 1000.3 μg/mL (comparable to the MIC value of *Rosmarinus officinalis*, 91.3–1113.3 μg/mL, but lower than those of ciprofloxacin as positive control, 2.5–62.2 μg/mL) (Hussain et al., 2011). Citrals (geranial and neral) and citronellal were demonstrated to be responsible for the antibacterial and antifungal activities of the EO (Mimica-Dukic et al., 2004). Petroleum ether, chloroform, ethyl acetate, and *n*-butanol extracts showed moderate to strong antibacterial activity against *Sarcina lutea* (zone inhibition (ZI) diameters range: 10.7–19.3 mm), *Staphylococcus aureus* (ZI range: 10.0–16.3 mm), and *Bacillus cereus* (ZI range: 8.0–14.0 mm). The corresponding ZI ranges of standard antibiotics against the aforementioned strains were 28.0–29.0 (amoxicillin) and 32.7–36.6 (penicillin). Petroleum ether and ethyl acetate extracts of the plant demonstrated the strongest antibacterial activities against *Sarcina lutea.* RA, caffeic, and *p*-coumaric acids are the main compounds of the ethyl acetate and *n*-butanol extracts while the petroleum ether and chloroform extracts, due to their non-polar nature, are very poor in these phenolic acids (Canadanovic-Brunet et al., 2008). *M. officinalis* decoctions containing RA and lithospermic acid A were mostly active against *P. aeruginosa* (MIC=0.2, MBC=0.4), *Salmonella typhimurium* (MIC=0.2, MBC=0.4) and *Penicillium funiculosum* (MIC=0.1, MBC=0.2) (Carocho et al., 2015). The antimicrobial potency of *M. officinalis* decoctions on the mentioned microorganisms was comparable or higher than those of streptomycin (MIC=0.2, MBC=0.3 [*P. aeruginosa*]; MIC=0.25, MBC=0.5 [*S. typhimurium*]; MIC=0.2, MBC=0.25 [*P. funiculosum*]) and ampicillin (MIC=0.75, MBC=1.2 [*P. aeruginosa*]; MIC=0.4, MBC=0.75 [*S. typhimurium*]; MIC=0.2, MBC=0.5 [*P. funiculosum*]) as positive controls. Besides, sulfated terpenes from the hydro-alcoholic extract of *M. officinalis* showed moderate antimicrobial activity against *Candida albicans*, *Candida glabrata*, *Candida krusei* and *Aspergillus fumigatus* (fungal strains), and *S. aureus*, *E. coli*, *P. aeruginosa* and *Mycobacterium intracellulare* (bacterial strains) with MIC values more than 1.5 μg/mL, while the corresponding MIC of ciprofloxacin was 0.98 μg/mL (Tantry et al., 2014). These findings verify the traditional applications of *M. officinalis* in the treatment of fevers, wounds, scrofula and aphtha. In addition, products of this plant may serve as natural preservatives for food and pharmaceutical products.

5.12. Antiviral activity

Traditional application of *M. officinalis* leaves in patients bitten by rabid dogs implies an antiviral activity of this plant. Hydro-alcoholic and aqueous extracts of *M. officinalis* have been shown to exert significant anti-HSV-1 and anti-HSV-2 activities *in vitro* (Mazzanti et al., 2008; Nolkemper et al., 2006). An increase in the HSV-1 virion's density prior to its attachment to the host cells is the most likely mechanism of action for the antiviral activity of the aqueous extract of *M. officinalis* (Geuenich et al., 2008). However, hydroalcoholic extract of *M. officinalis* does not prevent the entry of HSV-2 into the cells, suggesting a mechanism of action subsequent to the virus penetration into the cell (Mazzanti et al., 2008). It is evident that RA plays a major role in the antiviral effects of the plant (Astani et al., 2014). Inhibition of HSV-1 and HSV-2 by *M. officinalis* EO has also been demonstrated *in vitro* using the plaque reduction assay. The EO affects the virus before adsorption, but not after penetration into the host cell indicating direct antiviral effect of the EO on herpes viruses (Schnitzler et al., 2008). In another study, different concentrations of *M. officinalis* EO were found to inhibit the proliferation of HSV-2 and this effect was inversely associated with the concentration of the virus. This latter study suggested the role of citral and citronellal in the antiviral activity due to the inhibition of protein synthesis in cells (Allahverdiyev et al., 2004). However, relatively high doses of the plant

EO were used in this latter study with concentrations ranging from 25 to 200 μg/mL. The results of two clinical studies (a multicentric study involving 115 patients and another subsequent double-blind placebo-controlled study involving 116 patients) revealed significant antiviral activity of a topical cream containing 1% dried extract of *M. officinalis* against HSV infections. The studies showed that treatment must be initiated in the very early stages of the infection to bear maximum efficacy (Wolbling and Leonhardt, 1994). In spite of the traditional application of *M. officinalis* in the prevention and treatment of rabies, anti-lyssavirus effects of this plant have not been adequately studied and there is a need for more studies on this effect of the plant. Moreover, exploring the possible virostatic effects of *M. officinalis* and its ingredients against hepatitis C virus (HCV) and HIV infections is recommended.

5.13. Antispasmodic

In different systems of traditional medicine, *M. officinalis* has been used as an antispasmodic agent, and as a remedy for gripe and hiccup. In an *in vivo* study, the relaxant effect of the EO of *M. officinalis* and its main component, citral, on rat ileum contractions was evaluated. Both EO and citral could inhibit the response to KCl, ACh and 5-hydroxytryptamine (5-HT) in a concentration-dependent manner, indicating spasmolytic effects (Sadraei et al., 2003). In 2005, a randomized double-blind placebo-controlled trial was conducted to explore the efficacy of a polyherbal preparation containing *M. officinalis* L., *Matricaria recutita* L. and *Foeniculum vulgare* M. var. *dulce* in the treatment of infantile colic. The results showed a significant improvement in breastfed infant colic within one week of treatment initiation (Savino et al., 2005).

These findings along with the traditional uses of *M. officinalis* suggest a possible role of this plant, especially its EO in reducing intestinal contractions as well as alleviating broncho-constrictive diseases such as asthma or COPD. These effects need to be evaluated in more details in future studies.

5.14. Anti-angiogenic effects

Angiogenesis is the process of forming new blood vessels from pre-existing ones. The role of pathological and deregulated angiogenesis in cancer development is well established. The new blood vessels supply the tumor tissue with nutrients and facilitate cancer metastasis (Al-Husein et al., 2012; Carmeliet et al., 2000). Abnormalities of angiogenesis can be implicated in the pathogenesis of some diabetic manifestations like diabetic retinopathy and nephropathy, and fetal problems (Martin et al., 2003). Hydroalcoholic extract of *M. officinalis* (100 mg/kg/day) has been reported to inhibit laser-induced choroidal neovascularization (CNV) development in a dose-dependent fashion in rats through inhibition of vascular endothelial growth factor (VEGF) and MMP-9 (Lee et al., 2014). Moreover, a polyherbal anti-angiogenic formulation containing *M. officinalis* has been shown to reduce mRNA levels of angiogenic factors VEGF-A, -B, -C, -D, fibroblast growth factor-2 (FGF-2), and MMPs (MMP-2 and MMP-9), whereas mRNA levels of angiogenic inhibitors in differentiated cells were increased. MMP-2 and MMP-9 activities were also decreased in treated cells (Hong et al., 2011). The plant also inhibits adipose tissue growth and reduces body weight gain in mice via inhibiting angiogenesis (Kim et al., 2010; Yoon and Kim, 2011). RA could inhibit several steps of angiogenesis including proliferation, migration, adhesion and tube formation in human umbilical vein endothelial cells (HUVEC) in a dose-dependent manner. RA also reduces intracellular ROS levels, VEGF expression and IL-8 release from endothelial cells (Huang et al., 2006). In addition, RA suppresses retinal neovascularization in a mouse model of retinopathy through cell cycle arrest, an effect that could be partly explained by the increased expression of the cell cycle regulatory protein $p21^{WAF1}$ (Kim et al., 2009).

5.15. Antiepileptic activity

Methanol and aqueous extracts of *M. officinalis* have been reported to exert potent antiepileptic activity in two animal models of epilepsy namely maximal electroshock seizure (MES) and pentylenetetrazole (PTZ) seizures (Bhat et al., 2012). Additionally, pretreatment with hydro-alcoholic extract of the plant positively affects prevention of the seizure symptoms induced by PTZ injection in rats (Gorgich et al., 2012). While applied doses in both of the above-mentioned *in vivo* studies could be extrapolated to human according to allometric scaling principles, confirmation of the efficacy and safety requires accurate clinical evaluation. Gallic acid has been reported to protect against kainic acid (KA)-induced seizure through reducing PGE2 production in KA-stressed PC12 cells (Huang et al., 2012). Although the above-mentioned findings are in harmony with the traditional use of *M. officinalis* in the treatment of epilepsy, the results need to be supported by experiments using lower doses of *M. officinalis* extracts. Details of *in vitro* and *in vivo* pharmacological effects of *M. officinalis* are presented in Table 3.

6. Pharmacokinetics

There are few pharmacokinetic studies on the extracts of *M. officinalis*. Previous studies mostly focused on hydroxycinnamic acid derivatives especially RA. RA has no affinity for intestinal monocarboxylic acid transporter (MCT, a family of plasma membrane transporters that carry molecules having one carboxylate group across biological membranes) in human intestinal epithelial Caco-2 cell monolayers. RA is absorbed via paracellular diffusion, as a result of the negative effect of ester group on interactions with MCT (Halestrap, 2013; Konishi and Kobayashi, 2005). Konishi and Kobayashi (2005) examined the absorption characteristics of RA by measuring its passage through Caco-2 cell monolayers using an HPLC-electrochemical detector (ECD) coupled to a coulometric detection system. The results suggested that the majority of RA is metabolized by gut microbiota into *m*-coumaric acid and hydroxylated phenylpropionic acids. These metabolites are then transported by MCT and distributed within the body (Konishi et al., 2005; Konishi and Kobayashi, 2005). RA is also metabolized to methyl-RA, caffeic acid and ferulic acid. RA and its metabolites are present in plasma and urine, predominantly as conjugated forms such as glucuronide or sulfate (Baba et al., 2004, 2005). Metabolism of RA may be altered by the presence of other factors such as dietary phenolics, food intake, disease states and drugs. In addition, it is evident that plant extracts with a high diversity of phenolic compounds may have higher bioavailability compared with isolated compounds. For instance, Fale et al. (2013) showed that co-administration of RA with flavonoids such as luteolin and apigenin (both being present in *M. officinalis*) can result in an increase in the bioavailability of RA in Caco-2 cell monolayers (Fale et al., 2013). Therefore, the flavonoid content of *M. officinalis* should be considered when studying absorption of the extract and especially RA.

In a randomized controlled trial in healthy individuals (in fasted state and fed state), serum levels of RA was measured using a coulometric detection method with HPLC coupled to an electrochemical detector, after oral administration of a single dose of *M. officinalis* extract containing 100, 250, or 500 mg RA. Serum concentration of total RA peaked after 1 h in fasted state, with maximum serum concentrations (C_{max}) of 72.22 nM and 162.20 nM for extracts containing 250 mg and 500 mg RA, respectively. The area

under the curve (AUC) for intact RA was 832.13 nmol h/L. In addition, a significant increase in the mean AUC_{Total} (1.3 times) and a delay in time to reach maximum serum concentration (T_{max}) were observed with food intake (Noguchi-Shinohara et al., 2015). Additionally, RA was absorbed percutaneously and distributed through the blood to skin, muscle and bone tissues after topical application on the rat skin (Ritschel et al., 1989). Data are still too limited for the assessment of brain bioavailability of RA and other hydroxycinnamic acids. The intact forms of *M. officinalis* triterpenes, oleanolic acid and ursolic acid, have been shown to be recovered in blood and tissues after dietary intake of these compounds in mice. Furthermore, prolongation of the intake period was found to be associated with accumulated levels of these triterpenes in brain, heart, liver, kidney, colon, and bladder tissues (Yin et al., 2012). A number of animal experiments have studied the metabolism and pharmacokinetic properties of citral, an oxygenated monoterpene of *M. officinalis* EO. Citral was found to be almost completely absorbed orally as a result of its extreme volatility (Diliberto et al., 1988). The primary route of metabolism for citral has been suggested to be conversion to the corresponding acid species possibly by the action of aldehyde dehydrogenases (ALDH) (Boyer and Petersen, 1991). Urine is the major route of elimination of citral, followed by feces and expired volatiles (Diliberto et al., 1988). Orally administered citral has been found to be rapidly metabolized and excreted (with approximately 50% of the oral dose excreted within 24 h) as metabolites, including several acids and a biliary glucuronide: 3-hydroxy-3,7-dimethyl-6-octenedioic acid; 3,8-dihydroxy-3,7-dimethyl-6-octenoic acid; 3,9-dihydroxy-3,7-dimethyl-6-octenoic acid; (*E*)- and (*Z*)-3,7-dimethyl-2,6-octadienedioic acid; 3,7-dimethyl-6-octenedioic acid; and (*E*)-3,7-dimethyl-2,6-octadienoic acid (Diliberto et al., 1990). After dermal exposure, much of the applied dose of EO is not absorbed possibly due to the high polarity. This can support the findings of a clinical trial conducted by Burns et al. (2011) in which no significant difference in the incidence of agitation was found between *M. officinalis* EO massage aromatherapy, placebo and donepezil groups. A relatively small part of citral was eliminated through urine but most of the compound was excreted in feces, suggesting a role for the first-pass metabolism through the skin (Diliberto et al., 1988). Above-mentioned observations imply the necessity of conducting additional studies to evaluate the bioavailability of different extracts of *M. officinalis*. In particular, due to numerous neurological effects of the plant, brain distribution and bioavailability of the extracts and their main bioactive ingredients (including RA, and triterpenes such as oleanolic acid and ursolic acid) should be taken into consideration. It seems that microbial metabolites of RA may partially account for its pharmacological activities, since the majority of RA is metabolized by gut micribiota. Interestingly, antiviral effects observed with topical application of *M. officinalis* have been shown to be mainly attributed to RA which has an acceptable percutaneous absorption.

7. Safety

7.1. In vitro studies

Astani et al. reported a maximum noncytotoxic concentration of 150 μg/mL for aqueous extract of *M. officinalis* using neutral red assay (Astani et al., 2012). The EO showed toxic effects on HEp-2 cells in concentrations above 100 mg/mL using trypan blue exclusion method (Allahverdiyev et al., 2004). It also exhibited neurotoxicity at a concentration of 0.1 mg/mL in a neuronal viability assay using a primary neuronal culture system (Mahita et al., 2014). No hepatotoxicity was observed when *M. officinalis* decoctions were incubated with the primary culture of porcine liver cells (PLP2) at concentrations up to 400 μg/mL (Carocho et al., 2015). Citral was found to be able to displace [(3)H]17beta-estradiol from isolated alpha- and beta-human estrogen receptors, but did not show estrogenic activity on the estrogen-responsive human cell line Ishikawa-Var I at levels below its cytotoxic concentration. Citral did not exhibit androgenic and anti-androgenic activity in yeast (Howes et al., 2002).

7.2. In vivo studies

Bounihi et al. reported no toxicity following oral administration of *M. officinalis* EO at doses of 300 and 2000 mg/kg in rats (Bounihi et al., 2013). Aqueous and methanolic extracts of the plant also did not exert any toxicity or behavioral change at a dose level of 2000 mg/kg in Swiss albino mice (Bhat et al., 2012). Similarly, no genotoxic or mutagenic effects were reported for aqueous (100 mg/kg) and alcoholic extracts (at doses of 250 or 500 mg/kg) of the plant in animal studies (de Carvalho et al., 2011). Several *in vivo* studies have pointed to a possible synergism between citral and androgens. Cutaneous application of citral on adolescent rats for 10 days induced initial benign prostatic hyperplasia lesions. Citral enhanced the acinar hyperplasia after 30 days. After 3 months of treatment, involvement of the stromal component was observed. Moreover, intermittent (every 4th day) application of citral induced atypical prostatic hyperplastic changes in the ventral prostate after 30 days (Engelstein et al., 1996). Nonetheless, citral could not stimulate typical estrogenic responses of uterine hypertrophy or increase uterine vascular permeability at levels below its cytotoxic concentration (Howes et al., 2002).

7.3. Human studies

Based on the available clinical trials, oral administration of *M. officinalis* has been reported to be relatively well-tolerated when taken for up to 8 weeks. Evidence regarding the topical administration of the plant also suggests minimal side effects by up to 10 days of application. A randomized controlled trial demonstrated that a dose of *M. officinalis* extract containing 500 mg RA per day is safe in humans (Noguchi-Shinohara et al., 2015). In another randomized double-blind placebo-controlled multicenter study among healthy volunteers, consumption of coated combination tablets (Songha Night®) containing 80 mg *M. officinalis* and 120 mg *Valeriana officinalis* extracts was reported to be safe and well-tolerated, and there was no case of serious adverse events or significant changes in laboratory tests, physical examination and rating of well-being (Cerny and Schmid, 1999). Moreover, findings of a prospective open-label 15-day study did not show any adverse effects after oral administration of *M. officinalis* (600 mg/ day) (Cases et al., 2011). The results of two other clinical studies showed no statistical difference in the frequency of adverse effects between treatment and placebo groups at any assessed time point (Wolbling and Leonhardt, 1994).

Contradictory to the above-mentioned findings, some other studies have reported adverse effects. One randomized controlled trial reported the occurrence of vomiting, dizziness, wheezing, agitation, abdominal pain and nausea after consumption of *M. officinalis* extract (60 drops/day). However, the difference in the frequency of side effects between the plant extract and placebo was not significant (Akhondzadeh et al., 2003). Other reported side effects included increased appetite (Alijaniha et al., 2015), headache, EEG changes (at the high dose of 1200 mg), reduced alertness (with a dose of 900 mg), increased intraocular pressure, palpitation and thyroid hormone inhibition (Ulbricht et al., 2005).

In topical application, local reddening, contact dermatitis, burning sensation, paresthesia, residual pigmentation and dermal irritation were observed (Ulbricht et al., 2005). Thus, care should

Table 3
Pharmacological activities reported from the leaves of *M. officinalis* in detail.

Activity	Dosage form/ type of extract	Effective concentrations/ dosages/route of administration	Method of extraction	Model	Tested living system/organ/cell	Result*	References
Anti-anxiety	Methanol, water, ethyl acetate extracts	0–4 mg/mL	Maceration	*In vitro*	Rat brain GABA-T	The best results have been observed for methanol extract. IC_{50}s were 0.55, 0.82 and 2.55 mg/mL for extracts respectively	Awad et al. (2009)
	Hydroalcoholic extract (Cyracos®)	240 mg/kg and 360 mg/kg chronic oral administration	Maceration with ethanol 70 °C	*In vivo*	C57BL/6 mice	Reduction in anxiety-like reactivity in the elevated plus maze dose-dependently	Ibarra et al. (2010)
	Ethanolicextract	300 mg/kg subacute oral (10-day course) administration	Maceration	*In vivo*	Wistar rats	Anti-anxiety effects comparable with benzodiazepines	Taiwo et al. (2012)
	Aqueous extract of *M. officinalis* and *Passiflora caerulea*	200 mg/kg oral administration	Infusion	*In vivo*	Male mice	Decrease in plasma levels of corticosterone	Feliú-Hemmelmann et al. (2013)
Antidepressant effect	Methanolic and aqueous extracts	3.75, 7.5, 15, 30 and 60 μg/mL	Maceration	*In vitro*	–	Mild inhibition of MAO-A with IC_{50}s of 19.3 and 48.2 (μg/mL), respectively	Lopez et al. (2009)
	Ethanolic extract	subacute doses:100 and 300 mg/kg for male rats 30, 100 and 300 mg/kg for female rats	Maceration	*In vivo*	Wistar rats	Decrease in Immobility time in FS test	Taiwo et al. (2012)
	Aqueous extract	25, 75, 150, 300 mg/kgsub-chronic administration	Maceration	*In vivo*	Mice	Reduction in immobility along with an increase in climbing behavior similar to imipramine in FS test	Emamghoreishi and Talebianpour (2009)
	EO	300 mg/kg	Maceration	*In vivo*	Mice	Increase in swimming behavior in FS test	Emamghoreishi and Talebianpour (2009)
Neuroprotective effects	Pretreatments of cells with methanolic extract	60 and 80 μg/mL	Maceration	*In vitro*	PC12 cells	Protection of PC12 cells against H_2O_2 toxicity by the MTT and LDH assays.	Lopez et al. (2009)
	Aqueous and methanolic extracts	20–120 μg/mL	Maceration	*In vitro*	PC12 cells	Reduction in the percentage of ROS formation, methanolic extract being more effective	Lopez et al. (2009)
	Ethanolic extract and its acidic fraction of	10 μg/mL of ethanolic extract 1 μg/mL of acidic fraction	Maceration	*In vitro*	PC12 cells	Protection against Aβ-induced oxidative changes and cell death	Sepand et al. (2013)
	Aqueous extract	10 μg/mL	Maceration	*In vitro*	Primary neurons of hippocampal culture	Decrease in apoptotic neuronal death by 20%	Hassanzadeh et al. (2011)
	EO	10 μg/mL	Not mentioned	*In vitro*	Human primary neurons	Protection against hypoxia in cultured neurons	Bayat et al. (2012)
	EO	100 mg/kg	Not mentioned	*In vivo*	Male Sprague–Dawley rats	Decrease in caspase3 activity and TUNEL-positive cells and Hippocampal neuronal damage	Bayat et al. (2012)
Effects on mood, cognition and memory	Ethanolic extract, EO, decoction	Ethanolic extract: 0.5 mg/mL EO: 1 mg/mL decoction: 5 mg/mL	Maceration, hydrodistillation, decoction	*In vitro*	–	AChE inhibition of the EO, ethanolic extract and decoction: 65.3%, 17.8%and 53.1%, respectively	Ferreira et al. (2006)
	Ethanolic extract	–	Medium pressure solid liquid extraction	*In vitro*	–	AChE inhibition Activity of 1.72 ± 0.16 μg equivalents of physostigmine/mg of the extract	Dastmalchi et al. (2009)
	Ethanolic extract	Intra-peritoneal injections, 200 mg/kg	Maceration	*In vivo*	Male albino Wistar rats	Enhancement of learning and memory in naïve rats and amelioration in scopolamine-induced learning deficit	Soodi et al. (2014)

Table 3 (*continued*)

Activity	Dosage form/ type of extract	Effective concentrations/ dosages/route of administration	Method of extraction	Model	Tested living system/organ/cell	Result*	References
Cardiovascular effects	Ethanolic extract	100 and 200 mg/kg	Maceration	*In vivo*	Male adult Sprague Dawley rats	Decrease in heart rates and percentages of incidence of VPB, VT, and VF	Akhondali et al. (2015)
	Aqueous extract	0.038, 0.38, 3.8 and 38 mg	Maceration in water 70 °C	*In vivo*	Male rats	Cardiac rate (CR) reduction	Gazola et al. (2004)
	Aqueous extract	400 mg	Infusion	*In vivo*	male Wistar rats	Mild protection against reperfusion-induced lethal ventricular arrhythmias	Joukar et al. (2014)
	Aqueous extract	1–1000 mg/mL	Decoction	*In vivo*	Male Wistar rats	Inducing concentration-dependent relaxation in phenylephrine precontracted thoracic aorta rings	Ersoy et al. (2008)
	Aqueous extract	One week oral administration of 50, 100 and 200 (mg/kg/day)	Infusion	*In vivo*	Male Wistar rats	Prolongation of QRS, QTc, TpTe and JT intervals in ECG	Joukar and Asadipour (2015)
Cytotoxic effects	EO, citral	4.6; 9.2; 18.4 or 36.8 μg/mL for EO 4.2; 8.4; 16.9; 28.1 or 33.7 μg/mL for citral	Hydrodistillation	*In vitro*	GBM cell lines (U87 and A172)	Reduction in the number of viable cells in cell lines in a dose-dependent manner	Queiroz et al. (2014)
	EO	Dilutions of $0.02–0.5 \times 10^3$	Hydrodistillation	*In vitro*	Human cancer cell lines (MCF-7,A549, Caco-2, K562, HL-60) and a mouse cell line (B16F10)	Inhibition in the viability of cells	de Sousa et al. (2004)
	Hydroethanolic extract	–	Maceration	*In vitro*	Colon carcinoma cells (HT-29 and T84)	Inhibition of the proliferation of HT-29 and T84 cells with IC_{50} of 346 and 120 μg/mL, respectively as well as apoptosis induction	Weidner et al. (2015)
	Decoction	8 mg/mL	Decoction	*In vitro*	Human tumor cell lines (mainly HepG2 and MCF-7)	Growth inhibition against HepG2 and MCF-7 with GI_{50} values of 67 and 51 μg/mL respectively	Carocho et al. (2015)
	Ethanolic extract	72 h of treatment with 5–1,000 μg/mL	Maceration	*in vitro*	Human Colon Cancer Cell Line (HCT-116)	Reducing cell proliferation to 40% in NR and MTT assays	Encalada et al. (2011)
	Dichloromethane fraction of methanol extract	50 mg/mL	Maceration	*In vitro*	Leukemia cell lines (Jurkat and K562)	Induction of apoptosis in Jurkat and K562 by 85.66 and 65.04 respectively	Ebrahimnezhad Darzi and Amirghofran (2013)
	Hydro-alcoholic extract	Up to 100 μg/mL	Soxhlet	*In vitro*	Human cancer cell lines (A549, MCF-7, Caco-2, HL-60, K562)	Rosmarinic acid, caffeic acid and luteolin had potent cytotoxic activity (IC_{50} values of; 34.6, 41.1 and 62.4 μg/mL, respectively)	Tantry et al. (2014)
	Ethanolic extract	Intraperitoneal injection of 500 mg/kg for 2 weeks	Maceration	*In vivo*	CF-1 male mice	Antigenotoxic/antimutagenic properties	de Carvalho et al. (2011)
Anti-inflammatory and antinociceptive	Aqueous extract	50, 100, 200 and 400 mg/kg administered by gavage	Infusion	*In vivo*	Rats	Decrease in inflammagen-induced paw edema	Birdane et al. (2007)

effects							
	Aqueous extract	50, 100, 200 and 400 mg/kg administered by gavage	Infusion	*In vivo*	Mice	Decrease in the nociceptive response	Birdane et al. (2007)
	Ethanolic extract	Oral administration of 3–1000 mg/kg	Maceration	*In vivo*	Mice	Dose-related antinociceptive effects with ID_{50} of 241.9 mg/kg, inhibition the early and the late phases of formalin-induced licking	Guginski et al. (2009)
	Ethanolic extract	Oral administration of 10–1000 mg/kg	Maceration	*In vivo*	Mice	Dose-dependent inhibition of glutamate-induced pain(ID_{50} = 198.5 mg/kg)	Guginski et al. (2009)
	EO	4 weeks orally administration of 0.04 mg/day	Hydrodistillation	*In vivo*	Male Wistar rats	Inhibition of both of formalin-induced nociception in diabetic rats	Hasanein and Riahi (2015)
	EO	Oral administration of 200 and 400 mg/kg	Hydrodistillation	*In vivo*	Male Wistar rats	Reduction and inhibition of edema induced by carrageenan (61.76% and 70.58%) and edema induced by experimental trauma (91.66% and 94.44%)	Bounihi et al. (2013)
Hypoglycemic effects	Ethanolic extract	6 weeks of oral administration of 200 mg/kg/day	Maceration	*In vivo*	C57BL/6 mice	Reduction in fasting blood glucose (14%) and insulin resistance (35%)	Weidner et al. (2014)
	EO	4 weeks of orally administration of 0.02 and 0.04 mg/day	Hydrodistillation	*In vivo*	Male Wistar rats	Decrease in levels of plasma glucose and body weight	Hasanein and Riahi (2015)
Hypolipidemic effects	Ethanolic extract	21-day orally administration of 25, 50 and 75 mg/kg	Maceration	*In vivo*	Male Wistar rats	Decrease in liver enzymes levels in serum in hypercholesterolemic rats	Zarei et al. (2014)
	Ethanolic extract	6 weeks of oral administration of 200 mg/kg/day	Maceration	*In vivo*	C57BL/6 mice	Reduction in plasma triacylglycerol, LDL/VLDL cholesterol and nonesterified fatty acids in insulin-resistant obese mice	Weidner et al. (2014)
	Aqueous extract	Oral use of 2 g/kg every day for 28	Decoction	*In vivo*	Swiss albino rats	Reduction in total lipid, cholesterol, and liver enzyme levels in serum, and LPO levels in liver in hyperlipidemic rats increase in GSH levels	Bolkent et al. (2005)
	EO	2 weeks of oral administration of 12.5 mg/day	Hydrodistillation	*In vivo*	Human APOE2 transgenic mice	Reduction in plasma triglyceride (TG) concentrations in mice.	Jun et al. (2012)
Antimicrobial effects	EO	50% and 20% solutions in n-hexane	Hydrodistillation	*In vitro*	*P. aeruginosa, E. coli, S. enteritidis, S. typhi,* and *Shigella*	Antibacterial activity	Mimica-Dukic et al. (2004)
	Petroleum ether and ethyl acetate extracts	10 mg/mL	Maceration	*In vitro*	*Sarcina lutea*	Potent antibacterial activities	Canadanovic-Brunet et al. (2008)
	Decoction	–	Decoction	*In vitro*	*P. aeruginosa* and *S. typhi murium*, and *P. funiculosum*	Antibacterial activities	Carocho et al. (2015)
Antiviral activity	Hydroalcoholic extract	0.025–1 mg/mL	Not mentioned	*In vitro*	Vero cells	Anti-HSV-2 activity with the maximum inhibiting effect (60%) of 0.5 mg/mL	Mazzanti et al. (2008)
	EO	Pretreatment with dilutions of 0.002% and lower concentrations	Not mentioned	*In vitro*	Monkey kidney cells	Inhibition of plaque formation of both HSV-1 and HSV-2 in a dose-dependent manner	Schnitzler et al. (2008)
	EO	25, 50, 100, 150 and 200 μg/mL	Vapor distillation	*In vitro*	HEp-2 cell line	Inhibition of replication of HSV-2	Allahverdiyev et al. (2004)

Table 3 (*continued*)

Activity	Dosage form/ type of extract	Effective concentrations/ dosages/route of administration	Method of extraction	Model	Tested living system/organ/cell	Result*	References
Antispasmodic	EO, citral	2.5–75 ng/mL		*In vivo*	Male Wistar rats	Inhibition of the response to KCl, ACh and 5-HT in a concentration-dependent manner	Sadraei et al. (2003)
Antiepileptic activity	Methanolic and aqueous extract	250 and 500 mg/kg	Soxhlet	*In vivo*	Swiss Albino mice	Inhibition of Convulsion of two doses for MES and PTZ models: methanol extract: 66.75%/80.56% and 12.22%/25.53%. Aqueous extract:46.59%/64.61% and 29.59%/62.24%	Bhat et al. (2012)
	Hydro-alcoholic extract	50 and 100 mg/kg	Soxhlet	*In vivo*	Male Wistar rats	Decrease in mortality Rate by 37.5% and 12.5% respectively compared to 12.5% in the group treated by Phenytoin.	Gorgich et al. (2012)

be exercised with respect to prolonged and high-dose intake of *M. officinalis*-containing products until in-depth evidence from chronic toxicity and dose-escalation studies is provided.

8. Future perspectives and conclusions

M. officinalis is a medicinal plant that has been long used in various ethno-medical systems. The present review summarizes the botany, traditional uses, phytochemistry, pharmacology and toxicity of *M. officinalis.* Previous *in vivo* investigations and *in vitro* studies have revealed that *M. officinalis* possesses many biological activities such as anxiolytic, neuroprotective, antiinflammatory, antinociceptive, cardiovascular, antimicrobial, antioxidant and cytotoxic properties. While such activities can generate hypothesis for potential therapeutic effects of *M. officinalis*, proof-of-concept trials are warranted to verify such effects in clinical practice. Hitherto, evidence from clinical trials has been provided for the anxiolytic, antiviral and antispasmodic activities of this plant as well as its effects on mood, cognition and memory. The current clinical evidence suggests that the daily oral dose of 600 mg of *M. officinalis* extract is possibly safe and effective in the treatment of anxiety, mood and cognition problems. Moreover, topical administration of formulations containing 1% extract of *M. officinalis* would be effective in the treatment of very early stages of HSV infections. These activities have been shown to be mainly attributed to RA, the major constituent of *M. officinalis* extracts and citral, the main component of its EO. Nevertheless, mechanisms of actions, clinical effectiveness and proper dosage for the other ethno-medical uses and pharmacological activities of this plant would need to be studied. Furthermore, in spite of the large body of scientific evidence regarding the medicinal properties of *M. officinalis,* there are still several gaps in our understanding of the applications of this plant. The first gap, as referred in the above sections, is that some of the pharmacological effects of *M. officinalis* in *in vitro* and *in vivo* studies have been obtained with doses of the plant extract that may be high for clinical practice. For instance, doses of *M. officinalis* extract applied to evaluate hypolipidemic (e.g. administration of 2 g/kg of extract in mice), antidepressant (e.g. administration of 300 mg/kg EO in mice), *anti*-genotoxic and antimutagenic (e.g. administration of 500 mg/kg of extract in mice) activities of *M. officinalis* are too high for replication in clinical studies. It is also important to mention that a common mistake in translating animal doses to humans is the use of isometric scaling, which involves a simple conversion of animal dose to humans by body weight (a milligram per kilogram basis). This can lead to underestimation of the toxicity or overestimation of the human dose. Allometric scaling, which involves body surface area instead of body weight, is more appropriate due to the fact that larger animals normally have a slower metabolic rate and therefore require a smaller drug dose (Wojcikowski and Gobe, 2014). Accordingly, although administration of plant extracts in the order of 4–5 g/day is not uncommon in humans, any medical application with such doses should be preceded by careful safety studies.

Secondly, despite extensive traditional uses of *M. officinalis* as an exhilarating and anti-depressant medicine, only few studies using limited *in vivo* models have been conducted to evaluate these effects. Therefore, exploring various neurobiological mechanisms underlying anti-depressant activities of this plant using additional standard animal models of depression would be necessary. Moreover, there is a need for proof-of-concept clinical trials to validate the efficacy of *M. officinalis* as an adjunct to standard of care treatments for depression. Likewise, many other traditional uses of the plant have not received enough attention. A number of studies have revealed cytotoxic activities of *M. officinalis*

against several human cancerous cell lines. The plant also exhibits *anti*genotoxic and antimutagenic effects *in vivo*, although the tested doses were far too high to be replicated in clinical practice. Noteworthy, before any anti-tumor or anti-cancer effect could be attributed to *M. officinalis*, sufficient pre-clinical and clinical evidence should be obtained on the capacity of this plant, or its ingredients, to reduce tumor size, increase survival, or improve the outcomes of cancer. Currently, such evidence is scant and most of the data have been reported from cell culture studies, mostly employing monolayer cultures, instead of 3-dimensional or spheroid models, of tumor cells. Moreover, some of the previous studies on the cytotoxic effects of *M. officinalis* were limited in providing information on selective toxicity of this plant and its impact on the viability of non-cancerous cells. Until such data are provided, any interpretation on the utility of *M. officinalis* as a cytotoxic agent should be made with extreme caution.

Thirdly, the traditional use of *M. officinalis* is mostly combined with other medicinal plants. For instance, as mentioned before, there are around 400 compound prescriptions containing *M. officinalis* in ITM. Therefore, possible interactions and synergistic effects of this plant with other ingredients of poly-herbal preparations should be investigated. In this context, although the safety and well tolerability of the plant have been supported by experimental and clinical studies, potential interactions with components of poly-herbal preparations as well as antidepressant, anti-anxiety and mood-stabilizing drugs remain unknown.

Fourthly, hydroxycinnamic acids, in particular RA, are potent compounds that may account for several therapeutic activities of *M. officinalis* leaves, and mainly occur in hydrophilic extracts. EO of the leaves and its main component citral also contribute to the plant's pharmacological effects, particularly antibacterial and antiviral properties. However, there is little, if any, information on the phytochemical and pharmacological characteristics of *M. officinalis* seeds. Moreover, there is an infraspecific taxon of the species (*Melissa officinalis* subsp. *inodora* Bornm.) which may differ in its phytochemical compounds and pharmacological properties, thus necessitating comparative studies on this infraspecific taxon.

Fifthly, several studies addressed absorption, distribution and metabolism of a few active ingredients present in *M. officinalis.* However, data on the pharmacokinetic aspects of the whole extracts of the plant are scarce and the penetration capacity of plant's ingredients into the central nervous system is largely unknown.

Finally, although the majority of clinical studies reported no or minor adverse events following administration of *M. officinalis,* there remain concerns about some serious but not statistically significant observed side effects e. g. increased intraocular pressure and thyroid hormone inhibition which necessitate further investigations and caution in the administration of *M. officinalis* containing preparations to subjects with ocular and thyroid disorders.

Overall, although promising clinical evidence for the efficacy of *M. officinalis* in the treatment of anxiety, HSV infections, and mood and cognition problems exists, data regarding the other ethnomedical uses of this plant is too preliminary and mostly fails to explain the exact cellular and molecular mechanisms of action and the respective active compounds. Therefore, future studies should be focused on investigating mechanisms of actions and pharmacokinetics of the extracts and active compounds of *M. officinalis* based on a variety of appropriate animal models and realistic dosages. Further research should also consider clinical efficacy and safety of the plant extracts and active compounds in the treatment of depression, cancer, bacterial infections, epilepsy and other reported traditional uses of *M. officinalis.* This review provided information for the development and utilization of ethno-medical knowledge in order to develop efficient and safe *M. officinalis*-based pharmaceuticals.

References

Soltani, A., 2012. Encyclopedia of Traditional Medicine: Medicinal Plants. Andisheavar, Tehran.

Abdellatif, F., Hassani, A., 2015. Chemical composition of the essential oils from leaves of Melissa officinalis extracted by hydrodistillation, steam distillation, organic solvent and microwave hydrodistillation. J. Mater. Environ. Sci. 6 (1), 207–213.

Abdelwahed, A., Bouhlel, I., Skandrani, I., et al., 2007. Study of antimutagenic and antioxidant activities of gallic acid and 1,2,3,4,6-pentagalloylglucose from *Pistacia lentiscus*. Confirmation by microarray expression profiling. Chem. Biol. Interact. 165, 1–13.

Abuhamdah, S., Huang, L., Elliott, M.S., et al., 2008. Pharmacological profile of an essential oil derived from *Melissa officinalis* with anti-agitation properties: focus on ligand-gated channels PubMed PMID: 18284819. J. Pharm. Pharmacol. 60 (3), 377–384.

Abu-Shanab, B., Adwan, G., Jarrar, N., et al., 2006. Antibacterial activity of four plant extracts used in Palestine in folkloric medicine against methicillin-resistant *Staphylococcus aureus*. Turk. J. Biol. 30, 195–198.

Adsersen, A., Gauguin, B., Gudiksen, L., et al., 2006. Screening of plants used in Danish folk medicine to treat memory dysfunction for acetylcholinesterase inhibitory activity. J. Ethnopharmacol. 104, 418–422.

Afifi, F.U., Abu-Irmaileh, B., 2000. Herbal medicine in Jordan with special emphasis on less commonly used medicinal herbs. J. Ethnopharmacol. 72 (1–2), 101–110.

Agata, I., Kusakabe, H., Hatano, T., Nishibe, S., Okudam, T., 1993. Melitric Acids A and B, New Trimeric Caffeic Acid Derivatives from *Melissa officinalis*. Chem. Chem. Pharm. Bull. 41 (9), 1608–1611.

Ahwazi Arjani., A.A., 1877. Kamel al-Sina'ah al-Tibbiyah (The Perfect Art of the Medicine). Saadat Press. vol 3, p. 408.

Akhondali, Z., Dianat, M., Radan, M., 2015. Negative Chronotropic and Anti-dysrhythmic Effects of Hydroalcoholic Extract of Lemon Balm (*Melissa officinalis* L.) on CaCl2-Induced Arrhythmias in Rats. Electron. physician 7, 971–976.

Akhondzadeh, S., Noroozian, M., Mohammadi, M., et al., 2003. *Melissa officinalis* extract in the treatment of patients with mild to moderate Alzheimer's disease: a double blind, randomised, placebo controlled trial. J. Neurol. Neurosurg. Psychiatry 74, 863–866.

Albala, K., 2011. Food Cultures of the World Encyclopedia. ABC-CLIO, California.

Al-douri, N.A., 2000. A survey of medicinal plants and their traditional uses in Iraq. Pharm. Biol. 38 (1), 74–79.

al-Ghassani, 1990. Hadiqat Al-Azhar fi Mahiyat Al-'Ushb wa Al-'Uqar (Garden of Flowers in the Explanation of the Character of Herbs and Drugs). Dar Al-Gharb Al-Islami, Beirut.

Al-Husein, B., Abdalla, M., Trepte, M., et al., 2012. Antiangiogenic therapy for cancer: an update. Pharmacotherapy 32, 1095–1111.

Alijaniha, F., Naseri, M., Afsharypuor, S., et al., 2015. Heart palpitation relief with *Melissa officinalis* leaf extract: double blind, randomized, placebo controlled trial of efficacy and safety. J. Ethnopharmacol. 164, 378–384.

Al-Khalil, S., 1995. A survey of plants used in Jordanian traditional medicine. Pharm. Biol. 33 (4), 317–323.

Allahverdiyev, A., Duran, N., Ozguven, M., et al., 2004. Antiviral activity of the volatile oils of *Melissa officinalis* L. against Herpes simplex virus type-2. Phytomedicine 11, 657–661.

Amor, I.L.-B., Boubaker, J., Sgaier, M.B., et al., 2009. Phytochemistry and biological activities of *Phlomis* species. J. Ethnopharmacol. 125, 183–202.

Aqili Khorasani, M.H., 1992. Makhzan al-Adwiah (Drug Treasure). (Reprinted from a copy which was printed in Calcutta dated in) 1844. Enqelab-E Eslami Publishing And Educational Organization, Tehran.

Argyropoulos, D., Müller, J., 2014. Changes of essential oil content and composition during convective drying of lemon balm (*Melissa officinalis* L.). Ind. Crops Prod. 52, 118–124.

Ashori, A., Hamzeh, Y., Amani, F., 2011. Lemon balm (*Melissa officinalis*) Stalk: chemical composition and fiber morphology. J. Polym. Environ. 19, 297–300.

Astani, A., Reichling, J., Schnitzler, P., 2012. *Melissa officinalis* extract inhibits attachment of herpes simplex virus in vitro. Chemotherapy 58 (1), 70–77.

Astani, A., Navid, M.H., Schnitzler, P., 2014. Attachment and penetration of acyclovir-resistant herpes simplex virus are inhibited by *Melissa officinalis* extract. Phytother. Res. 28, 1547–1552.

Awad, R., Muhammad, A., Durst, T., et al., 2009. Bioassay-guided fractionation of lemon balm (*Melissa officinalis* L.) using an *in vitro* measure of GABA transaminase activity. Phytother. Res. 23, 1075–1081.

Baba, S., Osakabe, N., Natsume, M., et al., 2004. Orally administered rosmarinic acid is present as the conjugated and/or methylated forms in plasma, and is degraded and metabolized to conjugated forms of caffeic acid, ferulic acid and *m*-coumaric acid. Life Sci. 75 (2), 165–178.

Baba, S., Osakabe, N., Natsume, M., et al., 2005. Absorption, metabolism, degradation and urinary excretion of rosmarinic acid after intake of Perilla frutescens extract in humans. Eur. J. Clin. Nutr. 44 (1), 1–9.

Ballard, C.G., O'Brien, J.T., Reichelt, K., et al., 2002. Aromatherapy as a safe and effective treatment for the management of agitation in severe dementia: the results of a double-blind, placebo-controlled trial with *Melissa*. J. Clin. Psychiatry 63, 553–558.

Barros, L., Dueñas, M., Dias, M.I., et al., 2013. Phenolic profiles of cultivated, *in vitro* cultured and commercial samples of *Melissa officinalis* L. infusions. Food Chem. 136, 1–8.

Bayat, M., Azami Tameh, A., Hossein Ghahremani, M., et al., 2012. Neuroprotective

properties of *Melissa officinalis* after hypoxic-ischemic injury both *in vitro* and *in vivo*. Daru. J. Pharm. Sci. 20, 1–10.
Bhat, J.U., Nizami, Q., Aslam, M., et al., 2012. Antiepileptic activity of the whole plant extract of *Melissa officinalis* in swiss albino mice. Int. J. Pharm. Sci. Res. 3, 886–889.
Birdane, Y., Buyukokuroglu, M., Birdane, F., et al., 2007. Anti-inflammatory and antinociceptive effects of *Melissa officinalis* L. in rodents. Rev. Med. Vet. 158, 75–81.
Blumenthal, M., Goldberg, A., Brinckmann, J., 2000. Herbal Medicine: Expanded Commission E Monographs.: Integrative Medicine Communications. Newton, MA, pp. 230–232.
Bolkent, S., Yanardag, R., Karabulut-Bulan, O., et al., 2005. Protective role of *Melissa officinalis* L. extract on liver of hyperlipidemic rats: a morphological and biochemical study. J. Ethnopharmacol. 99, 391–398.
Bonet, M.A., Parada, M., Selga, A., et al., 1999. Studies on pharmaceutical ethnobotany in the regions of L'Alt Emporda and Les Guilleries (Catalonia, Iberian Peninsula). J. Ethnopharmacol. 68 (1–3), 145–168.
Bounihi, A., Hajjaj, G., Alnamer, R., et al., 2013. *In* vivo potential anti-inflammatory activity of *Melissa officinalis* L. essential oil. Adv. Pharmacol. Sci. 2013, 1–7.
19 Boyer, C.S., Petersen, D.R., 1991. The metabolism of 3,7-dimethyl-2,6-octadienal (citral) in rat hepatic mitochondrial and cytosolic fractions. Interactions with aldehyde and alcohol dehydrogenases. Drug. Metab. Dispos. 19 (1), 81–86, PubMed PMID: 1673427.
Braidy, N., Matin, A., Rossi, F., et al., 2014. Neuroprotective effects of rosmarinic acid on ciguatoxin in primary human neurons. Neurotox. Res. 25, 226–234.
British Herbal Pharmacopoeia. London, British Herbal Medicine Association, 1996.
Burns, A., Perry, E., Holmes, C., et al., 2011. A double-blind placebo-controlled randomized trial of *Melissa officinalis* oil and donepezil for the treatment of agitation in Alzheimer's disease. Dement. Geriatr. Cogn. Disord. 31, 158–164.
Canadanovic-Brunet, J., Cetkovic, G., Djilas, S., et al., 2008. Radical scavenging, antibacterial, and antiproliferative activities of *Melissa officinalis* L. extracts. J. Med. Food 11, 133–143.
Caniova, A., Brandsteterova, E., 2001. HPLC analysis of phenolic acids in *Melissa officinalis*. J. Liq. Chrom. Rel. Technol. 24, 2647–2659.
Cao, H., Chen, X., Jassbi, A.R., et al., 2015. Microbial biotransformation of bioactive flavonoids. Biotechnol. Adv. 33, 214–223.
Carmeliet, P., Jain, R.K., 2000. Angiogenesis in cancer and other diseases. Nature 407, 249–257.
Carnat, A., Carnat, A., Fraisse, D., et al., 1998. The aromatic and polyphenolic composition of lemon balm (*Melissa officinalis* L. subsp. *officinalis*) tea. Pharm. Acta Helv. 72, 301–305.
Carocho, M., Barros, L., Calhelha, R.C., et al., 2015. *Melissa officinalis* L. decoctions as functional beverages: a bioactive approach and chemical characterization. Food funct. 6, 2240–2248.
de Carvalho, N.C., Correa-Angeloni, M.J., Leffa, D.D., et al., 2011. Evaluation of the genotoxic and *anti*genotoxic potential of *Melissa officinalis* in mice. Genet. Mol. Biol. 34, 290–297.
Cases, J., Ibarra, A., Feuillere, N., et al., 2011. Pilot trial of *Melissa officinalis* L. leaf extract in the treatment of volunteers suffering from mild-to-moderate anxiety disorders and sleep disturbances. Med. J. Nutr. Metab. 4, 211–218.
Castellano, J.M., Guinda, A., Delgado, T., et al., 2013. Biochemical basis of the antidiabetic activity of oleanolic acid and related pentacyclic triterpenes. Diabetes 62, 1791–1799.
Cerny, A., Schmid, K., 1999. Tolerability and efficacy of valerian/lemon balm in healthy volunteers (a double-blind, placebo-controlled, multicentre study). Fitoterapia 70, 221–228.
Chashti, M.A.K., 1884. Exir-e-Azam, 2 ed. Nami Monshi Nolkshur, Delhi, vol 1, p. 48.
Cogan, T., 1636. The Haven of Health. Printed By Anne Griffin, Londen, p. 39.
Cummings, J.L., 2000. Cholinesterase inhibitors: a new class of psychotropic compounds. Am. J. Psychiatry 157, 4–15.
Cunningham, M.L., Fairlamb, A.H., 1995. Trypanothione reductase from Leishmania donovani. purification, characterisation and inhibition by trivalent antimonials. Eur. J. Biochem. 230, 460–468.
Dai, J., Mumper, R.J., 2010. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules 15, 7313–7352.
Dastmalchi, K., Dorman, H.D., Oinonen, P.P., et al., 2008. Chemical composition and *in vitro* antioxidative activity of a lemon balm (*Melissa officinalis* L.) extract. LWT – Food Sci. Technol. 41, 391–400.
Dastmalchi, K., Ollilainen, V., Lackman, P., et al., 2009. Acetylcholinesterase inhibitory guided fractionation of *Melissa officinalis* L. Bioorg. Med. Chem. 17, 867–871.
Dawson, B.S., Franich, R.A., Meder, R., 1988. Essential oil of *Melissa officinalis* L. subsp. altissima (Sibthr. et Smith) Arcang. Flavour Fragr. J. 3, 167–170.
De Oliveira, N.C., Sarmento, M.S., Nunes, E.A., et al., 2012. Rosmarinic acid as a protective agent against genotoxicity of ethanol in mice. Food Chem. Toxicol. 50, 1208–1214.
Di Stasi, L.C., Oliveira, G.P., Carvalhaes, M.A., et al., 2002. Medicinal plants popularly used in the Brazilian Tropical Atlantic Forest. Fitoterapia 73 (1), 69–91.
Diliberto, J.J., Usha, G., Birnbaum, L.S., 1988. Disposition of citral in male Fischer rats. Drug. Metab. Dispos. 16 (5), 721–727, PubMed PMID: 2906597.
Diliberto, J.J., Srinivas, P., Overstreet, D., et al., 1990. Metabolism of citral, an alpha, beta-unsaturated aldehyde, in male F344 rats. Drug. Metab. Dispos. 18 (6), 866–875, PubMed PMID: 1981530.
Ebrahimnezhad Darzi, S., Amirghofran, Z., 2013. Dichloromethane fraction of *Melissa officinalis* induces apoptosis by activation of intrinsic and extrinsic pathways in human leukemia cell lines. Immunopharmacol. Immunotoxicol. 35, 313–320.
Egamberdieva, D., Mamadalieva, N., Khodjimatov, O., et al., 2013. Medicinal plants from Chatkal Biosphere Reserve used for folk medicine in Uzbekistan. Med. Aromat. Plant Sci. Biotechnol. 7 (1), 56–64.
Ellis, J.M., 2005. Cholinesterase inhibitors in the treatment of dementia. J. Am. Osteopath. Assoc. 105, 145–158.
Elnakish, M.T., Hassanain, H.H., Janssen, P.M., et al., 2013. Emerging role of oxidative stress in metabolic syndrome and cardiovascular diseases: important role of Rac/NADPH oxidase. J. Pathol. 231, 290–300.
Emamghoreishi, M., Talebianpour, M., 2009. Antidepressant effect of *Melissa officinalis* in the forced swimming test. Daru. J. Pharm. Sci. 17, 42–47.
Encalada, M.A., Hoyos, K.M., Rehecho, S., et al., 2011. Anti-proliferative effect of *Melissa officinalis* on human colon cancer cell line. Plant Food Hum. Nutr. 66, 328–334.
Engelstein, D., Shmueli, J., Bruhis, S., et al., 1996. Citral and testosterone interactions in inducing benign and atypical prostatic hyperplasia in rats. Comp. Biochem. Physiol. C. Pharmacol. Toxicol. Endocrinol. 115 (2), 169–177.
Erkan, N., Ayranci, G., Ayranci, E., 2008. Antioxidant activities of rosemary (*Rosmarinus officinalis*L.) extract, blackseed (*Nigella sativa* L.) essential oil, carnosic acid, rosmarinic acid and sesamol. Food Chem. 110, 76–82.
Ersoy, S., Orhan, I., Turan, N.N., et al., 2008. Endothelium-dependent induction of vasorelaxation by *Melissa officinalis* L. ssp. *officinalis* in rat isolated thoracic aorta. Phytomedicine 15, 1087–1092.
Estomba, D., Ladio, A., Lozada, M., 2006. Medicinal wild plant knowledge and gathering patterns in a Mapuche community from North-western Patagonia. J. Ethnopharmacol. 103 (1), 109–119.
European Pharmacopoeia, 1999. 3rd ed., Suppl. 2000. Strasbourg, Council of Europe.
Falé, P.L., Ascensão, L., Serralheiro, M.L., 2013. Effect of luteolin and apigenin on rosmarinic acid bioavailability in Caco-2 cell monolayers. Food Func. 4 (3), 426–431.
Feliú-Hemmelmann, K., Monsalve, F., Rivera, C., 2013. *Melissa officinalis* and Passiflora caerulea infusion as physiological stress decreaser. Int. J. Clin. Exp. Med. 6, 444.
Fernandes, R., 1973. Melissa officinalis. In: Tutin, T.G., Heywood, V.H., Burges, N.A., Valentine, D.H., Walters, S.M., Webb, D.A. (Eds.), Flora Europaea. University Press, Cambridge, p. 162.
Ferreira, A., Proenca, C., Serralheiro, M.L., et al., 2006. The *in vitro* screening for acetylcholinesterase inhibition and antioxidant activity of medicinal plants from Portugal. J. Ethnopharmacol. 108, 31–37.
Fotakis, G., Timbrell, J.A., 2006. *In vitro* cytotoxicity assays: comparison of LDH, neutral red, MTT and protein assay in hepatoma cell lines following exposure to cadmium chloride. Toxicol. Lett. 160, 171–177.
Furtado, M.A., de Almeida, L.C., Furtado, R.A., et al., 2008. Antimutagenicity of rosmarinic acid in Swiss mice evaluated by the micronucleus assay. Mutat. Res. 657, 150–154.
Furtado, R.A., de Araujo, F.R., Resende, F.A., et al., 2010. Protective effect of rosmarinic acid on V79 cells evaluated by the micronucleus and comet assays. J. Appl. Toxicol. 30 (3), 254–259.
Gülçin, İ., 2006. Antioxidant activity of caffeic acid (3, 4-dihydroxycinnamic acid). Toxicology 217, 213–220.
Gazola, R., Machado, D., Ruggiero, C., et al., 2004. *Lippia alba*, *Melissa officinalis* and *Cymbopogon citratus*: effects of the aqueous extracts on the isolated hearts of rats. Pharmacol. Res. 50, 477–480.
Geuenich, S., Goffinet, C., Venzke, S., et al., 2008. Aqueous extracts from peppermint, sage and lemon balm leaves display potent anti-HIV-1 activity by increasing the virion density. Retrovirology 5, 27.
Gorgich, E., Komeili, G., Zakeri, Z., et al., 2012. Comparing anticonvulsive effect of *Melissa officinalis* hydro-alcoholic extract and phenytoin in rat. Health Scope 2012, 44–48.
Grieve, M., 1971. A Modern Herbal: the Medicinal, Culinary, Cosmetic and Economic Properties, Cultivation and Folk-lore of Herbs, Grasses, Fungi, Shrubs, & Trees With all their Modern Scientific Uses. Courier Corporation, p. 76.
Guginski, G., Luiz, A.P., Silva, M.D., et al., 2009. Mechanisms involved in the antinociception caused by ethanolic extract obtained from the leaves of *Melissa officinalis* (lemon balm) in mice. Pharmacol. Biochem. Behav. 93, 10–16.
Halestrap, A.P., 2013. Monocarboxylic acid transport. Compr. Physiol. 3 (4), 1611–1643, PubMed PMID: 24265240.
Hammond, G.B., Fernandez, I.D., Villegas, L.F., et al., 1998. A survey of traditional medicinal plants from the Callejon de Huaylas, Department of Ancash, Peru. J. Ethnopharmacol. 61 (1), 17–30.
Han, H., Yi, Y., Li, L., et al., 2009. Antifungal active triterpene glycosides from sea cucumber *Holothuria scabra*. Yao Xue Xue Bao 44, 620–624.
Hanlidou, E., Karousou, R., Kleftoyanni, V., et al., 2004. The herbal market of Thessaloniki (N Greece) and its relation to the ethnobotanical tradition. J. Ethnopharmacol. 91 (2–3), 281–299.
Hasanein, P., Riahi, H., 2015. Antinociceptive and antihyperglycemic effects of *Melissa officinalis* essential oil in an experimental model of diabetes. Med. Princ. Pract. 24, 47–52.
Hassanzadeh, G., Pasbakhsh, P., Akbari, M., et al., 2011. Neuroprotective properties of *Melissa officinalis* L. Extract against ecstasy-induced neurotoxicity. Cell J. 13, 25–30.
Heitz, A., Carnat, A., Fraisse, D., et al., 2000. Luteolin 3′-glucuronide, the major flavonoid from *Melissa officinalis* subsp. *officinalis*. Fitoterapia 71, 201–202.
Heleno, S.A., Martins, A., Queiroz, M.J.R., et al., 2015. Bioactivity of phenolic acids: metabolites *versus* parent compounds: a review. Food chem. 173, 501–513.
Hong, Y., Kim, M.Y., Yoon, M., 2011. The anti-angiogenic herbal extracts Ob-X from

Morus alba, *Melissa officinalis*, and *Artemisia capillaris* suppresses adipogenesis in 3T3-L1 adipocytes. Pharm. Biol. 49, 775–783.
Howes, M.J., Houghton, P.J., Barlow, D.J., et al., 2002. Assessment of estrogenic activity in some common essential oil constituents. J. Pharm. Pharmacol. 54 (11), 1521–1528.
Huang, H.L., Lin, C.C., Jeng, K.C., et al., 2012. Fresh green tea and gallic acid ameliorate oxidative stress in kainic acid-induced status epilepticus. J. Agric. Food Chem. 60, 2328–2336.
Huang, L., Abuhamdah, S., Howes, M.J., et al., 2008. Pharmacological profile of essential oils derived from *Lavandula angustifolia* and *Melissa officinalis* with anti-agitation properties: focus on ligand-gated channels. J. Pharm. Pharmacol. 60 (11), 1515–1522, PubMed PMID: 18957173.
Huang, S.S., Zheng, R.L., 2006. Rosmarinic acid inhibits angiogenesis and its mechanism of action *in vitro*. Cancer Lett. 239, 271–280.
Hussain, A.I., Anwar, F., Nigam, P.S., et al., 2011. Antibacterial activity of some Lamiaceae essential oils using resazurin as an indicator of cell growth. LWT – Food Sci. Technol. 44, 1199–1206.
Ibarra, A., Feuillere, N., Roller, M., et al., 2010. Effects of chronic administration of *Melissa officinalis* L. extract on anxiety-like reactivity and on circadian and exploratory activities in mice. Phytomedicine 17, 397–403.
Ibn Beytar, Z., 2001. Al-Jamee Le-Mofradaat al- Adwiah wal- Aghziyah (Comprehensive Book in Simple Drugs and Foods). Dar- Al-Kotob Al-Ilmiyah, Beirut.
Ibn sina, H., 1984. Resalat fi-al-Adviyt ol Qalbiye (The treatise on cardiac drugs). Al-Maahadal-Torath Al-Elmi Al-Arabi, Damascus, pp. 221–282.
Ibn Sina, H., 1987. A. Al-Qanun fi'l-Tibb (Canon of Medicine). I.H.M.M.R Printing Press, New Delhi.
Ibragić, S., Salihović, M., Tahirović, I., et al., 2014. Quantification of some phenolic acids in the leaves of *Melissa officinalis* L. from Turkey and Bosnia. Bull. Chem. Technol. Bosnia Herzeg. 42, 47–50.
Iranian herbal pharmacopoeia (Vol. 1). Tehran: Ministry of Health Publication. pp.141–121.
Ivanova, D., Gerova, D., Chervenkov, T., et al., 2005. Polyphenols and antioxidant capacity of Bulgarian medicinal plants. J. Ethnopharmacol. 96 (1–2), 145–150.
J., Evelyn, 1699. Acetaria: A Discourse Of Sallets. ⟨http://www.bookrags.com/ebooks/15517/1.html#gsc.tab=0⟩.
Jäger, A.K., Gauguin, B., Adsersen, A., et al., 2006. Screening of plants used in Danish folk medicine to treat epilepsy and convulsions. J. Ethnopharmacol. 105, 294–300.
Jang, S.M., Yee, S.T., Choi, J., et al., 2009. Ursolic acid enhances the cellular immune system and pancreatic beta-cell function in streptozotocin-induced diabetic mice fed a high-fat diet. Int. Immunopharmacol. 9, 113–119.
Javadi, B., Emami, S.A., 2015. Avicenna's contribution to mechanisms of cardiovascular drugs. Iran. J. Basic Med. Sci. 18, 721–722.
Javadi, B., Iranshahy,, M., Emami, S.A., 2015. Anticancer Plants in Islamic Traditional Medicine. In: Saad, M. (Ed.), Complementary Therapies for the Body, Mind and Soul. In Tech, Croatia, p. 119.
Jorjani, S.E., 1976. Saeedi Sirjani, A.A. (Ed.), Zakhireh Kharazmshahi (A photo print of the manuscript dated 1206 A.D) 3. The Iranian Culture Foundation, Tehran, p. 462.
Joukar, S., Asadipour, H., 2015a. Evaluation of *Melissa officinalis* (Lemon Balm) effects on heart electrical system. Res. Cardiovasc. Med. 4, 27013.
Joukar, S., Zarisfi, Z., Sepehri, G., et al., 2014. Efficacy of *Melissa officinalis* in suppressing ventricular arrhythmias following ischemia-reperfusion of the heart: a comparison with amiodarone. Med. Princ. Pract. 23, 340–345.
Joukar, S., Asadipour, H., Sheibani, M., et al., 2015b. The effects of *Melissa officinalis* (lemon balm) pretreatment on the resistance of the heart to myocardial injury. Pharm. Biol. 12, 1–9.
Jun, H.J., Lee, J.H., Jia, Y., et al., 2012. *Melissa officinalis* essential oil reduces plasma triglycerides in human apolipoprotein E2 transgenic mice by inhibiting sterol regulatory element-binding protein-1c-dependent fatty acid synthesis. J. Nutr. 142, 432–440.
Karaman, S., Kocabas, Y.Z., 2001. Traditional medicinal plants of K. Maras (Turkey). Sciences 1 (3), 125–128.
Karthik, D., Arunkumar, E., Viswanathan, P., et al., 2012. Rosmarinic acid treatment alleviates fibrotic changes in the myocardium induced in a rat model of insulin resistance. Asian Pac. J. Trop. Dis. 2, S920–S926.
Kennedy, D.O., Scholey, A.B., Tildesley, N.T., et al., 2002. Modulation of mood and cognitive performance following acute administration of *Melissa officinalis* (lemon balm). Pharmacol. Biochem. Behav. 72, 953–964.
Kennedy, D.O., Wake, G., Savelev, S., et al., 2003. Modulation of mood and cognitive performance following acute administration of single doses of *Melissa officinalis* (Lemon balm) with human CNS nicotinic and muscarinic receptor-binding properties. Neuropsychopharmacology 28, 1871–1881.
Kennedy, D.O., Little, W., Scholey, A.B., 2004. Attenuation of laboratory-induced stress in humans after acute administration of *Melissa officinalis* (Lemon Balm). Psychosom. Med. 66, 607–613.
Kihara, T., Shimohama, S., 2004. Alzheimer's disease and acetylcholine receptors. Acta Neurobiol. Exp. 4 (1), 99–105, PubMed PMID: 15190684.
Kihara, T., Shimohama, S., Sawada, H., et al., 1997. Nicotinic receptor stimulation protects neurons against beta-amyloid toxicity. Ann. Neurol. 42 (2), 159–163.
Kim, J.H., Lee, B.J., Kim, J.H., et al., 2009. Rosmarinic acid suppresses retinal neovascularization via cell cycle arrest with increase of p21(WAF1) expression. Eur. J. Pharmacol. 615, 150–154.
Kim, M.Y., Park, B.Y., Lee, H.S., et al., 2010. The anti-angiogenic herbal composition Ob-X inhibits adipose tissue growth in obese mice. Int. J. Obes. 34, 820–830.
Kim, S.-K., Himaya, S., 2012. Triterpene glycosides from sea cucumbers and their biological activities. Adv. Food Nutr. Res. 65, 297–319.
Komarov, V.L., 1977. Flora of the USSR. Flora of the USSR XXI. Keter Publishing House Jerusalem Ltd.
Konishi, Y., Kobayashi, S., 2005b. Transepithelial transport of rosmarinic acid in intestinal Caco-2 cell monolayers. Biosci., Biotechnol., Biochem. 69 (3), 583–591.
Konishi, Y., Hitomi, Y., Yoshida, M., et al., 2005a. Pharmacokinetic study of caffeic and rosmarinic acids in rats after oral administration. J. Agric. Food Chem. 53 (12), 4740–4746.
Kultur, S., 2007. Medicinal plants used in Kirklareli Province (Turkey). J. Ethnopharmacol. 111 (2), 341–364.
Leask, A., 2007. TGFbeta, cardiac fibroblasts, and the fibrotic response. Cardiovasc. Res. 74 (2), 207–212.
Lee, E.K., Kim, Y.J., Kim, J.Y., et al., 2014. *Melissa officinalis* extract inhibits laser-induced choroidal neovascularization in a rat model. PLoS One 9, e110109.
Leśniewicz, A., Jaworska, K., Żyrnicki, W., 2006. Macro-and micro-nutrients and their bioavailability in polish herbal medicaments. Food Chem. 99 (4), 670–679.
Liu, Q., Zhao, B., 2004. Nicotine attenuates beta-amyloid peptide-induced neurotoxicity, free radical and calcium accumulation in hippocampal neuronal cultures. Br. J. Pharmacol. 141 (4), 746–754.
Lopez, V., Martin, S., Gomez-Serranillos, M.P., et al., 2009. Neuroprotective and neurological properties of *Melissa officinalis*. Neurochem. Res. 34, 1955–1961.
Lu, J., Zheng, Y.L., Wu, D.M., et al., 2007. Ursolic acid ameliorates cognition deficits and attenuates oxidative damage in the brain of senescent mice induced by D-galactose. Biochem. Pharmacol. 74, 1078–1090.
Luno, V., Gil, L., Olaciregui, M., et al., 2014. Antioxidant effect of lemon balm (*Melissa officinalis*) and mate tea (*Ilex paraguensys*) on quality, lipid peroxidation and DNA oxidation of cryopreserved boar epididymal spermatozoa. Andrologia 47 1004–11.
Macia, M.J., Garcia, E., Vidaurre, P.J., 2005. An ethnobotanical survey of medicinal plants commercialized in the markets of La Paz and El Alto, Bolivia. J. Ethnopharmacol. 97 (2), 337–350.
Mahita, M., Abuhamdah, R., Howes, M.-J., et al., 2014. Identification of a novel GABAA receptor channel ligand derived from *Melissa officinalis* and *Lavandula angustifolia* essential oils. European. J. Med. Plants 4 (7), 810.
Martin, A., Komada, M.R., Sane, D.C., 2003. Abnormal angiogenesis in diabetes mellitus. Med. Res. Rev. 23, 117–145.
Mazzanti, G., Battinelli, L., Pompeo, C., et al., 2008. Inhibitory activity of *Melissa officinalis* L. extract on Herpes simplex virus type 2 replication. Nat. Prod. Res. 22, 1433–1440.
Meftahizade, H., Lotfi, M., Moradkhani, H., 2010. Optimization of micropropagation and establishment of cell suspension culture in *Melissa officinalis* L. Afr. J. Biotechnol. 9, 4314–4321.
Melo, A., Monteiro, L., Lima, R.M., et al., 2011. Oxidative stress in neurodegenerative diseases: mechanisms and therapeutic perspectives. Oxid. Med. Cell. Longev. 2011, 1–14.
Mencherini, T., Picerno, P., Scesa, C., et al., 2007. Triterpene, antioxidant, and antimicrobial compounds from *Melissa officinalis*. J. Nat. Prod. 70, 1889–1894.
Merzouki, A., Ed-derfoufi, F., Molero Mesa, J., 2000. Contribution to the knowledge of Rifian traditional medicine. II: Folk medicine in Ksar Lakbir district (NW Morocco). Fitoterapia 71 (3), 278–307.
Mimica-Dukic, N., Bozin, B., Sokovic, M., et al., 2004. Antimicrobial and antioxidant activities of *Melissa officinalis* L. (Lamiaceae) essential oil. J. Agric. Food Chem. 52, 2485–2489.
Misharina, T., Samusenko, A., 2008. Antioxidant properties of essential oils from lemon, grapefruit, coriander, clove, and their mixtures. Appl. Biochem. Microbiol. 44, 438–442.
Moradkhani, H., Sargsyan, E., Bibak, H., et al., 2010. *Melissa officinalis* L., a valuable medicine plant: a review. J. Med. Plants Res. 4, 2753–2759.
Mulkens, A., Kapetanidis, I., 1986. Flavonoids of the leaves of *Melissa officinalis* L. (Lamiaceae). Pharm. Acta Helv. 62, 19–22.
Mustafa, B., Hajdari, A., Krasniqi, F., et al., 2012. Medical ethnobotany of the Albanian Alps in Kosovo. J. Ethnobiol. Ethnomed. 8, 1–14.
Neves, J.M., Matos, C., Moutinho, C., et al., 2009. Ethnopharmacological notes about ancient uses of medicinal plants in Tras-os-Montes (northern of Portugal). J. Ethnopharmacol. 124 (2), 270–283.
Noguchi-Shinohara, M., Ono, K., Hamaguchi, T., et al., 2015. Pharmacokinetics, safety and tolerability of *Melissa officinalis* extract which contained rosmarinic acid in healthy individuals: a randomized controlled trial. PloS One 10, e0126422.
Nolkemper, S., Reichling, J., Stintzing, F.C., et al., 2006. Antiviral effect of aqueous extracts from species of the Lamiaceae family against Herpes simplex virus type 1 and type 2 *in vitro*. Planta Med. 72, 1378–1382.
Oliveira, S.G., de Moura, F.R., Demarco, F.F., et al., 2012. An ethnomedicinal survey on phytotherapy with professionals and patients from Basic Care Units in the Brazilian Unified Health System. J. Ethnopharmacol. 140 (2), 428–437.
Orhan, I., Aslan, M., 2009. Appraisal of scopolamine-induced antiamnesic effect in mice and *in vitro anti* acetylcholinesterase and antioxidant activities of some traditionally used Lamiaceae plants. J. Ethnopharmacol. 122 (2), 327–332.
Osbaldeston, T.A., 2000. Dioscorides De Materia Medica. IBIDIS Press, Johannesburg, South Africa.
Park, J.-I., Bae, H.-R., Kim, C.G., et al., 2014. Relationships between chemical structures and functions of triterpene glycosides isolated from sea cucumbers. Front. Chem. 2, 1–14.
Patel, K.C., Pramanik, S., Patil, V.C., 2014. Ayurvedic approach with a prospective to treat and prevent alzheimers and other cognitive diseases. World J. Pharm.

Pharm. Sci. 3, 234–252.
Patora, J., Klimek, B., 2002. Flavonoids from lemon balm (*Melissa officinalis* L., Lamiaceae). Acta Pol. Pharm. 59, 139–144.
Pereira, R.P., Boligon, A.A., Appel, A.S., et al., 2014. Chemical composition, antioxidant and anticholinesterase activity of *Melissa officinalis*. Ind. Crops Prod. 53, 34–45.
Pieroni, A., 2000. Medicinal plants and food medicines in the folk traditions of the upper Lucca Province, Italy. J. Ethnopharmacol. 70 (3), 235–273.
Pieroni, A., Quave, C.L., Villanelli, M.L., et al., 2004. Ethnopharmacognostic survey on the natural ingredients used in folk cosmetics, cosmeceuticals and remedies for healing skin diseases in the inland Marches, Central-Eastern Italy. J. Ethnopharmacol. 91 (2–3), 331–344.
Proestos, C., Chorianopoulos, N., Nychas, G.-J., et al., 2005. RP-HPLC analysis of the phenolic compounds of plant extracts. Investigation of their antioxidant capacity and antimicrobial activity. J. Agric. Food Chem. 53, 1190–1195.
Queiroz, R.M., Takiya, C.M., Guimaraes, L.P., et al., 2014. Apoptosis-inducing effects of *Melissa officinalis* L. essential oil in glioblastoma multiforme cells. Cancer Invest. 32, 226–235.
Rains, J.L., Jain, S.K., 2011. Oxidative stress, insulin signaling, and diabetes. Free Radic. Biol. Med. 50, 67–75.
Rexhepi, B., Mustafa, B., Hajdari, A., et al., 2013. Traditional medicinal plant knowledge among Albanians, Macedonians and Gorani in the Sharr Mountains (Republic of Macedonia). Genet. Resour. Crop Evol. 60 (7), 2055–2080.
Ritschel, W.A., Starzacher, A., Sabouni, A., Hussain, A.S., Koch, H.P., 1989. Percutaneous absorption of rosmarinic acid in the rat. Methods Find. Exp. Clin. Pharmacol. 11 (5), 345–352.
Ruberto, G., Baratta, M.T., 2000. Antioxidant activity of selected essential oil components in two lipid model systems. Food Chem. 69, 167–174.
Šarić-Kundalić, B., Dobeš, C., Klatte-Asselmeyer, V., et al., 2011. Ethnobotanical survey of traditionally used plants in human therapy of east, north and northeast Bosnia and Herzegovina. J. Ethnopharmacol. 133 (3), 1051–1076.
Saeb, K., Gholamrezaee, S., 2012. Variation of essential oil composition of *Melissa officinalis* L. leaves during different stages of plant growth. Asian Pac. J. Trop. Biomed. 2 (2), S547–S549.
Saad, B., Said, O., 2011. Greco-Arab and Islamic Herbal Medicine: Traditional System, Ethics, Safety, Efficacy, and Regulatory Issues. John Wiley & Sons, United States.
Sadraei, H., Ghannadi, A., Malekshahi, K., 2003. Relaxant effect of essential oil of *Melissa officinalis* and citral on rat ileum contractions. Fitoterapia 74, 445–452.
Salah, S.M., Jäger, A.K., 2005. Screening of traditionally used Lebanese herbs for neurological activities. J. Ethnopharmacol. 97, 145–149.
Sari, A., Ceylan, A., 2002. Yield characteristics and essential oil composition of lemon balm (*Melissa officinalis* L.) grown in the aegean region of Turkey. Turk. J. Agric. . 26, 217–224.
Savino, F., Cresi, F., Castagno, E., et al., 2005. A randomized double-blind placebo-controlled trial of a standardized extract of *Matricariae recutita*, *Foeniculum vulgare* and *Melissa officinalis* (ColiMil) in the treatment of breastfed colicky infants. Phytother. Res. 19, 335–340.
Schnitzler, P., Schuhmacher, A., Astani, A., et al., 2008. *Melissa officinalis* oil affects infectivity of enveloped herpesviruses. Phytomedicine 15, 734–740.
Scholey, A., Gibbs, A., Neale, C., et al., 2014. Anti-stress effects of lemon balm-containing foods. Nutrients 6, 4805–4821.
Sepand, M.R., Soodi, M., Hajimehdipoor, H., et al., 2013. Comparison of neuroprotective effects of *Melissa officinalis* total extract and its acidic and non-acidic fractions against A beta-induced toxicity. Iran. J. Pharm. Res. 12, 415–423.
Shakeri, A., Khakdan, F., Soheili, V., Sahebkar, A., Rassam, G., Asili, J., 2014. Chemical composition, antibacterial activity, and cytotoxicity of essential oil from *Nepeta ucrainica* L. *spp. kopetdaghensis*. Ind. Crops Prod. 58, 315–321.
Slemmer, J.E., Shacka, J.J., Sweeney, M.I., et al., 2008. Antioxidants and free radical scavengers for the treatment of stroke, traumatic brain injury and aging. Curr. Med. chem. 15, 404–414.
Solas, M., Puerta, E., Ramirez, M.J., 2015. Treatment options in Alzheimer's disease: the GABA story. Curr. Pharm. Des. 21 (34), 4960–4971.
Somova, L.I., Shode, F.O., Mipando, M., 2004. Cardiotonic and antidysrhythmic effects of oleanolic and ursolic acids, methyl maslinate and uvaol. Phytomedicine 11, 121–129.
Somova, L.O., Nadar, A., Rammanan, P., et al., 2003. Cardiovascular, antihyperlipidemic and antioxidant effects of oleanolic and ursolic acids in experimental hypertension. Phytomedicine 10, 115–121.
Soodi, M., Naghdi, N., Hajimehdipoor, H., et al., 2014. Memory-improving activity of *Melissa officinalis* extract in naive and scopolamine-treated rats. Res. Pharm. Sci. 9, 107–114.
Sosa, V., Moline, T., Somoza, R., et al., 2013. Oxidative stress and cancer: an overview. Ageing Res. Rev. 12, 376–390.
de Sousa, A.C., Alviano, D.S., Blank, A.F., et al., 2004. *Melissa officinalis* L. essential oil: antitumoral and antioxidant activities. J. Pharm. Pharm. 56, 677–681.
Sousa, A.C., Gattass, C.R., Alviano, D.S., et al., 2004. *Melissa officinalis* L. essential oil: antitumoral and antioxidant activities. J. Pharm. Pharmacol. 56, 677–681.
Sun, Z.K., Yang, H.Q., Chen, S.D., 2013. Traditional Chinese medicine: a promising candidate for the treatment of Alzheimer's disease. Transl. Neurodegener. 2, 1–7.
Taiwo, A.E., Leite, F.B., Lucena, G.M., et al., 2012. Anxiolytic and antidepressant-like effects of *Melissa officinalis* (lemon balm) extract in rats: influence of administration and gender. Indian J. Pharmacol. 44, 189–192.
Takeda, H., Tsuji, M., Inazu, M., et al., 2002a. Rosmarinic acid and caffeic acid produce antidepressive-like effect in the forced swimming test in mice. Eur. J. Pharmacol. 449, 261–267.
Takeda, H., Tsuji, M., Matsumiya, T., et al., 2002b. Identification of rosmarinic acid as a novel antidepressive substance in the leaves of *Perilla frutescens* Britton *var.* acuta Kudo (*Perillae Herba*). Nihon Yakurigaku Zasshi 22, 15–22.
Tantry, M.A., Bhat, G.A., Idris, A., et al., 2014. Sulfated triterpenes from Lemon balm. Helv. Chim. Acta 97, 1497–1506.
Tene, V., Malagon, O., Finzi, P.V., et al., 2007. An ethnobotanical survey of medicinal plants used in Loja and Zamora-Chinchipe, Ecuador. J. Ethnopharmacol. 111 (1), 63–81.
Testai, L., 2015. Flavonoids and mitochondrial pharmacology: a new paradigm for cardioprotection. Life Sci. 15, 68–76.
The Plant List, 2013. Version 1.1. Published on the Internet; ⟨http://www.theplantlist.org/⟩ (accessed 1st January).
Thimmappa, R., Geisler, K., Louveau, T., et al., 2014. Triterpene biosynthesis in plants. Annu. Rev. Plant Biol. 65, 225–257.
Tonekaboni, S.M.M., 2007. Tohfe Hakim Momen. ITMRC Press, Tehran.
Tsai, F.S., Peng, W.H., Wang, W.H., et al., 2007. Effects of luteolin on learning acquisition in rats: involvement of the central cholinergic system. Life Sci. 80, 1692–1698.
Tsai, F.S., Cheng, H.Y., Hsieh, M.T., et al., 2010. The ameliorating effects of luteolin on beta-amyloid-induced impairment of water maze performance and passive avoidance in rats. Am. J. Chin. Med. 38, 279–291.
Turhan, M., 2006. Hand book of herbal plants, chapter 4. Meliss. Off. 3, 184–245.
Ulbricht, C., Brendler, T., Gruenwald, J., et al., 2005. Lemon balm (*Melissa officinalis* L.): an evidence-based systematic review by the Natural Standard Research Collaboration. J. Herb. Pharmacother. 5 (4), 71–114.
Ulubelen, A., Topcu, G., Kolak, U., 2005. Labiatae flavonoids and their bioactivity. Stud. Nat. Prod. Chem. 30, 233–302.
Van den Berg, T., Freundl, E., Czygan, F.C., 1997. *Melissa officinalis subsp. altissima*: characteristics of a possible adulteration of lemon balm. Pharmazie 52 (10), 802–808.
Vitalini, S., Tome, F., Fico, G., 2009. Traditional uses of medicinal plants in Valvestino (Italy). J. Ethnopharmacol. 121 (1), 106–116.
Vogl, S., Picker, P., Mihaly-Bison, J., et al., 2013. Ethnopharmacological *in vitro* studies on Austria's folk medicine–an unexplored lore *in vitro* anti-inflammatory activities of 71 Austrian traditional herbal drugs. J. Ethnopharmacol. 149, 750–771.
Wake, G., Court, J., Pickering, A., et al., 2000. CNS acetylcholine receptor activity in European medicinal plants traditionally used to improve failing memory. J. Ethnopharmacol. 69, 105–114.
Weidner, C., Wowro, S.J., Freiwald, A., et al., 2014. Lemon balm extract causes potent antihyperglycemic and antihyperlipidemic effects in insulin-resistant obese mice. Mol. Nutr. Food Res. 58, 903–907.
Weidner, C., Rousseau, M., Plauth, A., et al., 2015. *Melissa officinalis* extract induces apoptosis and inhibits proliferation in colon cancer cells through formation of reactive oxygen species. Phytomedicine 22, 262–270.
WHO Monographs on Selected Medicinal Plants. 1994, Vol. 2. Essential Medicines and Health Products Information Portal; ⟨http://apps.who.int/medicinedocs/en/d/Js4927e/18.html⟩.
Wojcikowski, K., Gobe, G., 2014. Animal studies on medicinal herbs: predictability, dose conversion and potential value. Phytother. Res. 28 (1), 22–27.
Wolbling, R.H., Leonhardt, K., 1994. Local therapy of herpes simplex with dried extract from *Melissa officinalis*. Phytomedicine 1, 25–31.
Wood, M., Bégnoche, F.B., Light, P.D., 2015. Traditional Western Herbalism and Pulse Evaluation: A Conversation. Lulupress, United States.
Yin, M.C., Lin, M.C., Mong, M.C., et al., 2012. Bioavailability, distribution, and antioxidative effects of selected triterpenes in mice. J. Agric. Food Chem. 60 (31), 7697–7701.
Yoon, M., Kim, M.Y., 2011. The anti-angiogenic herbal composition Ob-X from *Morus alba*, *Melissa officinalis*, and *Artemisia capillaris* regulates obesity in genetically obese ob/ob mice. Pharm. Biol. 49, 614–619.
Zarei, A., Changizi Ashtiyani, S., Taheri, S., et al., 2014. Comparison between effects of different doses of *Melissa officinalis* and atorvastatin on the activity of liver enzymes in hypercholesterolemia rats. Avicenna J. Phytomed. 4, 15–23.
Zeraatpishe, A., Oryan, S., Bagheri, M.H., et al., 2011. Effects of *Melissa officinalis* L. on oxidative status and DNA damage in subjects exposed to long-term low-dose ionizing radiation. Toxicol. Ind. Health 27, 205–212.