Industrial Crops and Products 53 (2014) 6–15

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Antioxidant capacity and phenolic contents of some Mediterranean medicinal plants and their potential role in the inhibition of cyclooxygenase-1 and acetylcholinesterase activities

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ARTICLE INFO

Article history:
Received 28 October 2013
Received in revised form
28 November 2013
Accepted 2 December 2013

Keywords:
Cyclooxygenase-1
Acetylcholinesterase
Medicinal plants
Phenolic compounds
Antioxidant activities

ABSTRACT

Extracts of nine medicinal plants were screened for their anti-inflammatory activity using the cyclooxygenase-1 assay and their acetylcholinesterase inhibitory effect. The antioxidant activity was assessed by four methods: free radicals DPPH• (1,1-diphenyl-2-picrilhydrazyl), nitric oxide assay, β-carotene bleaching test and metal chelating power. The amounts of different phenolic compounds were also determined. *Myrtus communis* (leaves), *Pistacia lentiscus* (leaves) and *Globularia alypum* (flowers) presented the highest amounts of total phenolic compounds while the concentrations of total flavonoids, flavonols, proanthocyanidins and total tannins varied with plant species. *Marrubium vulgare* (leaves) gave the best inhibitory activity of the enzyme Cox-1 with an IC_{50} of 0.082 mg/ml which was statistically not different from the standard indomethacin (0.061 mg/ml). The best anti-acetylcholinesterase activity was exhibited by the leaf extracts of *M. communis*, *P. lentiscus* and *Eryngium maritimum*, 92.38, 73.84 and 65.34%, respectively. In the DPPH assay, *P. lentiscus* and *M. communis* presented the best activity and their inhibitions were not different from each other ($^*p < 0.05$) but were significantly different from the pure standards rutin and BHA. Among the tested plants, *Scilla maritima* presented the best nitric oxide scavenging activity. In the β-carotene assay, extracts of *M. communis* leaves and fruits and *P. lentiscus* leaves were the most potent with 63.60, 47.61 and 43.02%, respectively. Metal chelating activity assay showed that *E. maritimum* leaves and stem and *M. communis* leaves had the best chelating power, 49.78, 32.32 and 35.98%, respectively.

1. Introduction

Non-steroid anti-inflammatory drugs (NSAIDs) are of huge therapeutic benefit in the treatment of inflammatory diseases since they are widely used for the treatment of pain, inflammation and fever (Vonkeman and van de Laar, 2010). The main mechanism of action of these drugs is believed to be the inhibition of the cyclooxygenase enzymes: the constitutive cyclooxygenase-1 (Cox-1) and the inducible cyclooxygenase-2 (Cox-2) (Ulbrich et al., 2002). Cyclooxygenase (Cox) is a bi-functional enzyme that first catalyzes the addition of two molecules of oxygen to arachidonic acid to form the hydroperoxide prostaglandin G_2 (PGG_2), then reduces the hydroperoxide to the alcohol, PGH_2, by a peroxidase activity. Prostaglandins (PGs) are important biological mediators of inflammation, originating from biotransformation of arachidonic acid catalyzed by cyclooxygenase (Gierse et al., 2008). The most common side effects associated with all currently available NSAIDs are gastrointestinal haemorrhagia and ulceration (Dannhardt et al., 2000). These side effects during anti-inflammatory therapy are caused by interference with the physiological properties of prostaglandins (Ulbrich et al., 2002). During inflammatory responses, the activation of phospholipase A_2 induces the mobilization of fatty acids, in particular arachidonic acid from the membrane lipid pool (Fiorucci et al., 2001; Fawole et al., 2010). Arachidonic acid is then oxidized by Cox-1 or Cox-2 enzymes, leading to the production of prostaglandins (Fiorucci et al., 2001). In addition, oxidants such as reactive oxygen species (ROS) generated from activated neutrophils and macrophages have been reported to play an important role in the pathogenesis of various pain-related diseases, including neurodegenerative disorders, like Alzheimer's disease (AD) (Gibson and Huang, 2005; Fawole et al., 2010). This disease is frequent in elderly people, as a result of malfunctioning of different biochemical pathways. The drugs approved for the AD therapy act by counteracting the

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http://dx.doi.org/10.1016/j.indcrop.2013.12.008

Table 1
Botanical and common names, families, voucher specimens, plant parts and medicinal properties of the investigated plants.

Family	Species	Local name	Voucher specimen	Used part	Traditional uses
Myrtaceae	*Myrtus communis*	Chilmoune	D-PH-2013-37-6 D-PH-2013-37-12	Leaves Fruits	Antiseptic, disinfectant drug and hypoglycemic effects, antimicrobial, tonic and balsamic agent, Inhibit xanthine oxidase activity, anti-inflammatory activity
Apiaceae	*Eryngium maritimum*	Tabelyatut	D-PH-2013-37-1 D-PH-2013-37-16	Leaves Stem	Diuretic, antiscorbutic, a cytotonic, a urethritis remedy, a stone inhibitor, an aphrodisiac, an expectorant, an anthelmintic, antinociceptive and anti-inflammatory activity. Used for snakebites, fevers, or female reproductive disorders
Anacardiaceae	*Pistacia lentiscus*	Amadagh	D-PH-2013-37-5	Leaves	Antibacterial, and antiulcer agent, treatment of eczema, diarrhea and throat infections, anti-inflammatory, antipyretic and insecticidal activities
Globulariaceae	*Globularia alypum*	Taselgha	D-PH-2013-37-7 D-PH-2013-37-17	Leaves Flowers	Hypoglycemic, laxative and diuretic agent, treatment of rheumatism, arthritis, hemorrhoids. Anti-inflammatory activity
Lamiaceae	*Marrubium vulgare*	Marruyet	D-PH-2013-37-2	Leaves	Treatment of gastroenterical and respiratory diseases, antinociceptive, anti-inflammatory, hypoglycemic and insecticidal effects. Tonic, aromatic, expectorant, diaphoretic and diuretic activities
Liliaceae	*Scilla maritima*	Lebsel wuchen	D-PH-2013-37-14	Bulb	Treatment of heart insufficiency, edema and bad kidney performance, memory-enhancement and rodenticide. Anti-inflammatory activity, cardiotonic, diuretic

acetylcholine deficit, that is, they try to enhance the acetylcholine level in the brain (Heinrich and Teoh, 2004). Acetylcholine is involved in the signal transfer in the synapses. After being delivered in the synapses, acetylcholine is hydrolyzed giving choline and acetyl group in a reaction catalyzed by the enzyme acetylcholinesterase. The molecular basis of the Alzheimer drugs used so far, take advantage of their action as acetylcholinesterase inhibitors (AChEIs) (Heinrich and Teoh, 2004; Ferreira et al., 2006) but these drugs have been reported to have their adverse effects including gastrointestinal disturbances, hepatotoxicity, nausea, vomiting, diarrhea, dizziness and problems associated with bioavailability (Schulz, 2003; Mukherjee et al., 2007), which increases the interest in finding better acetylcholinesterase inhibitors from natural resources.

Considering the developing increasing demand for plant-derived drugs, the nine selected plants: *Pistacia lentiscus* (leaves), *Myrtus communis* (leaves and fruits), *Globularia alypum* (leaves and flowers), *Marrubium vulgare* (leaves), *Eryngium maritimum* (leaves and stem) and *Scilla maritima* (bulb) could be further assessed and utilized in view of their health benefit effects where some of them are reported in Table 1. Moreover, according to our knowledge, there are no reports on the acetylcholinesterase and cyclooxygenase-1 inhibitory activities of the studied species in Algeria. Therefore, the aims of the present investigation were to screen for antioxidant capacities in the medicinal plant extracts, and to determine their inhibitory effects on the acetylcholinesterase and cyclooxygenase-1 enzymes. To elucidate their oxidative actions, the extracts were subjected to a range of *in vitro* tests, including the metal chelating power, the ability to scavenge DPPH radical, the β-carotene bleaching test and the nitric oxide assay. The amounts of antioxidant components (total phenolic compounds, flavonoids, flavonols, proanthocyanidins and total tannins) from the crude plant extracts were also determined.

2. Material and methods

2.1. Chemicals

All chemicals and reagents were of analytical grade and were supplied from Sigma–Aldrich Química S. A. (Sintra, Portugal) and from Sigma (represented by Algerian Chemical Society, Setif, Algeria).

2.2. Plant materials and samples preparation

Nine medicinal plants were harvested in 2009 from two locations, in remote areas in the suburbs of Taghzouit and Aboudaou, Bajaia City (Algeria). The various data (local name, medicinal uses, used parts of plant, method of preparation and administration) were collected from local inhabitants having knowledge of the curative properties of these plants. Botanical identification was made by the member of laboratory of Botany (Faculty of Life and Nature Sciences, University Abderrahmane Mira of Bejaia) according to "Nouvelle flore de l'Algérie et des regions desertiques et Meridionales" (Quezel and Santa, 1963). Voucher specimens (Table 1) were deposited at the Herbarium of Natural History Museum of Aix-en-Provence, France. Fresh leaves, flowers and stems were air-dried in shade at room temperature and the bulbs of *S. maritima* were peeled, ground and then frozen and lyophilized immediately. After drying and lyophilization, plant material was ground to a fine powder (diameter < 250 μm) using an electric mill (IKA^R A11 basic, Germany) and 1 g of this powder was exhaustively extracted by maceration with 10 ml of methanol, at room temperature for 24 h. In all cases, the solutions were filtered and concentrated to dryness under reduced pressure in a rotary evaporator (40 °C). Dry extracts were stored at −20 °C until used.

2.3. Determination of the amounts of phenolic compounds

2.3.1. Total phenolics content

Total phenolic compounds content was assayed using the Folin–Ciocalteu reagent, following Singleton and Rossi method (1965). An aliquot (1 ml) of diluted sample extract (0.3 mg/ml) was added to 500 μl of the Folin–Ciocalteu reagent and 6 ml of water. The mixture was shaken and allowed to stand for 5 min, before addition of 1.5 ml of Na_2CO_3 (20%). An aliquot of 1.9 ml of distilled water was added and mixed thoroughly. After incubation in dark for 2 h, the absorbance at 760 nm was read *versus* the prepared blank. Total phenolic content of plant parts was expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW) through the calibration curve with gallic acid ($y = 0.9397x$; $R^2 = 0.998$).

2.3.2. Total flavonoids content

Determination of the flavonoids content was achieved using the method described by Huang et al. (2004) by addition of