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Asian Pacific Journal of Tropical Biomedicine

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

Document heading

Antioxidant activities and total phenol content of *Inula viscosa* extracts selected from three regions of Morocco

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PEER REVIEW

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Comments

This is a valuable research work in which authors have demonstrated the greatest antioxidant properties of *I. viscosa* extracts in all systems of assays.
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ABSTRACT

Objective: To investigate antioxidant activity, total phenolic and flavonoid content of ethanol (E) and ethyl acetate (A) extracts of *Inula viscosa* aerial parts selected from three regions of Morocco (Imouzzer, Sefrou and Taounate).

Methods: Antioxidants properties were measured by three different test systems of assay namely free radical scavenging activities against 2,2-diphenyl-1-picrylhydrazyl, total antioxidant capacity and iron-reducing capacity. Total phenolic content was measured by Folin-Ciocalteu reagent.

Results: All the extracts showed significant antioxidant activities and contained important levels of phenols. The ethanol extract (0.3 mg/mL) from Sefrou showed the greatest antioxidant capacity in the three systems of assay, which was probably due to its high content of polyphenols (274.39±6.94) mg gallic acid equivalent/g dry extract. Total flavonoid content was found equal for all extracts.

Conclusions: Our results of antioxidant assays were justified and partially supported the popular usage of the tested plants. The high antioxidant activity found in the plant from Sefrou and its great biomass in this region suggested that *Inula viscosa* is a good source of natural antioxidants compounds which might have benefits for health.

KEYWORDS

Inula viscosa, Folk medicine, Flavonoids contents, Polyphenols, DPPH, Reducing power

1. Introduction

Oxidative stress is characterized as an imbalance between the production of reactive species and antioxidant defense activity. Its enhanced state has been associated with many of the chronic diseases such as cancer, diabetes, neurodegenerative and cardiovascular diseases[1]. Health care systems are becoming more and more expensive, therefore, we have to introduce herbal medicine systems in our health care[2]. In recent years, the research of natural antioxidants[3] as alternative sources to synthesis antioxidants has emerged and the exploitation of the various secondary metabolites of the plant was highlighted. These substances are able to reduce free radicals like superoxide, peroxyl, alkoxylate and hydroxyl[4].

Plant consists of a set of organs whose growth depends on the environmental conditions in which it develops, including the intercepted light energy, water and available nutrients drawn from the soil. The genus *Inula* including more than 100 species[5] are widely distributed in the Mediterranean area. In Morocco, *Inula viscosa* (L.) Aiton (*I. viscosa*) [*Dittrichia viscosa* (L.)], locally called "Bageraman", is an herbaceous perennial Mediterranean plant of the family Asteraceae which was largely known and used topically in folk medicine to treat animal's injuries. The plant

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Article history:
Received 6 Nov 2014
Received in revised form 20 Nov, 2nd revised form 24 Nov, 3nd revised form 27 Nov 2014
Accepted 15 Dec 2014
Available online 23 Dec 2014

has been used to treat diabetes and inflammation in North African traditional medicine[6], to treat tuberculosis, anemia and as cataplasm for rheumatic pain in Jordan[7] and it has been used for its antiseptic, skin inflammations properties and gastroduodenal disorder treatment in Spain[8,9].

It is well known that phenolics and flavonoids are important antioxidant substances obtained from most natural plants. Thus, this study was planned to evaluate the antioxidant properties of Moroccan *I. viscosa* extracts from three different regions. The choice of solvent was showed to have a significant influence on the concentration of antioxidants[10,11]. Thus, we investigated the ethanol and ethyl acetate extracts. As widely recommended, the antioxidant effect was assessed by three *in vitro* methods: 2,2-diphenyl-1-picrylhydrazyl (DPPH), phosphomolybdenum and reducing power assays. Further experiments were conducted to determine phenol and flavonoid contents. The results of this study will allow us to optimize the possibility of exploiting this plant to produce antioxidants agents to be used in the global effort to combat free radical damages.

2. Materials and methods

2.1. Reagents and standards

DPPH, butylated hydroxytoluene (BHT), ammonium molybdate, sodium phosphate, sulphuric acid, gallic acid, FeL_3, $K_3Fe(CN)_6$ and Folin-Ciocalteu reagent (FCR) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium carbonate was purchased from Prolabo (Paris, France). All the other chemicals and solvents used were of analytical grade.

2.2. Plants materials and preparation of extracts

Plants were collected in June to July 2012 from the regions of Imouzzer, Sefrou and Taounate, and dried for a week at ambient temperature and then ground. Identification was confirmed by professor Amina Bari, botanist (Department of Biological Sciences, Faculty of Science, Sidi Mohammed Ben Abdellah University, Fes, Morocco). Voucher specimens (ADV14101) were deposited in the Department of Biological Sciences, Sidi Mohammed Ben Abdellah University, Fes, Morocco. Ethanol and ethyl acetate extraction was performed at the ratio of 10% (w/v) for 3 h under agitation for plant powder[12], then the mixture was filtered through a filter paper (Whatman No. 1) and concentrated in vacuo at 45 ℃ to obtain an oily, dry, green paste, then stored at 4 ℃ for further use.

2.3. DPPH scavenging activity

The hydrogen atoms or electrons donation ability of the plant extracts and some pure compounds were measured from the bleaching of a purple-coloured methanol solution of DPPH[13]. Briefly, 1 mL of a 0.01 mmol/L solution of DPPH radical in methanol was added to 4 mL of the extract at different concentrations. The absorbance of the resulting solution was measured after 30 min in dark at 514 nm with a spectrophotometer (Selecta, E.U.). The percentage inhibition of activity was calculated as:

$$\%\text{Inhibition}=\frac{A_{blank}-A_{sample}}{A_{blank}}\times 100$$

BHT and ascorbic acid was used as positive control and the concentration providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentage plotted against the extract concentration.

2.4. Iron (III) to Iron (II) capacity

The reductive capacity of the extract was determined using ferric to ferrous iron reduction assay as determined spectrophotometrically from the formation of Perl's Prussian blue coloured complex[14]. Briefly, 1 mL of each sample, in methanol, was mixed with 2.5 mL of phosphate buffer (0.2 mol/L, pH 7.0) and 2.5 mL of potassium hexacyanoferrate $K_3Fe(CN)_6$ solution. After 30 min incubation at 50 ℃, aliquots (2.5 mL) of trichloroacetic acid (10%) were added to the mixture. Then, 2.5 mL of this solution was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%), and the absorbance was measured at 700 nm. BHT standard was used for comparison.

2.5. Determination of total antioxidant capacity

The assay was based on the reduction of Mo (VI) to Mo (V) and subsequent formation of a green phosphate/Mo(V) complex in acid pH[13]. A total volume of 0.3 mL extract dissolved in methanol was added to 3 mL of reagent solution (0.6 mol/L sulphuric acid, 28 mmol/L sodium phosphate and 4 mmol/L ammonium molybdate). The mixtures were incubated at 95 ℃ for 90 min then cooled to room temperature. The absorbance was measured at 695 nm. The total antioxidant activity was expressed as the number of equivalence of ascorbic acid and BHT.

2.6. Determination of total phenolic compounds content

The total phenolic content was determined using the FCR[15]. The reaction mixture contained 100 μL of methanolic solution (1 mg/mL) of extract, 0.5 mL of FCR, 1.5 mL of 20% (w/v) sodium carbonate and 10 mL of distilled water. After 2h of reaction at ambient temperature, the absorbance was measured at 765 nm and used to calculate the phenolic contents, using gallic acid as a standard. Then the total phenolic contents were expressed in term of gallic acid equivalents (mg GAE/g dry extract).

2.7. Total flavonoids contents

The flavonoid contents of the ethanolic and ethyl acetate extracts of *I. viscosa* were assessed using the method of Lamaison and Carnat based on the formation of a complex flavonoid-aluminium[16]. Briefly, 1 mL of diluted sample (20 μg/mL) was mixed with 1 mL of 2% aluminium chloride methanolic solution, after incubation for 10 min at room temperature. The absorbance was read at 430 nm and the flavonoids content was expressed in μg quercetin equivalent (QE) per mg of dry extract.

2.8. Statistical analysis

The measurements of total phenolic compounds, total flavonoids and DPPH radical-scavenging activity, total antioxidant and reducing capacity were carried out for three replicates. The results are expressed as mean±SD.

3. Results

3.1. Extraction yield

As shown in Table 1, the most extractive solvent was ethanol. The highest extraction yield of *I. viscosa* aerial part was the extract from