South African Journal of Botany 94 (2014) 114–121

Contents lists available at ScienceDirect

South African Journal of Botany

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

Phenolic profile, biological activities and fraction analysis of the medicinal halophyte *Retama raetam*

Saada Mariem [a,*], Falleh Hanen [a], Jalleli Inès [a], Snoussi Mejdi [b], Ksouri Riadh [a]

[a] Laboratoire des Plantes Extrêmophiles, Centre de Biotechnologie de Borj-Cédria, BP 901, 2050 Hammam-lif, Tunisie
[b] Laboratoire de Traitement et de Recyclage des Eaux, Centre de Recherches et des Technologies des Eaux (CRTE), Technopole de Borj-Cédria, BP 273, Soliman 8020, Tunisie

ARTICLE INFO

Article history:
Received 25 February 2014
Received in revised form 5 May 2014
Accepted 12 June 2014
Available online 10 July 2014

Edited by AR Ndhlala

Keywords:
Retama raetam
Biological activities
Fractionation
RP-HPLC

ABSTRACT

Retama raetam is a medicinal and aromatic plant present in the humid to the arid bioclimatic regions of Tunisia. In this work, we investigated *R. raetam* shoots antioxidant and antimicrobial activities and its natural antioxidant contents obtained from four fractions (petroleum ether, acetone 60%, ethyl acetate and water). Results showed that the ethyl acetate fraction exhibits the highest antioxidant activity as compared to the other ones. In fact, IC_{50} values of ethyl acetate extract were equal to 33.5, 500 and 1380 μg/ml (DPPH and ABTS radicals scavenging activity and reducing power, respectively). Accordingly, this fraction presented the highest total polyphenol and flavonoid contents (401 mg GAE/g DR and 33.21 mg CE/g DR, respectively). Moreover, RP-HPLC analysis showed that syringic acid and coumarin were the major phenolic compounds. Furthermore, this moderately polar fraction showed considerable antibacterial properties against human pathogen strains especially against *Escherichia coli* and *Bacillus cereus*. Finally, fractionation allows the identification of *R. raetam* most active molecules and therefore the optimization of their utilization. Our findings pointed out the appropriate solvent for extracting *R. raetam* potent phenolics which might provide a rich and novel source of natural antioxidants as food additives replacing synthetic ones in food industry.

1. Introduction

In healthy individuals, there is an equilibrium between the natural antioxidative defense system and the reactive oxygen species (ROS), generated from both living organisms and exogenous sources. Once the equilibrium is disrupted, the ROS excess induces oxidative damage to various biomolecules including: protein, lipid, DNA and RNA associated with cell structural damage, tissue injury and gene mutation (Velazquez et al., 2005). This oxidative damage plays a causative role in aging, as well as several degenerative diseases, such as heart disease, hypertension, cognitive dysfunction and cancer (Ksouri et al., 2010, 2011). In order to protect foods and humans against oxidative damage caused by free radicals, synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) were chemically synthesized (Moure et al., 2001). However, consumers' concern has come to focus on the toxicity and potential health hazards of synthetic antioxidants (Moure et al., 2001).

Natural antioxidants, especially plant phenolics, flavonoids, tannins and anthocyanidins are proved to be a safe alternative (Mohsen and Ammar, 2009). Putative therapeutic effects of many traditional medicines may be ascribed to the presence of these natural antioxidants, which are able to scavenge oxygen radicals and inhibit peroxidation (Maksimovic et al., 2005). With this respect, numerous crude extracts, from plants rich in phenolic, are of interest in the food industry, because they can retard lipid oxidative degradation and thereby improve the quality and nutritive value of food (Kahkonen et al., 1999). Accordingly, some studies have shown that the increased dietary intake of natural antioxidants like flavonoids may act as potent candidates in preventing diseases related to oxidative stress, such as cancer, atherosclerosis, aging and rheumatoid arthritis (Behera et al., 2008).

Evaluation of antioxidant and antiradical activities of fruits, vegetables, and other plant products cannot be carried out accurately by any single universal method or extraction solvent system (Ksouri et al., 2008). As a matter of fact, several studies showed that polyphenol contents differed with solvent polarities and different solvent systems should be used for extraction of polyphenols from plant materials (Chavan et al., 2001). In this context, Trabelsi et al. (2010) found that extraction solvent systems (pure or mixture) of varying polarities differ significantly in their extraction capacity and selectivity for leaf phenolic content and

Abbreviations: DPPH, 2,2-diphenyl-1-picrylhydrazyl; IC_{50}, inhibition concentration at 50%; EC_{50}, effective concentration at which the absorbance was 0.5; ATCC, American Type Culture Collection; GAE, gallic acid equivalents; CE, catechin equivalents; BCBT, β-carotene bleaching; AA, antioxidant activity; DR, dry residue; BHT, butylated hydroxytoluene; SD, standard deviation; TAC, total antioxidant capacity; RT, retention time; RP-HPLC, reverse phase high performance liquid chromatography.

* Corresponding author. Tel.: +216 79325848; fax: +216 79 325638.
E-mail addresses: saadamariem@hotmail.com, saadamariem@gmail.com (S. Mariem), Hanenfalleh@yahoo.fr (F. Hanen), jallali_ines@yahoo.fr (J. Inès), snmejdi@yahoo.fr (S. Mejdi), riadh.ksouri@cbbc.rnrt.tn (K. Riadh).

http://dx.doi.org/10.1016/j.sajb.2014.06.010
0254-6299/

antioxidant activity evaluation in the halophyte *Limoniastrum monopetalum*. Consequently, the solubility of phenolic compounds is actually governed by the type of solvent used, the degree of polymerization of phenolics, as well as by the interaction of phenolics with other food constituents (Djeridane et al., 2006).

In Tunisia, a considerable diversity of species with multiple interests including therapeutic practices occurs, and a number of them have not been subject to chemical investigations. For example *Retama raetam* is a famous herb drug in Tunisia. In fact, in traditional medicine, *R. raetam* is used for the treatment of several diseases such as eczema, diabetes and hypertension and its shoots are also used as an antidote against snake bites (Maghrani, 2005). Research undertaken on gender *Retama* showed that the aqueous extract of *R. raetam* had diuretic and hypoglycemic effects (Maghrani, 2005). Even more, oral administration of 20 mg/kg of this aqueous extract reduced significantly the glucose levels in diabetic rat bloods (Maghrani, 2005). Moreover, the administration of *R. raetam* aqueous extracts induces a decrease in the triglyceride concentrations in the plasma of normal and diabetic rats and leads to a significant decrease in weight (Maghrani, 2005). However, there is a paucity of information regarding the estimation of biological activities of different *R. raetam* fractions and the identification of the most active phenolic compounds responsible of these activities. Therefore, the present study was designed to estimate the antioxidant and antimicrobial activities of *R. raetam* fractions (hexane, acetone 60%, ethyl acetate and water). Additionally, phenolic contents (total polyphenols, flavonoids, condensed tannins) were also estimated as well as the identification by RP-HPLC of the main phenolic compounds.

2. Material and methods

2.1. Plant sampling and extract preparation

R. raetam samples were collected from Sabkha of Soliman (30 km from the capital of Tunis; 36°42′50″ N and 10°24′31″ E; superior semi-arid bioclimatic stage; mean annual rainfall: 500–600 mm) in December 2010. Plants were identified by the botanist of the Biotechnology Center of Borj-Cedria (CBBC), and a voucher specimen [F-RE 27] was deposited at the Herbarium of the Laboratory of Extremophile Plants (at CBBC). Aerial parts were collected during the vegetative stage, rinsed with distilled water then air-dried for two weeks and ground to a fine powder in a Mettler AE 200 (Dangoumau type) grinder. Extraction was performed using solvents of increasing polarity (petroleum ether, ethyl acetate, acetone/water 60/40, V/V and water) using a simple a beaker for the solid–liquid extraction and a separatory funnel for the liquid–liquid extraction. At each extraction step, 50 g of plant material was mixed with 250 ml of solvents. First, the samples were extracted by petroleum ether to remove lipophilic compounds and then the resulting residues were extracted with acetone 60% (Fig. 1). The acetonic extract was further portioned in separating funnel using ethyl acetate and water, yielding four fractions (petroleum ether, acetone 60%, ethyl acetate and water).

2.2. Chemical reagents

Folin–Ciocalteu reagent, sodium carbonate anhydrous (Na_2CO_3), gallic acid, sodium nitrite ($NaNO_2$), aluminum chloride hexahydrate ($AlCl_3$, $6H_2O$), vanillin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium hydroxide de (NaOH), trichloroacetic acid iron, chloride anhydrous ($FeCl_3$) and catechin were purchased from Fluka (Buchs, Switzerland). ABTS single reagent, β-carotene, Tween 40, linoleic acid and butylated hydroxytoluene (BHT) were purchased from Sigma-Aldrich (GmbH, Sternheim, Germany). Sulfuric acid (H_2SO_4), potassium ferricyanide $K_3Fe(CN)_6$ and Muller Hinton medium were purchased from Merck (Darmstadt, Germany).

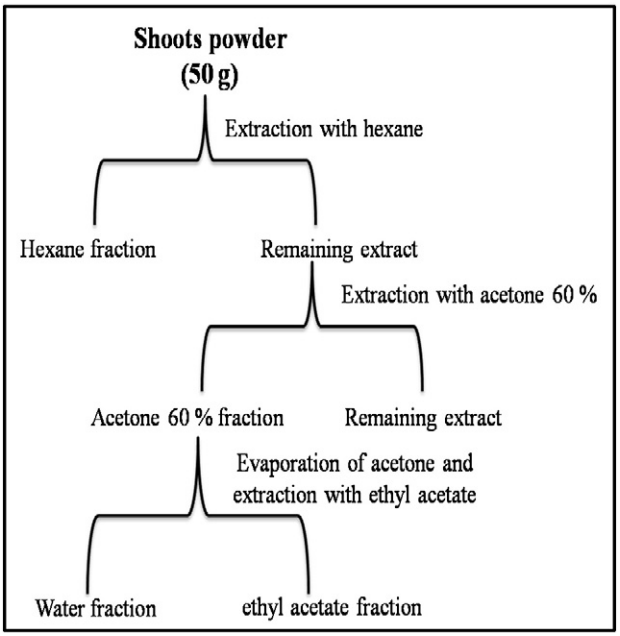

Fig. 1. Extraction procedure of *Retama raetam* shoots.

2.3. Colorimetric quantification of antioxidants

2.3.1. Total phenolic content

Colorimetric quantification of total polyphenols was determined, as described by Dewanto et al. (2002). An aliquot (0.125 ml) of appropriately diluted sample extract was mixed with 0.5 ml distilled water and 0.125 ml of Folin–Ciocalteu reagent. After 3 min, 1.25 ml of Na_2CO_3 solution (7%) was added and the final volume was made up to 3 ml with distilled water. The absorbance of the resulting solution was measured at 760 nm, after incubation for 90 min. The phenol contents were expressed in terms of milligram gallic acid equivalent per gram of dry residue (mg GAE/g DR). Triplicate measurements were taken for all samples.

2.3.2. Total flavonoid content

Total flavonoids were measured colorimetrically according to Dewanto et al. (2002). An aliquot of suitable diluted samples was added to 0.075 ml of $NaNO_2$ and mixed for 6 min, before adding 0.15 ml of a freshly prepared $AlCl_3$ (10%). After 5 min, 0.5 ml of NaOH (1 M) solution was added. The final volume was adjusted to 2.5 ml with distilled water and thoroughly mixed. Absorbance of the mixture was determined at 510 nm. Total flavonoid content was expressed as mg catechin per gram of DR (mg CE/g DR). All samples were analyzed in triplicate.

2.3.3. Total condensed tannin assay

The analysis of condensed tannins (Proanthocyanidins) was carried out according to the method of Sun et al. (1998). Three milliliters of 4% methanolic vanillin solution and 1.5 ml of concentrated H_2SO_4 were added to 0.05 ml of suitably diluted sample. The mixture was allowed to stand for 15 min, and the absorbance was measured at 500 nm. The amount of total condensed tannins was expressed as mg (+)-catechin equivalent/g DR. All samples were analyzed in three replications.

2.4. Assessment of antioxidant activities

2.4.1. Evaluation of total antioxidant capacity

An aliquot (0.1 ml) of fraction extracts was combined to 1 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated in a thermal