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Total phenol and flavonoid content, antioxidant and cytotoxicity assessment of Algerian *Launaea glomerata* (Cass.) Hook.f. extracts

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ABSTRACT: El-Oued province (southeast Algeria), is located in a medicinal plant-rich area; *Launaea glomerata* (Cass.) Hook. f. is one among them which is a perennial herb spread widely in the arid regions of the Mediterranean Sea. The selection of the studied plant corresponds perfectly to the scientific needs due two reasons, firstly because these samples are used by the Algerian population as herbal remedies for primary health care, secondly, for the lack of published data on it. The aim of this investigation is to provide new data on quantities of phenols, which were estimated at 25.81 mg GAE/g extract and flavonoids (49.13 mg RE/g extract), and the determination of antioxidant activities by three ways (DPPH, CAT, ABTS), the results of IC_{50} equals to 98.07 mg TE/g extract for DPPH• and 286.5 mg eq. AG/g for ABTS assays, noted that the best inhibition was by the ABTS root. We also conducted a test for the inhibitory ability of extract against cancer cells tested on both human hepatocellular carcinoma (HePG2) and colon cells (HCT116), the results were negative. The data obtained in this work can be useful for the pharmaceutical industry, also used in the Algerian medicinal herbs database.

Keywords: *Launaea glomerata*; Medicinal plant; Antioxidant activity; Antitumor; Phenols; Flavonoids.

1. INTRODUCTION

Medicinal plants have been used in chemotherapy due to their organic properties especially the secondary metabolites. Recently many studies have been directed to identifying and isolating new important therapeutic compounds from plants for specific diseases [1-3].

Launaea (Asteraceae family) is one of the most common genus in the Algerian Saharan regions, where the genus *Launaea* in Algerian flora is represented by nine species, namely, *L. acanthoclada*, *L. angustifolia*, *L. anomala*, *L. arborescens*, *L. cassiniana*, *L. glomerata*, *L. nudicaulis* and *L. querceaifolia* [2]. The present study was carried out on, *Launaea glomerata*, which belongs to the compound family (local name “Harchaia”), a perennial herb that is widespread in arid regions of the Mediterranean.

These genera were used in folk medicine to treat some illness like stomach and dermatological diseases, also it has potential anti-tumor, pesticide, antimicrobial and cytotoxic activities [4]. There are many bioactive compounds in plants, such as alkaloids, tannins, flavonoids, sterols, terpenes, etc., which are noted to have a major role in nutrition, physiology and disease control [1, 5-7]. In view of this importance, we made a preliminary detection of *Launaea glomerata* and it was found that it is rich in all those bioactive compounds.

In this study, we have focused on the measurement of the antioxidant activity of the methanol extract of the aerial parts of the plant by three methods antioxidant activity namely, catalase activity (CAT), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), and 2,2-diphenyl-1-picrylhydrazyl radical scavenging capacity assays (DPPH•) in addition to testing its inhibitory capacity on the proliferation of hepatic and colon cancer cells, in addition to the quantitative estimation of phenolic and flavonoid content.

2. MATERIALS AND METHODS

2.1. Plant material and extraction

The aerial parts of *Launaea glomerata* plant (Fig. 1), were collected in February 2018, from El-Oued Province, Algeria (33.263678 N and 6.899561 E), the identification of those samples was confirmed at Biology Laboratory, El-Oued University, Algeria under code number RO-041. The samples were washed many times with distilled water to remove dust, next, the sample were ground to a fine powder using an agate mortar and pestle, and subsequently passed through 150 µm mesh sieve and stored in airtight glass container. The extraction was done by cold maceration using methanol-water (1:4) to extract the polar compounds. The dried and crude methanolic extract was obtained through distillation in a rotary evaporator at 45°C.

Figure 1. Photo of *Launaea glomerata*.

2.2. Total phenol content determination

The phenolic content was estimated using spectrophotometry, utilizing the Singleton-Rossi method using the Folin-Ciocalteu reagent [8]. The gallic acid is employed as the reference phenol at a wavelength (λ = 765 nm). Gallic acid stock solution was diluted in methanol with known concentrations to create a standard curve. For estimating the phenols in the plant extract, 1 ml of the extract was mixed with 0.5 ml of Folin-Ciocalteu reagent (diluted ten times with water), and then, the mixture was left 5 min in the darkness. 2 ml of

sodium carbonate (7.5%), were added yo this latter, and were stirred in a tube and put in the darkness at temperature room for 30 min. In the end, the absorbance of the solution obtained was read at the wavelength (λ = 765 nm). The results were calculated according to gallic acid's standard curve and expressed as mg gallic acid equivalents (GAE)/g extract. The results were calculated by triplicates (means ± SD, n = 3).

2.3. Flavonoid content determination

Total flavonoid content was quantitatively estimated by the aluminium chloride method utilizing the UV-VIS spectrophotometer, by providing a series of concentrations of rutin, which is used to construct the standard reference curve. [9]. To quantify the flavonoids in the plant extract, 1 ml of the plant extract was mixed with 1 ml of aluminum trichloride ($AlCl_3$, 2%). The tube was shaken well, then, left for an hour in the dark until the color turned yellow. The absorbance was measured at 420 nm. Total flavonoid content was expressed as mg rutin equivalents (RE) per g of plant extract. The results were calculated by triplicates (means ± SD, n = 3).

2.4. Antioxidant properties

Antioxidant substances can reduce free radicals and improve shelf life by inhibiting the lipid peroxide process that is one of the main causes of a breakdown of food and pharmaceutical products during processing and storage [10]. DPPH• is an organic compound used to measure the antioxidants activity of plant extracts [11]. DPPH is generally used as a reagent to assess the antioxidant activity of eliminating free radicals; it is a stable free radical that accepts an electron or hydrogen to convert a stable molecule [10]. The 2,2 -azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) also forms a relatively stable free radical, the color of which disappears in its no-radical. Herein, the antioxidant activity was determined applying three methods, DPPH, CAT, and ABTS assays. All results obtained were calculated by triplicates (means ± SD, n = 3).

2.4.1. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

Free radical scavenging ability of the extracts was tested by (DPPH•) radical scavenging assay as described by Sirivibulkovit with some modifications [12, 13], ascorbic acid was used as reference. About 1 ml of the prepared concentrations was put in measuring cell. To this amount, two hundred microliter of methanol, then 800 µl of DPPH solution (4 mg/100 ml of methanol) were added, and then, the reaction mixture was vortexed thoroughly and left in the dark at room temperature (30°C) for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm.

The percentage of inhibition (%DPPH) of free radicals (DPPH•) was calculated using the obtained absorbance values with mathematical calculations applying the following equation (1).

$$\% \text{ DPPH radical scavenging activity}=\{(A_0 - A_1)/A_0\}\times 100 \quad (1)$$

where A_0 is the absorbance of the control, and A_1 is the absorbance of the extractives/standard.

2.4.2. Total antioxidant capacity (Phosphomolybdenum method)

Total antioxidant capacity was estimated by the phosphomolybdenum method. An aliquot of 0.1 ml of the sample was combined with 1 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) and stirred well. After an incubation of 90 min at 95°C, samples were cooled to room temperature. Then absorbance of the mixture was measured at 695 nm using a UV spectrophotometer. The total antioxidant capacity of each sample was expressed as gallic acid equivalent. Experiments were performed in triplicate [14].

2.4.3. ABTS radical scavenging assay

The free radical scavenging activity of plant samples was determined by ABTS radical cation decolorization assay. $ABTS^{\cdot +}$ cation radical was produced by the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate (1:1), stored in the dark at room temperature for 12-16 h before use. $ABTS^{\cdot +}$ solution was then diluted with methanol to obtain an absorbance of 0.700 at 734 nm. About 1 ml of ABTS solution was mixed with 50 µl of plant extract in test tubes that were shaken then left in the dark for 10 to 30 min at room temperature (30°C). Then, the absorbance was measured at λ = 734 nm utilizing a UV spectrophotometer [13]. Butylated hydroxytoluene (BHT) is a phenolic antioxidant used as a standard substance to graph the reference curve. The inhibition rate (% ABTS) of the extract is determined by the following equation (2).

$$\% \text{ ABTS radical scavenging activity} = \{(A_0 - A_1)/A_0\} \times 100 \qquad (2)$$

where A_0 is the absorbance of the control, and A_1 is the absorbance of the extractives/standard.

2.5. MTT cytotoxicity assay

The cytotoxic activity of the plant extract was tested against both HCT116 [ATCCCCL-247^{TM}] (colon cancer) and HePG2 [ATCCHB-8065TM] (human hepatocellular carcinoma). The assay was carried out utilizing (3-(4,5-dimethyl thiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT). MTT is cleaved by mitochondrial enzyme dehydrogenase of viable cells, yielding a measurable purple product formazan. This formazan production is directly proportional to the viable cell number and inversely proportional to cytotoxicity level [15].

3. RESULTS AND DISCUSSION

3.1. Estimation of the total content of phenols and flavonoids

In this study, methanolic extract of dark green color and viscous texture is obtained from the aerial parts of *Launaea glomerata* with a yield of 3%. Phenolic compounds are one of the by-products of metabolism, although the role of these plant bio-factors is not yet entirely clear, phenolic compounds are important for the survival of plants in their environment [16]. Usually, phenols are synthesized by plants during their normal growth in response to stressful conditions, and appear in many vital activities that benefit humans when consumed. Indeed, many foods, herbs and medicines derived from plants are rich in phenolic compounds which can prevent, treat or cure coronary heart disease and carcinogenicity [17, 18].

Epidemiological studies have also shown that regular consumption of foods rich in phenols such as grains, legumes, oilseeds and their products can protect against the risk of cardiovascular disease, diabetes type 2, gastrointestinal cancers and other disorders [19].

Plant tissues contain a variety of compounds with antioxidant properties due to the phenolic compounds, they contain one or more "hydroxyl" groups carrying the aromatic characteristic which makes them reducing agents, hence their importance in the beneficial effects on human health [20]. Phenolic compounds are one of the main secondary receptors of great physiological and morphological importance in plants [21]. Flavonoids are the most studied group of polyphenols in foods. In this study, the total content of flavonoids was determined by the interaction of the latter with aluminum, where they form a pink complex with tertiary aluminum through a 4-keto group and an adjacent hydroxyl group [17]. Flavonoids and anthocyanins are a group of polyphenols found in most plants that have a wide range of biological functions, including distinct roles in stress prevention [21].

Table 1. Comparative study of the results of phenols and flavonoids of *L. glomerata* with *L. procumbens* and *L. taraxacifolia* (same genus).

	Total phenolic content (mg GAE/g extract)	**Total phenolic content (mg GAE/g dry plant)**	**Total flavonoid content (mg quercetin/g extract)**	**Total flavonoid content (mg quercetin/g dry plant)**
L. glomerata	25.81 ± 0.14	25.69	49.13 ± 0.09	49.24
L.procumbens [22]	432.8 ± 2.93	-	13.98 ± 0.87	-
L. taraxacifolia [23]	-	32.27	-	56.95

L. glomerata: Launaea glomerata. L. procumbens: Launaea procumbens.

Our results were compared with another study carried out on the same genus *L. procumbens* due the unavailability of data in literature on the *L. glomerata*. The results for the phenols were very different, maybe due to the difference in the collection period, *L. glomerata* was collected in February before one month to its flowering, knowing that during this period the plant is at its highest activity and is rich in phenolic compounds and flavonoids. In addition, *L. glomerata* (the dry plant) were compared to *L. taraxacifolia* of the same genus (Table 1).

We can noticed that the disparity in results may due to the difference in time of recorded, as well as by others several factors such as geographical location, photosynthesis and temperature, in addition, the type of solvent, the method and the extraction conditions play an important role in estimating the amount of phenols and flavonoids in the plant [24].

3.2. Antioxidant activity results

The need to identify natural and safe alternative sources of dietary antioxidants, especially of plant origin, has increased dramatically in recent years. Antioxidants have been widely used as food additives to provide protection against oxidative degradation of food, which can protect the human body from free radicals and the effects of ROS, andalso delay the progression of many chronic diseases.Interest in antioxidants has increased in recent years due to their ability to immunize the body against invading germs and to kill them. They also protect the body from common diseases of the century, as well as DNA damages, and inhibit the action of free radicals. The main role of antioxidants is to prevent the chain propagation of these free radicals resulting from oxidation. Free radicals are in fact responsible for genetic mutation and molecular transformation controlled by the natural antioxidant defense system of organisms [25]. All aerobic organisms have antioxidant defenses, including enzymes and antioxidant food ingredients, to remove or repair damaged molecules [26]. Antioxidant compounds can eliminate free radicals, and increase the shelf life of nutrients by delaying the process of lipid peroxidation which is one of the main causes of degradation of food and pharmaceutical products during processing and storage [26]. In order to test the antioxidant activity of the studied plant extracts, the following tests were carried out using three methods: the first one is (DPPH) (2,2-diphenyl-1-picrylhydrazyl) which is an organic compound used to measure the antioxidant power of plant extracts. It is reduced by antioxidant compounds in plant extracts, and changes from purple to pale yellow [25]. It has been widely used to assess the free radical scavenging efficiency of various antioxidants. In this test, the antioxidant is able to reduce the stable radical (DPPH) to yellow-colored diphenylpycrylhydrazine. The method is based on the reduction of the radical (DPPH) in the alcoholic solution in the presence of antioxidants which give hydrogen due to the formation of the non-radical form (DPPH-H) in the reaction. (DPPH•) is commonly used as a reagent to assess the free radical scavenging activity of antioxidants. It is a stable free radical and accepts an electron or a hydrogen to become a stable

molecule [26]. The second one is the radical (ABTS) [2,2'-azino-bis (3-ethylbenzothiazolin-6-sulfonic acid), which is a relatively stable free radical, which removes discoloration in its non-radical form. This method is based on the reduction of the color of the methanolic solution of free radicals of ABTS from greenish blue to colorless ; this, given the gain of an electron from another antioxidant compound [26]. Flavonoids and plant phenols in general, considered to be antioxidants, are known to be very effective in removing free radicals. Polyphenols and flavonoids are also used for the prevention and treatment of various diseases mainly related to free radicals [27]. Therefore, there should be antioxidant activity, due to the presence of these compounds in the studied plant.The results of the measurement of the antioxidant activity of plant extracts by the three methods (ABTS, DPPH, CAT) are summarized in Table 2.

Table 2. Values of antioxidant activity via the 3 methods (DPPH, ABTS, CAT) for *L. glomerata.*

Plant	DPPH (mg TE/g extract)	ABTS (mg TE/g extract)	CAT (mg EqAG/g extract)
L. glomerata	80.56	93.65	83.1

Through the results of table (03), we notice that the highest inhibition value is obtained with the free radical (ABTS), and that the values of the inhibition of free radicals, (DPPH) and (CAT), are close. The results of the IC_{50} values for the test (DPPH) for the plants are summarized in Table 3.

Table 3. IC_{50} values for the test (DPPH) of *L. glomerata.*

	Ascorbic acid	*L. glomerata*
IC_{50} (µg/ml)	62.29	286.559
ARP	0.0160	0.003489

ARP = $1/IC_{50}$

By comparing the IC_{50} value for ascorbic acid and the studied plant, the IC50 value for *L. glomerata* is high compared to that for ascorbic acid. By comparing the IC_{50} value for (BHT) and the studied plant shown in Table 4, the results are close. We conclude that radical inhibition of free radicals (ABTS) is better than radical inhibition (DPPH).

Table 4. IC50 values for the (ABTS) test for *L. glomerata.*

	BHT	*L. glomerata*
IC_{50} (µg/ml)	79.50	98.07
ARP	0.0126	0.010

ARP = $1 / IC_{50}$

3.3. Antitumor activity

According to many literature studies, the polyphenols content polyphenol content of many foods like fruits, vegetables and herbal remedies can interfere with several cell signaling pathways [28], based on this, the experiment was done on two types of cells ;Human liver cancer cells (HePG2) and colon cancer cells (HCT116). Where this study of cytotoxic activity test (in vitro bioassay on human tumor cell lines) was conducted and determined by the Bioassay - Cell Culture Laboratory, National Research Centre, Cairo Egypt.

3.3.1. Human hepatic cancer cells HePG2

The sample's concentration varies from (100 to 0.78 µg/ml) using the (MTT) test. The results are presented in Table 5.

Table 5. Results of the hepatocellular carcinoma test for alcoholic extracts of *L. glomerata.*

Plant	LC_{90} (µg/ml)	LC_{50} (µg/ml)	Remarks
L. glomerata	>100	>100	12.3% at 100 ppm
DMSO	>100	>100	1% at 100 ppm
Negative test	>100	>100	0%

LC_{50}: lethal concentration of the sample causing the death of 50% of the cells in 48 hours. LC_{90}: lethal concentration of the sample which causes the death of 90% of the cells in 48 hours.

3.3.2. Colon cancer cells HCT116

The concentration of the sample varies from (100 to 0.78 µg/ml) using the MTT test. The results are presented in Table 6.

Table 6. Results of tests on colon cancer cells for alcoholic extracts of *L. glomerata.*

Plant	LC_{50} (µg/ml)	LC_{90} (µg/ml)	Remarks
L. glomerata	>100	>100	1.3% at 100 ppm
DMSO	>100	>100	1% at 100 ppm
Negative test	>100	>100	0%

LC_{50}: lethal concentration of the sample causing the death of 50 % of the cells in 48 hours. LC_{90}: lethal concentration of the sample which causes the death of 90 % of the cells in 48 hours.

These results showed no response or efficacy of the methanolic extract from the aerial part of this plant, both against hepatocellular carcinomas and against colon cells. These tests against the two cancer cells were the first time done on this plant neither in Algeria, nor in any other country, according to our search in the literature. Experience also indicates that these factors show a biological response to certain serum blood concentrations, insufficient to demonstrate this response in vitro; this also indicates that the evaluation of their bioavailability should not be done in the same way as that of plant extracts in the laboratory, and biological tests of certain animal organisms should be addressed. As is well known, the most recent chemical drugs to treat cancerous tumors are generally, expensive, toxic, and less effective, therefore, it is necessary to consider in more detail the factors derived from natural sources, traditionally described, for the prevention and treatment of cancerous tumors. In addition, other clinical trials are also needed to validate the benefits of these agents, alone or with concomitant treatment [22].

4. CONCLUSION

The present investigation regarding the study of the methanolic extract of the *Launaea glomerata* plant, which there are not many phytochemistry studies about it. The results obtained on this plant show that this plant contained significant amounts of phenols (25.81 mg GAE/g extract) and flavonoids (49.13 mg RE/g extract). The antioxidant activity was evaluated by using three methods DPPH (80.56 mg TE/g extract), CAT (83.1 mg EqAG/g extract) and ABTS (93.65 mg TE/g extract), which considered as valuable results. As for

the hepatocellular and colon carcinoma tests, the extract showed no activity against two types of human hepatocellular carcinoma (HePG2) and colon cancer (HCT116). In addition and due to the importance of this plant according to the results obtained, we will try to examine this plant in depth by introducing it into the world of medical treatments in Algeria, and thus take advantage of its effective elements.

Authors' Contributions: IC and NB conceived and designed the experiment. SB and MM studied and analyzed the data. HD helped sample preparation and data collection. MM and NB wrote the manuscript. AR performed the proof reading and final editing. All authors read and approved the final manuscript.

Conflict of Interest: The authors declare that they have no conflict of interest.

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