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Antioxidant activity of solvent extracts of *Inula viscosa* from Morocco

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Abstract

The antioxidative activity of *Inula viscosa* was evaluated by using a DPPH test system to suggest it as a new potential source of natural antioxidants. The amounts of total phenolics and flavonoids in the solvent extracts (diethyl ether and ethyl acetate) were determined spectrometrically. Furthermore, The DPPH scavenging activity of extracts increased in the order ascorbic acid < diethyl ether extract < ethyl acetate extract. Finally, a relationship was observed between the antioxidant activity potential and total phenolic and flavonoid levels of the extract.

Keywords: Inula viscosa, Phenolic, Flavonoid; antioxidant.

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1. Introduction

Aromatic and medicinal plants are known to produce certain bioactive molecules possessing various properties including antimicrobial and antioxidant properties [1].

Oxidative process is one of the most important routes for producing free radicals in foods, drugs and even in living systems [2].

Free radicals which have one or more unpaired electrons are produced during normal and pathological cell metabolites. They have been implicated in many diseases such as cancer, atherosclerosis, diabetes, neurodegenerative disorders, and this could be neutralized by the antioxidants from different medicinal plants [3].

Several substances from aromatic and medicinal plants have been shown to contain antioxidants and are under study. Antioxidant compounds like polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to

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degenerative diseases [4]. Studies have shown that increasing levels of flavonoids in the diet could decrease cancer and heart disease. The redox properties of flavonoids also allow themto act as reducing agents and in some cases they chelate transition metals [5].

Positive relationship were found between antioxidant activity and phenolic content for only a few plants *(Phoenix dactylifea, Ficus* sycomorus, *Zingiber officinale, Vitis vinifera, Olea europaea and Punica granatum)* screening of antioxidant activity and phenolic content of selected food items cited in the holly Quran [6]. Antioxidants are widely used as food additives to provide protection against oxidative degradation of foods by free radicals [7].

Inula viscosa (Asteraceae) is a medicinal plant widely used by Mediterranean population in folk medicine to treat different diseases [8]. It is known in Morocco under the common names: Terhal, Mâgrâmân or Amagramane.The antihypertensive effect of the aqueous extract obtained from *I. viscosa* leaves was studied in hypertensive rats in our university [9]. Previous researches on *I. viscosa* have revealed in this species the presence of flavonoids [10]. Furthermore, exract of the flavonoids of *I. viscosa* showed potent antifungal, anti-inflammatory and antioxydant powers [11-13].

I. viscosa has many uses, as anti-inflammatory [14], antipyretic, antiseptic [15,16]. Several studies have been published on extracts properties of *I. viscose* as antifungal [17], antioxidant [18] and antiulcerogenic [19]. Chemical analysis showed that *I. viscose* contains many biologically active compounds, including flavonoids and terpenoids [15].

Solvent extracts of *I. viscosa* exhibited high amounts of flavonoid components with high antiproliferative activity such as nepetin, 3-O-methylquercetin, 3,3′-di-O-methylquercetin, and hispidulin [20].

The aim of this work is to evaluate the antioxidative properties of the extracts of *I. viscosa*. Additionally, the total phenolic and flavonoid contents of diethyl ether and ethyl acetate extracts have been determined.

2. Materials and methods

2.1. Plant material

The aerial parts of *I. viscosa* were harvested in october 2009 (full bloom) from Al Hoceima, Morocco. Voucher specimens were deposited in the herbarium of Mohamed 1st University, Oujda, Morocco.

I. viscosa (L) is an annual plant, herbaceous, glandular and viscous, with a strong smell which belongs to the Asteraceae (Compositae). It can reach 50 cm to 1m high and present heads of yellow flowers atop many of the stem. The leaves are entire or dentate, acute, sinuate, the cauline amplexicaul more broadly lanceolate, rather large flower heads in long racemes pyramidal [21].

2.2. Preparation of the extracts

Boiling water extracts (100 ml) of plant samples obtained under reflux conditions (hydrodistillation process) were extracted three times (3 × 20 ml) with organic solvents (diethyl ether and ethyl acetate). Water extract residues were then extracted by boiling acidified water (2 N HCl) prior to liquid–liquid extraction. The diethyl ether and ethyl acetate extracts were filtered and concentrated under vacuum to obtain two extracts in yields of 0.10 and 0.18% (w/w) respectively. The organic solvent extracts were dried over anhydrous sodium sulfate and then stored in sealed glass vials at 4 to 5°C prior to analysis. Each extraction was performed in triplicate.

2.3. Determination of total phenolic contents

Total phenolic contents of the extracts were determined by using Folin–Ciocalteu reagent according to the method previously reported by Slinkard and Singleton (1977) [22], using caffeic acid as a standard, and as modified by Li et al. (2008). [23]. 200 µl of the diluted solution extract was mixed with 1 ml of Folin–Ciocalteu (diluted in distillated water) and the volumetric flask was vigorously shaken. After 4 min, 800 µl of Na_2CO_3 (75 mg/ml) solution was added and the mixture was allowed to stand for 45 min at room temperature. At the end of the incubation, the absorbance was measured at 760 nm. The same procedure was also applied to the standard solutions of caffeic acid, and a standard curve was obtained. The concentrations of phenolic compounds expressed as µg caffeic acid equivalent per mg of extract were calculated according to the standard caffeic acid graph. All experiments were carried out in triplicate, and caffeic acid equivalent values were reported as X (average) ± SD (standard deviation) of triplicates.

2.4. Determination of total flavonoids contents

Total flavonoid contents were determined using the Dowd method as adapted by Arvouet-Grand et al. (1994) [26]: 1 ml of 2% aluminium trichloride (AlCl3) in methanol was mixed with the same volume of extracts (200 µg). The absorption at 430 nm was measured after 10 min against a blank sample consisting of 1 ml methanol without $AlCl_3$. The concentrations of flavonoid compounds expressed as µg quercetin equivalent per mg of extract were calculated according to the standard quercetin graph. All experiments were carried out in triplicate, and quercetin equivalent values were reported as X ± SD of triplicates.

2.5. Antioxidant activity

The free radical-scavenging activities of solvent extracts were measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH) as described by Hatano et al. (1988) [24]; antioxidants react with the stable free

radical DPPH (deep violet color) and convert it to 1,1-diphenyl-2-picrylhydrazine with discoloration. Various concentrations (0.1 ml) of the diethyl ether extract (14 to 80 mg/l) and ethyl acetate extract (14 to 80 mg/l) in ethanol and water were added to 3.9 ml of a DPPH radical solution in ethanol (the final concentration of DPPH was 0.05 mM). The mixture was strongly shaken and left to stand at room temperature for 30 min in the dark. The absorbance was measured at 517 nm against a blank. The radical-scavenging activity was expressed as percentage of inhibition (I%) according to the following formula [25]:

2. $$I(\%)= 100* (A_{control} - A_{sample})/A_{control}$$

Where $A_{control}$ is the absorbance of the control reaction and A_{sample} is the absorbance of the test compound. The sample concentration providing 50% inhibition (IC50) was calculated from the graph of inhibition percentage against sample concentration. Tests were carried out in triplicate. Ascorbic acid was used as a positive control.

3. Results and discussion

3.1. Antioxidant activity, total phenolics and flavonoid contents

Free radical-scavenging capacity of the solvent extracts were measured by DPPH method. Both extracts of *I. viscosa* exhibited potential antioxidant activity. As shown in **Table 1**, free radical scavenging activity also increased with increasing concentration of solvent extracts.

Table 1. DPPH radical-scavenging of solvent extracts (Diethyl ether and Ethyl acetate) from *I. viscosa* measurated at different concentrations.

Sample	Antioxidant activities					
Diethyl ether	Extract concentration (µg/ml)	0.35	0.5	0.7	1.0	2.0
	Scavenging effect on DPPH (%)	21±3.2	31±2.1	44±2.4	55±4.7	75±5.2
Ethyle acetate	Extract concentration (µg/ml)	0.35	0.5	0.7	1.0	2.0
	Scavenging effect on DPPH (%)	29±4	50±3.8	68±4.5	85±3.6	87±2.2
Ascorbique acid	Concentrations (µg/ml)	0.2	0.35	0.5	1.0	2.0
	Scavenging effect on DPPH (%)	21±0.7	26±0.4	34±2.5	54±3.5	82±4.1

The results show that *I. viscosa* possesses strong antioxidant activity compared to ascorbic acid, the strongest activity (87%) was exhibited by the ethyl acetate extract at a concentration of 2 µg/ml. The same concentration gave 75 % for diethyl ether extract and 82 % for ascorbic acid. At a concentration

of 1 µg/ml, diethyl ether extract exhibited similar activity (55%) to ascorbic acid. The same concentration gave 85 % for ethyl acetate extract.

The ethyl acetate and the diethyl ether extract scavenged 50% DPPH free radical at the concentration of 0.5 and 0.85 µg/ml, respectively. DPPH scavenging abilities of ethyl acetate and diethyl ether extract were higher than that of the standard (ascorbic acid with an IC50 value of 0.97 µg/ml) (**Fig 1**). Thus, the DPPH scavenging effect increased in the order of ascorbic acid < diethyl ether extract < ethyl acetate extract.

These results may be due to hydroxyl groups existing in the chemical structure of phenolic compounds from *I. viscosa* extracts that can provide the necessary component as a radical scavenger [27-29].

It could be inferred from our results that there was a positive correlation between phenolic compounds content and antioxidant activity, as the higher activity of the ethyl acetate extract could be attributed to the higher content of phenolic compounds and flavonoids.

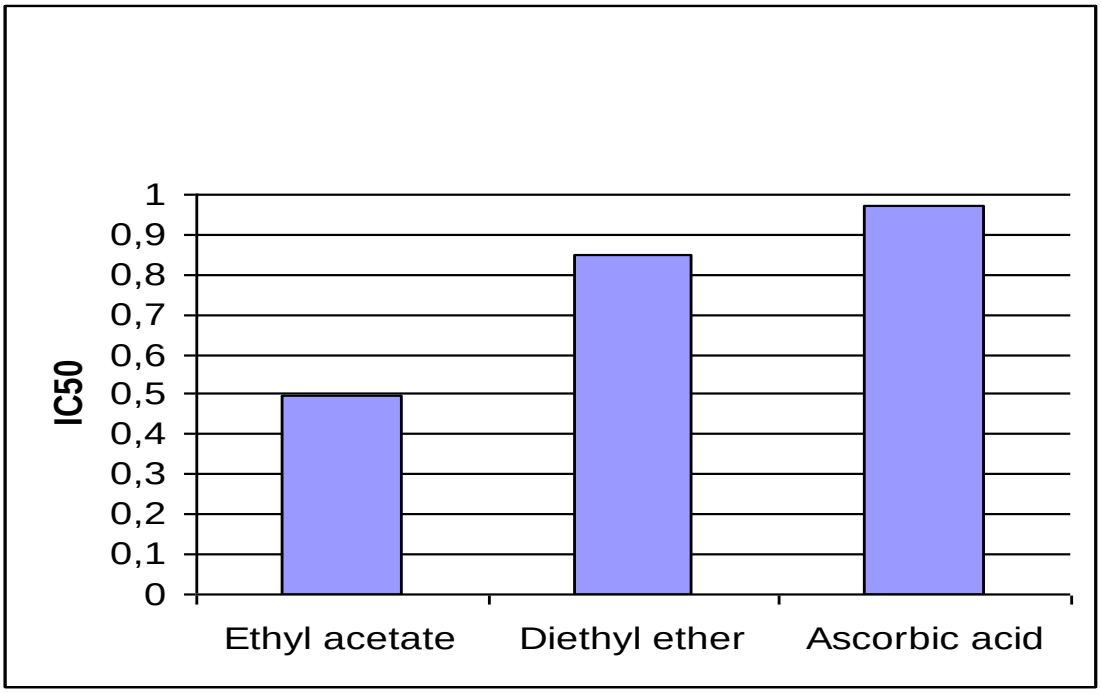

Fig 1. IC50 of solvent extracts (Ethyl acetate and Diethyl ether) of *I. viscosa* and ascorbic acid

For instance the ethyl acetate extract, which contain higher amount of phenolic compounds (**Table 2**) (441 µg/mg) than the diethyl ether (306 µg/mg), also exhibited a greater antioxidant activity. Similarly, the ethyl acetate extract was found to be richer in flavonoids (74 µg/mg) than the diethyl ether extract (32 µg/mg).

Table 2. Total phenol and flavonoid contents of *I. viscosa* solvent extracts.

	Solvent extracts	Total polyphenol content (µg CA/mg extract)	Total flavonoid content (µg quercetin/mg extract)
Inula viscosa	Diethyl ether	306 ± 8,5	32 ± 2,3
	Ethyle acetate	441 ± 1,2	74 ± 3,6

Conclusion

The solvent extracts of *I. Viscosa* were found to be effective antioxidants by in vitro assays, and can therefore be proposed as new potential sources of natural additives for the food and/or pharmaceutical industries. According to these results, there is a relationship between the total phenol content and antioxidant activity. Indeed, it is extremely important to point out that there is a positive correlation between the antioxidant activity potential and the amount of phenolic compounds in the extracts.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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