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Research Article

TOTAL PHENOL CONTENT, ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES OF *INULA VISCOSA* FROM GUELMA- ALGERIA

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ABSTRACT. In the present study, phenolic, flavonoid and tannins composition as well as antioxidant and anti-inflammatory activities of methanolic extract of *Inula viscosa* L. aerial parts selected from Guelma-Algeria were investigated. The total phenolic content analyzed using Folin–Ciocalteu's reagent, of the sample was 263.78 ± 1.23mg/g dry weight, expressed as gallic acid equivalents (GAE). The total flavonoid concentration, detected using aluminum chloride, was 86.274 ± 2.13mg quercetine quivalents (QE)/g dry weight. The total tannins content, detected using the Folin Denis reagent, was 29.59mg tannic acid/g dry weight. Antioxidant activity was evaluated by radical scavenging ability (DPPH method), the result showed significant antioxidant activity with an IC50 value of 1.36mg/L and important anti-inflammatory activity also. Our results of antioxidant and anti-inflammatory assays were justified and partially supported the popular usage of the tested plants. The high antioxidant activity found in the plant from Guelma and its great biomass in this region suggested that *Inula viscosa* is a good source of natural antioxidants and anti-inflammatory compounds which might have benefits for health.

Keywords: *Inula viscosa, polyphenols, flavonoids, tannins, anti-inflammatory, antioxidant.*

INTRODUCTION

Herbs have been used in many domains including medicine, nutrition, flavoring, beverages, dyeing, repellents, fragrances, cosmetics, smoking, and other industrial purposes. Since the prehistoric era, herbs have been the basis for nearly all medicinal therapy until synthetic drugs were developed in the nineteenth century [1,2].

Today, and despite the advances made in medicine, many people resort to plants for treatment, either because of inaccessibility to drugs prescribed by modern medicine, or because these plants have given very encouraging therapeutic results and with lesser side effects noticed during their use, or because they are less aggressive and less harmful for the body [3]. The search for new active pharmacological molecules via the screening of natural sources has led to the discovery of a large number of useful drugs that are beginning to play a major role in the treatment of many human diseases [4].

The flora of Algeria is rich of several thousand medicinal species, among this vast natural heritage, our choice was on the *Inula viscosa* L., commonly called "Magramen" is an herbaceous perennial Mediterranean plant of the *Asteraceae* family which has been used to treat diabetes and inflammation in North African traditional medicine [5].

Ethnobotanical study showed that the plant is very used in traditional medicine as an antiviral [6], antiseptic, antibacterial, healing [7,8] and antifungal [9].

Reactive oxygen species (ROS) and free radicals, such assuperoxide anion, hydrogen peroxide, and hydroxyl radical, are constantly formed in the human body by normal metabolic action. Their action is opposed by a balanced system of antioxidant defenses, including antioxidant compounds and enzymes. Upsetting this balance causes oxidative stress, which can lead to cell injury and death . Recently, much attention has been given to naturally antioxidants, which may play an important role in inhibiting both free radicals and oxidative chain reactions within tissues and membranes [11]. The health promoting effect of antioxidants from plants is thought to arise mainly from their protective effects by counteracting reactive oxygen species, which are believed to play a significant role in the etiology and pathogenesis of various chronic diseases, premature ageing, and the oxidative deterioration of cosmetics, foods, and pharmaceutical preparations [12].

Many phenolic compounds, particularly flavonoids, exhibit a wide range of biological effects, including antibacterial, antiviral, anti-inflammatory, antiallergic, anti-thrombotic, and vasodilatory actions [13].

The aim of our study is the investigation of phenolic, flavonoids and tannins composition as well as antioxidant and anti-inflammatory activities of methanolic extract of *Inula viscosa* L. aerial parts harvested from Guelma-Algeria were investigated.

MATERIALS AND METHODS

Plant material

Inula viscosa L. aerial parts were collected from Guelma (Algeria), in January 2021. The identification of the plant was done with the key to determining the flora of Quezel and Santa [14].

Specimens were kept at the Laboratory of Cryptogamy and Medical Botany, Department of Pharmacy, Faculty of Medicine, Annaba-Algeria.

Preparation of methanolic extract

Dry aerial parts (stem and leaf) of *Inula viscos*a L. have been ground and stored in glass bottles, hermetically sealed at low temperatures. 10g of the vegetable powder was macerated in 100mL of methanol with stirring for 24 hours at a temperature of 25 ± 2°C.

The extract obtained was filtered and evaporated to dryness under reduced pressure at 50°C on a rotavapor. The dry residue is taken up in 3mL of methanol and stored at -18°C until it is used [15].

The yield of the methanolic extracts was calculated by the following formula:

$$R(\%) = (M/M_0)\times 100.$$

With R(%): yield expressed in %; M: mass in grams of the resulting dry extract; M_0: mass in grams of the plant material to be treated.

Total phenolic content

The total phenolic content (TPC) of *Inula viscosa* L. extract was spectrophotometrically determined by Folin Ciocalteu's reagent assay using gallic acid for the preparation of calibration curve (1mg/mL) Singleton's method [16]. A suitable aliquot (200µL) of extract or standard solution was added to one milliliter of Folin Ciocalteu`s phenol reagent diluted 10 times was added to the mixture and shaken. After 5min. 800µl of 7,5% Na_2CO_3 solution were added to the mixture. After incubation for 30min. at room temperature, the absorbance was determined at 765nm with spectrophotometer (SHIMADZU UV-1202) against prepared reagent as blank. A total phenolic content in samples was expressed as mg gallic acid equivalents (GAE)/g dry weight. All samples were analyzed in triplicates.

Determination of total flavonoids content

The flavonoid content (TFC) of *Inula viscosa* L. extract was determined by aluminium trichloride ($AlCl_3$) method as described by Dewanto [17], quercetine was used as a reference compound (standard). The sample contained 500µL of extract dissolved in methanol 2mL distilled water and 50µL $NaNO_2$ (5%). After 6min, 1mL of Na_2CO_3 (1M) was added. The solutions were mixed well and the absorbance was measured against prepared reagent blank at 510nm by using spectrophotometer (SHIMADZU UV-1202). Total flavonoids in sample were expressed as mg quercetin equivalents (QE)/g dry weight. Samples were analyzed in triplicates.

Total tannins content

Total tannins content (TTC) of methanolic extract was measured using the Folin-Ciocalteu reagent assay, with minor modifications [18]. 100µL of *Inula viscosa* L. extract or standard solution of (tannic 10mg/L) was added to 0.5mL of Folin Denis reagent and 1mL of 0.5% sodium carbonate solution. The volume was made up for 5mL with distilled water and absorbance was measured against prepared reagent blank, at 775nm by using spectrophotometer (SHIMADZU UV-1202). Total tannins in sample were expressed as mg tannic acid equivalent (TAE)/g dry weight. All samples were analysed in triplicates.

Determination of antioxidant activity with 2,2-di-phenyl-1-picrylhydrazyl (DPPH) method

Free radical scavenging ability of the plant extract was measured from the bleaching of a purple-coloured methanol solution of DPPH [19].
Based on a pre-examination, ten concentrations of 0.3, 0.6, 0.9, 1.2, 1.5, 1.8, 2.1, 2.4, 2.7 and 3mg/mL of the extracting methanol. The DPPH solution was prepared by solubilizing 0.025g of DPPH in 1000mL of methanol.

Briefly, 1950µL of a 0,025 g/L solution of DPPH radical in methanol was added to 50µL of the extract at different concentrations. The mixture was shaken vigorously for 1min by vortexing and left to stand at room temperature in the dark for 30min. Absorbances were measured at 515nm with a spectrophotometer against a blank. Ascorbic acid was used for comparison as standard antioxidants. The percentage inhibition of activity was calculated as:

$$\% \text{ Inhibition} = [(A_{blank} - A_{sample})/A_{blank}] \times 100.$$

Where A_{blank} is the absorbance of the control and A_{sample} is the absorbance of the test extract.
IC50 value (mg/mL) is the concentration of the sample scavenging 50% of the DPPH radical.

In-vitro anti-inflammatory activity inhibition of albumin denaturation

Effect on protein denaturation assay was done according to the method described by Gambhire et al. [20], with some modifications as described in Gunathilake et al. [21].

The reaction mixture consisted on 2mL of egg albumin (from fresh hen's egg), 2.8mL of phosphate buffered saline (PBS, pH 6.4), and 2mL of methanolic extract at different concentrations (1.2, 1.6 and 2mg/mL), and the mixture was mixed, and was incubated in a water bath (37°C) for 10min, and then the reaction mixture was heated at 70°C for 5min. After cooling, the turbidity was measured at 660nm using a spectrophotometer (SHIMADZU UV-1202). Phosphate buffer solution was used as the control. The experiment was performed in triplicate.

Percent inhibition of protein denaturation was calculated as follows:

$$\% \text{ inhibition} = [(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100$$

Statistical Analysis

All data were expressed as means ± SD for at least three replications for each prepared sample. Statistical analysis was performed using one-sample *t*-test. The results are considered to be significant when $p < 0.05$.

RESULTS AND DISCUSSION

Extraction Yield

The yield, appearance and color of the methanolic extracts of the areal parts of *Inula viscosa* L. are shown in Table 1. These results show that the yield of the methanolic extract of the leaf is very important, with a percentage of 22.55%, and it is pasty green.

Table 1. *Extraction yield of Inula viscosa L., by methanol mixture*

	Extraction Solvent	**Color of the extract**	**Aspect**	**Yield in%**
Areal parts	Absolute methanol 99%	Green	Pasty	22.55%

The variation in yield and biological activity could be attributed to difference in the type and amount phytochemicals concentrated during growth of the plant. The variation in phytochemicals is an attribute of differences in soil, age, seasons, climate and type of vegetation among the ecological zones, phytochemical production in plants varies with the geographical location. Plant developmental stage influences secondary metabolism; defense compounds are generally more concentrated and diverse when plants are young and more "apparent" to herbivores, but they are known to decrease with age as structural defenses are developed [22].

Total phenolic, flavonoids and Tannins content evaluation

The total phenolic content of the crude methanolic extract of *Inula viscosa* L. was estimated by Folin–Ciocalteu reagent and expressed in gallic acid equivalents (GAE) and it was calculated from the linear regression equation of standard curve (y = 17.601x - 0.074; R^2 = 0.971). The results showed that the crude methanolic extract contains a high amount of phenols (263.78 ± 1.23 mg GAE/g).

The total flavonoids content of crude methanolic extract was determined via aluminum chloride colorimetric method and it was calculated from the linear regression equation of standard curve of quercetin (y = 0.530x + 0.092; R^2 = 0.976) and expressed as quercetin equivalent per gram of plant extract. The tested extract contains high amounts of flavonoids (86.274 ± 2.13 mg QE/g).

The total tannins content (TTC) of crude methanolic extract was measured using the Folin-Denis reagent assay and it was calculated from the linear regression equation of standard curve of tannic acid (y = 0.368x + 0.140; R^2 = 0.979) and expressed as tannic acid equivalent per gram of plant extract. The tested extract contains high amounts of tannins (29.591 ± 2.13 mg EAT/g).

The results suggest that phenolics, flavonoids and tannins are important components of the crude methanolic extract. These results are in accordance with previous findings reported in other *Asteraceae* species extracted with methanol [23].The results of total flavonoids and total phenolic contents can be different at the beginning and the end of the flowering period. The total flavonoids content can also depend on cultivars. In the studies of selected *Inula* species from Turkey, total phenolic content of three *Inula* species were ranging from (21.1 ± 0.8) to (190.9 ± 6.1)mg GAE/g extract [24], while our study showed a highest value of (263.78 ± 5.80)mg GAE/g close to the value found by Chahmi [25], in Morocco (274.39 ± 6.94)mg GAE/g.

Species of this genus contain terpenic compounds, especially sesquiterpene lactones, flavonoids, glycolipids and anthranilic acid derivatives [26,27].

DPPH scavenging activity

In this study, methanolic extract of *Inula viscosa* L. from Guelma- Algeria was investigated for its antioxidant activity with DPPH scavenging assay. The results showed an important antioxidant power of *Inula viscosa* L. extract compared to the standard product. Statistical analysis revealed that the difference between the IC50 values methanolic extract and positive control was not significant ($p > 0.05$).

The IC50 value was defined as the concentration of sample that scavenged 50% of the DPPH. A lower IC50 value means better efficiency of antioxidant activity of the sample. The results showed that antioxidant activity of the methanolic extract from Guelma-Algeria was superior to all samples tested from Morocco with an IC50 value at 1.36 mg/L which was close to the inhibition capacity of the positive control ascorbic acid (IC50 = 0.04 mg/L).

This extract is able to give electrons, which can react with free radicals to convert them to stable products and strongly inhibit radical chain the potency of *Inula viscosa* L. power reducing may be attributed to the presence of natural antioxidants such as phenolic compounds in the plants [28].

Concerning the reducing power and the total antioxidant capacity, the effectiveness of the methanolic extract may be explained by its high phenol and flavonoids contents. In this context, the results of our investigation are in accordance with those published

earlier, which mentioned that containing flavonoids and polyphenols may play an important role in reducing power [29] and they contribute significantly to the total antioxidant activity of many fruits such as red grape [30], star apple [31] and medicinal plants [32].

In vitro anti-inflammatory activity

The anti-inflammatory effect induced by natural products and non-steroidal compounds in heat treated (immunogenic) egg-albumin is proposed as a screening assay for the detection of anti-inflammatory compounds.

Denaturation of proteins is a well-documented cause of inflammation. The ability of *Inula viscosa* L. extract in inhibiting heat induced albumin denaturation was studied (Table 2). Maximum inhibition (44.44%) was observed from the concentration of 2mg/mL *Inula viscosa* L. methanolic extract followed by 35.43% (1.6mg/mL) and 18.16% 1.2mg/mL.

Acetylsallicylic acid (ASA), a standard anti-inflammation drug showed maximum inhibition (66.51%). This may be attributed to the ability of *Inula viscosa* L. methanolic extract to inhibit the release of lysosomal content of neutrophils at the site of inflammation. These neutrophils lysosomal constituents include bactericidal enzymes and proteinases, which upon extracellular release cause further tissue inflammation and damages [33,34].

Hence, the presence of bioactive compounds in the plant extract of *Inula viscosa* L. may contribute to its, antioxidant and anti-inflammatory activity.

Table 2. *Anti-inflammatory activity of Inula viscosa* L.

Concentration of methanolic extract (mg/mL)	% Inhibition of methanolic extract	% inhibition of acetylsallicylic acid
1.2	18.16	30.18
1.6	35.43	53.45
2	44.44	66.51

The present investigation has shown that strong antioxidant and anti-inflammatory properties were confirmed in the methanolic extract. These activities may be due to strong occurrence of polyphenolic compounds such as flavonoids, tannins, terpenoids, phenolics and saponins [35].

CONCLUSION

Inula viscosa species is a source of natural antioxidants and anti-inflammatories which may have many health benefits. The results of the present work have justified and partially supported the data in the literature and the popular use of the tested plant. Further studies are needed to extract and isolate the active components in the extract of *Inula viscosa* L. and to confirm their mechanisms of action.

Conflict of interest statement

We declare that we have no conflict of interest.

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