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Phytochemical screening, butyrylcholinesterase inhibitory activity and anti-inflammatory effect of some Tunisian medicinal plants

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ABSTRACT

In the present study, we have tested the butyrylcholinesterase (BChE) inhibitory activity of the petroleum ether, ethyl acetate and methanol aerial extracts of *Marrubium alysson*, *Peganum harmala* and *Retama raetam* at 10 and 1000 μg/mL. The anti-inflammatory activity was determined by the carrageenan-induced paw edema assay in rats. Total flavonoids and phenols and total pigment contents were also investigated.
The highest flavonoid contents were found in the methanolic extracts of *P. harmala* and the highest phenol content in the methanolic extract of *R. raetama*. The highest concentration of chlorophyll a and b and carotenoids were found in the leaves of *P. harmala*. The methanol extract of *Marrubium alysson* showed the best inhibitory activity against BChE (72.5 ± 2.07%) whereas the methanol extract of *Peganum harmala* had a moderate inhibitory effect (50.53 ± 1.98%) at the same concentration of 1 mg/ml. Methanol extracts of *M. alysson* and *P. harmala* had the highest anti-inflammatory activity.

1. Introduction

Due to economic conditions and availability, plants are the main medicinal source to treat infectious diseases in many developing countries (Sofowora, 1996). Medicinal plants have also been used for many centuries by a substantial part of the Tunisian population. Many of the plants have now been screened for the presence of natural antioxidants including tocopherols, vitamin C, carotenoids and phenolic compounds which are responsible for maintenance of health by reducing oxidative damage and protecting from coronary heart diseases and cancer (Yanga et al., 2002).

Actually, natural products in general and medicinal plants in particular are important sources of new active compounds that may become key molecules in new medical preparations. They are a high-impact source of new "lead-compounds" for new therapeutic agents despite the large development of biotechnology and combinatorial chemistry (Dall'Acqua, 2014). Several species of the genus *Corydalis* have for example been used in folk medicine in the treatment of memory dysfunction (Orhan et al., 2004). This study aims to valorize medicinal and aromatic plants of the Tunisian flora, in order to find new bioactive natural products. For this reason, we examined the *in vitro* butyrylcholinesterase inhibitory activity and anti-inflammatory activities of extracts obtained from *Retama raetam* L., *Peganum harmala* L. and *Marrubium alysson* L. It is for example indeed well known that the search for new cholinesterase inhibitors is still a promising approach for management of Alzheimer's disease (*e.g.*, Schneider, 2001; de Oliveira et al., 2011; Razik et al., 2016; Sun et al., 2016).

Retama raetam belongs to the family Fabacea, with a large distribution in East Mediterranean regions, North Africa and on the Canary Islands (Quezel and Santa, 1962). *Retama raetam* (Forsk.) Webb. locally named as "Rtem" had many traditional usages, as vermifuge, anthelmintic, antiseptic (Quezel and Santa, 1962) and antidiabetic, antioxidant, antibacterial, anticancer and anti-inflammatory (Eddouks et al., 2007; Edziri et al., 2008). *Peganum harmala* (Zygophyllaceae), the so-called "Harmal", is a perennial herbaceous glabrous plant that grows along the Mediterranean region in North Africa and the Middle East (Kuhn and Winston, 2000). *Peganum harmala* extracts have antioxidant,

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antibacterial and antiviral activities (Edziri et al., 2010; Khlifi et al., 2013). *Marrubium alysson* is a common plant in Tunisia and is used in traditional medicine in the form of a decoction as a remedy for asthma, diabetes and as a diuretic. Many studies demonstrated the antibacterial, antiviral and gastro-protective activities of *Marrubium alysson* extracts (Edziri et al., 2008, 2010; de Oliveira et al., 2011).

The aim of the present work is to evaluate the phytochemical screening, the butyrylcholinesterase inhibitory activity and anti-inflammatory activities of *Peganum harmala*, *Retama raetam* and *Marrubium alysson* extracts growing in Tunisia. It is also of interest to find whether there is any correlation between phenolic contents of these plant extracts and the studied activities.

2. Materials and methods

2.1. Plant materials

Plants were collected in the flowering season from Mahdia region (Tunisia). The plants were identified by Professor Mohamed Chaieb; botanist in the University of Science of Sfax (Tunisia). The voucher specimen of *Marrubium alysson* (MA 287), *Peganum harmala* (PA 068) and *Retama raetam* (RE 207) were deposited at the Herbarium of the Faculty of Pharmacy of the University of Monastir.

2.2. Plant extraction

Powdered plant material (500 g) from the aerial part (stem, flowers and leaves) of *M. alysson*, *P. harmala* and *R. raetam* were extracted for 3 h with 1 l of petroleum ether (PE), ethyl acetate (EA) and methanol (M) successively by Soxhlet extraction. Extracts of each solvent were evaporated under reduced pressure and the final residues were used for the bioassay. The plants, plant parts used in this study and their medicinal use are given in Table 1.

2.3. Determination of total phenolic content

The amount of total soluble phenolics in the extracts was determined spectrophotometrically according to the Folin–Ciocalteu method (Singleton and Rossi, 1965). The reaction mixture was prepared by mixing 0.1 ml of a 1 mg/ml water solution of the extract with 7.9 ml of distilled water, 0.5 ml of Folin–Ciocalteu's reagent and 1.5 ml of 20% sodium carbonate. After 2 h, the absorbance at 750 nm (UV-1800 spectrophotometer, Shimadzu, Kyoto, Japan) was read against the control that was prepared in a similar way but with distilled water instead of the extract. The total phenolic content was expressed as mg of gallic acid equivalents (GAE)/100 g of extract.

2.4. Determination of total flavonoids

Total flavonoids were determined using the colorimetric assay developed by Zhishen et al. (1999). A 1 mg/ml aliquot of extract was added to a 10 ml volumetric flask containing 4 ml of distilled H_2O. Then, 0.3 ml 5% NaNO was added, followed by 0.3 ml 10% $AlCl_3$ 5 min later. After 6 min, 2 ml of 1 M NaOH solution was added and the total volume was made up to 10 ml with distilled H_2O. The solution was well mixed and the absorbance was measured at 510 nm against the control that was prepared in the same way but with distilled water instead of the extract. The total flavonoid content was expressed as mg Catechin equivalents (CE) per/100 g of extract.

2.5. Determination of butyrylcholinesterase inhibitory activity

The BChE inhibitory activity of the extracts was determined by the spectrophotometric method of Ellman et al. (1961). Human serum BChE was used as enzyme source, while butyrylthiocholine chloride (Sigma, St. Louis, MO, USA) was employed as substrate for the reaction. The tested compound was initially dissolved in 10% dimethyl sulfoxide (DMSO) and diluted to various concentrations in sodium phosphate buffer (100 mM, pH 8.0) immediately before use. 5,5′-Dithio-bis(2-nitrobenzoic)acid (DTNB, Sigma, St. Louis, MO, USA) was used for the measurement of the cholinesterase activity. The hydrolysis of butyrylthiocholine chloride was monitored by the formation of the yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholines. Absorbance was measured at 410 nm immediately after adding the enzyme source to the reaction mixture using a spectrophotometer (Shimadzu UV-1240, Tokyo, Japan). Readings were done at 0 min, 2 min, 10 min and 15 min at room temperature and at 37 °C. The percentage of inhibition of BChE was determined by comparison of the reaction rates of the samples relative to blank sample (DMSO in phosphate buffer pH = 8) using the formula $(E - S)/E \times 100$, where E is the activity of enzyme without test sample and S is the activity of enzyme with test sample. The experiments were done in triplicate. Galanthamine, the anticholinesterase alkaloid-type of drug isolated from the bulbs of snowdrop (Galanthus sp.), was purchased from Sigma (St. Louis, MO, USA) and was used as reference.

2.6. Anti-inflammatory activity

2.6.1. Carrageenan-induced rat paw oedema

The anti-inflammatory activity was determined *in vivo* using the carrageenan-induced ratpaw oedema test (Winter et al., 1962; Bilici et al., 2002). Fasted Wistar rats of both sexes weighing 160–180 g were used. The animals were divided into six groups of six animals each. The control group received 2.5 ml/kg of saline, the standard group received the reference drug Lysine Acetylsalicylate (ASL, 300 mg/kg) and the test groups received different extracts of plants at a dose of 0.5 and 1 mg/kg. Thirteen minutes after intra-peritoneal administration of different substances, 0.05 ml of 1% of carrageenan suspension was injected to all animals in the right hind paw. The paw volume, up to tibiotarsal articulation, was measured using a plethysmometer. The measures were determined at 0 h (V_0: before oedematogenic agent injection) and 1, 2, 3, 4 h intervals later (V_T). The difference between V_T (1, 2, 3, 4 h) and V_0 was taken as the oedema value. The percentages of inhibition were calculated according to the following formula:

$$\%\text{inhibition} = \left[(V_T - V_0)_{\text{control}} - (V_T - V_0)_{\text{treated group}}\right] \times 100/(V_T - V_0)_{\text{control}}$$

Table 1
Traditional use of medicinal plants.

Plant name	Family	Plant part used	Medicinal use
Marrubium alysson L.	Lamiaceae	Aerial parts (leaves and stem)	Asthma, cardioprotective hypotensive and antidiabetic (Laonigro et al., 1979; Meyre-Silva and Cechinel-Filho, 2010; Mnonopi et al., 2012)
Peganum harmala L.	Zygophyllaceae	Aerial parts (leaves, stem and flowers)	Emmenagogue, lactogogue, antimicrobial, anticancer (Aghili, 2009; Edziri et al., 2010)
Retama raetam (Forsk.) Webb	Fabaceae	Flowers	Hyototensive, antidiabetic (Maghrani et al., 2005; Eddouks et al., 2007)