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Research Article

Antioxidant Activity of *Bellis Sylvestris Cyr.* Extracts

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ABSTRACT

In the present study, the different solvent extracts viz., ethanol and water of the medicinal plant *Bellis sylvestris* Cyr. (aerial parts) (Asteraceae), were evaluated for phytochemical analysis, and antioxidant activity. The polyphénols and flavonoids contents of *Bellis sylvestris* Cyr. extracts were determined. The highest phenolic content was obtained from ethanolic extract of *Bellis sylvestris* Cyr. (221.323±10.69 µg GAE/mg extract) and lowest in aqueous extract (125.09±9.08 µg GAE/mg extract). As well as the highest concentration of Total flavonoid content was detected in ethanolic extract (27.354±0.26 µg QE/mg extract), followed by the aqueous extract (18.618±0.19 µg QE/mg extract), Ethanolic extract exhibited the highest antioxidant capacity of DPPH(0.053 ± 0.000 mg/mL) followed by the ,aqueous extract of *Bellis sylvestris* (0.234 ± 0.012 mg/mL). All the test extracts showed potent antioxidant capacity in the β-carotene bleaching inhibition test with (85.775% ± 14.87) for ethanolic extract and (75.766% ± 2.51) for aqueous extract, in comparison with BHT(100%). In Conclusion, the present study concludes that the plant *Bellis sylvestris* Cyr. could be exploited for the isolation of bioactive compounds which could be a potential source for antioxidants.

INTRODUCTION

Plant species has remained a good source of anti-infective agents, which are cost-effective and have fewer side effects. Recently the WHO (World Health Organization) estimated that 80% of people worldwide rely on herbal medicines for some aspects [1].

The use of herbal medicine to manage various ailments is a common practice in developing countries, where most people depend on herbal drugs as the major form of treatment [2]. A vast proportion of the available higher plant species have not yet been screened for biologically active compounds; drug discovery from plants should remain an essential component in the search for new medicines.

Herbal medicines have recently attracted much attention as alternative medicines useful for treating or preventing lifestyle-related disorders, but relatively very little knowledge is available about their mode of action.

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There has been a growing interest in the analysis of plant products, which has stimulated intense research on their potential health benefits. So, it is anticipated that plants can provide potential bioactive compounds for the development of new "leads" to combat various diseases [1].

The genus *Bellis* includes eight to fourteen species mainly distributed throughout Europe and the adjacent Mediterranean regions, in addition to certain areas of northern and southern America, Hawaii, and New Zealand, where *B. perennis* L. have become naturalized as an escape from gardening. There are six species of *Bellis* inhabiting the Iberian Peninsula [3].

Bellis species are annual or perennial plants. This species has spathulate or oblanceolate leaves, campanulate involucrum, 1-2 seriate phyllaries, conical receptaculum, 1 seriate ray flowers, white or pink ligules, yellow disc flowers and obovate achenes. The genus has an important role as a source of medicinal plant. At the same time this species has commercial importance as a ornamental plant [4].

Most *Bellis* species are endemic to restricted regions and only *B. perennis* (the cornmon daisy), *B. annua,* and *B. sylvestris* are widespread in their distribution. *Bellis sylvestris* Cyril1, the autumn daisy, is native to the Mediterranean region. Flowering begins in autumn and reaches a maximum in winter [5].

Bellis sylvestris, the southern daisy, a stenomediterranean species belonging to the family of the *Asteraceae*,, is an officinal and edible plant. Its use is highly superimposable to that of another plant of the same genus, *Bellis perennis*. Young leaves are eaten as salad, while leaf and flowers are known for their diuretic, purgative and diaphoretic properties. They also have anti-inflammatory and astringent properties and have been used to treat common cold and infections of the upper respiratory tract in traditional medicine, and the compounds isolated from *Bellis sylvestris* were tested for their antimicrobial activity against some microorganisms associated with urinary tract infections (*Proteus mirabilis*, *Pseudomonas aeruginosa*, *Streptococcus aureus* and *Candida albicans*). The bacterial strains showed variable degrees of susceptibility to the compounds [6].

There is some variation in chromosome numbers in the *Bellis* group even though the diploid number (2n = 18) is most common. The chromosome number and morphology of *Bellis* species of Turkey were analyzed, and the chromosomes numbers are determined as *Bellis sylvestris* Cyr.2n: 36, *Bellis perennis* L.2n: 18 and *Bellis annua* L. 2n: 18 [4].

Four saponins have been isolated from the herbal parts of *Bellis sylvestris* and the same, together with three further compounds, from the underground parts. Among these were the common deacylsaponin of the *Bellis* genus, bellissaponin BS1, and a new saponin called besysaponin C12 [7].

In the present study, solvent extracts such as ethanol and water of *Bellis sylvestris* Cyr. were evaluated for the quantitative phyto-chemical analysis, in vitro antioxidant activity which may lead to the finding of more effective agent for the management of diseases and effective potential source of natural antioxidant that may help in preventing various oxidative stresses.

MATERIALS AND METHODS

1. Chemicals

Folin-Ciocalteu, aluminum chloride ($AlCl_3$), gallic acid, quercetin, 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH), gallic acid and tween 40 were purchased from Sigma Chemical Co. (St. Louis, MO). Linoleic acid, β-carotene and butylated hydroxytoluene (BHT) were obtained from Fluka Chemical Co. (Buchs, Switzerland).

2. Plant Material

Bellis sylvestris plant material was collected from Bougaa région, Wilaya of Sétif Northeast of Algeria .

3. Preparation of Plant Extract

3.1. Aqueous Extract

The aerial parts of plant material were cleaned with tap water, dried in the shade at room temperature for 2 weeks and ground into powder using an electric grinder. The aqueous extract was prepared by boiling 100g of *Bellis sylvestris* powder in distilled water for 15 minutes, the resulting mixture was filtered using Wattman filter paper and then evaporated in rotary vacuum evaporator at 45°C.

3.2. Ethanolic Extract

The ethanolic extract was obtained by maceration in water/ethanol mixture (20:80) for 24 h. The resultant extract was filtered through Whattman paper and the solvent was removed by rotary evaporator under reduced pressure at 45°C.

4. Determination of Total Polyphenol Content

Total phenolic content was determined using Folin-Ciocalteu method, according to Li and al, [8] with slight modifications. A volume of 100 µl of the extract was mixed with 500 µl of Folin–Ciocalteau (diluted 10% in distilled water). After 4 min, 400 µl of sodium carbonate solution Na_2CO_3 (75 g/l) was added to the mixture, the reaction mixture was incubated at room temperature for 1h 30 min and the absorbance of the mixture was measured at 760 nm, Gallic acid (20-140 mg/l) was used as standard for the calibration curve. The total polyphenols content was expressed as micrograms of gallic acid equivalents (GAE) per milligram of extract. All samples were analyzed in three replications.

5. Determination of Total Flavonoids Contents

The total flavonoids in plant extracts were determined using the aluminum trichloride

($AlCl_3$) method [9]. Briefly, 1 ml of 2% $AlCl_3$ in methanol was mixed with 1 ml of the extract. After incubation in dark at room temperature for 10 min, the absorbance of the reaction mixture was measured at 430 nm. Quercetin (1-40 mg/l) were used as standards for calibration curve and the total flavonoids content was expressed as micrograms quercetin equivalents (QE) per milligram of extract.

6. Evaluation of Antioxidant Activity

6.1. DPPH Free Radical-Scavenging Assay

The free radical scavenging activity of the extracts was measured by 2,2- diphenyl-1-picrylhydrazyl (DPPH) assay [10]. After dissolving the extracts, the solution of DPPH in methanol (0.04mg/ mL) was prepared and 1250 µL of this solution was added to 50µL of extracts solution at different concentration and kept in the dark for 30 minutes at room temperature. Then, the absorbance of this solution was measured at 517nm. All tests were performed in triplicate. Radical-scavenging activity was calculated using the following equation:

$$\text{Radical scavenging activity (\%)} = \left(\frac{A_{blank} - A_{sample}}{A_{blank}}\right) \times 100$$

A_{blank}: Absorbance of the control.
A_{sample}: Absorbance of extract.

6.2. β-carotene/Linoleic Acid Assay

In this test, the antioxidant capacity of the extracts was determined according to the method described by Kartal et al. [11]. The β-carotene solution was prepared by dissolving 0.5 mg β-carotene in 1 mL of chloroform, one milliliter of this solution was pipetted to a flask covered with aluminum foil. Then 25 µL of linoleic acid and 200 mg of tween 40 were added in the foil and the chloroform was evaporated using evaporator. After evaporation, 100 mL of distilled water saturated with oxygen was added. Then 2.5 mL of this mixture were transferred to test tubes, and 350 µL of the extracts (2mg/mL methanol) were added and the absorbance was reading at 490 nm after 1h, 2h, 6h, 24h and 48h respectively. The same procedure was repeated with butylated hydroxyl toluene (BHT) as a positive control and with distilled water as a negative control. The antioxidant activity of extracts was calculated using the following equation:

$$AA\% = \frac{A_{sample}}{A_{BHT}} \times 100$$

A_{sample}: Absorbance of the Extract.
A_{BHT}: Absorbance of positive control BHT.

Statistical Analyses

The results are expressed as the mean ± standard deviation. One-way analysis of variance (ANOVA) was performed to assess differences between groups.

RESULTS AND DISCUSSION

1. Total Polyphenols and Flavonoids Contents

Total phenolic content of *Bellis sylvestris* extracts was assessed using the Folin-Ciocalteu assay and the results was expressed as micrograms of gallic acid equivalents (GAE) per milligram dry weight of extracts and Total flavonoids content of *Bellis sylvestris* extracts was determined by the aluminum chloride colorimetric method. The total flavonoid content was expressed as micrograms quercetin equivalents per milligram dry weight of plant extract. The results presented in (Table 1). The highest phenolic content was obtained from ethanolic extract of *Bellis sylvestris* (221.323±10.69 µg GAE/mg extract) and lowest in aqueous extract (125.09±9.08 µg GAE/mg extract). The highest concentration of Total flavonoid conten was detected in ethanolic extract of *Bellis sylvestris* (27.354±0.26 µg QE/mg extract), followed by aqueous extract of *Bellis* (18.618±0.19 µg QE/mg extract).

Table 1: Total polyphenols and flavonoids content of *Bellis sylvestris* extracts.

Extract	Polyphenols	Flavonoids
	µg GAE/mg extract	µg QE/mg extract
AqE	125.09±9.08	18.618±0.19
EE	221.323±10.69	27.354±0.26

AqE : aqueous extract, EE : ethanolic extract, GAE: gallic acid equivalent, QE: quercetin equivalent. Each value represents the mean ± SD (n = 3).

2. Antioxidant Activity Evaluation

2.1. DPPH Radical Scavenging Activity

DPPH is a stable free radical and can be reduced in the presence of an antioxidant molecule. The ability of a molecule to donate a hydrogen atom to a radical determines its antioxidant potentials. DPPH accepts a hydrogen atom from an antioxidant and becomes a stable diamagnetic molecule. Radical scavenging activity using DPPH radical are shown in Table 2 and expressed as

IC_{50}. The highest DPPH radical scavenging ability was observed in ethanolic extract of *Bellis sylvestris* (0.053 ± 0.000 mg/mL).

Table 2: DPPH scavenging activity of *Bellis sylvestris* extracts and standards

Extracts	IC_{50}(mg/mL)
AqE	0.234 ± 0.012
EE	0.053 ± 0.000
Gallic acid	0.001 ± 0.000#
BHT	0.043 ± 0.003#

#: µg/ml. Each value represents the mean ± SD (n = 3).

2.2. β-carotene/Linoleic Acid Bleaching Assay

The antioxidant assay using the discoloration of β-carotene is widely used to measure the antioxidant activity of bioactive compounds. In this assay, oxidation of linoleic acid produces hydroperoxyl radicals evolving toward lipid hydroperoxides, conjugated dienes, and volatile by-products, which simultaneously attack the chromophore of β-carotene, resulting in bleaching of the reaction emulsion [12]. As shown in Table 3, all the extracts showed potent activity. The highest activity was found in ethanolic extract of *Bellis sylvestris* (85.775±14.87 %) folowed by aqueous extract of *Bellis sylvestris* (75.766±2.51%).

Table 3. Antioxidant activities of *Bellis sylvestris* extracts at 24 hours of incubation measured by β-carotene bleaching method.

Extracts	Inhibition %
AqE	75.766 ± 2.51
EE	85.775 ± 14.87
BHT	100 ± 3.972
H_2O	30.91 ± 3.864

Each value represents the mean ± SD (n = 3).

Free radicals are known to play a definite role in a wide variety of pathological manifestations. Antioxidants fight against free radicals and protect us from various diseases. They exert their action either by scavenging the reactive oxygen species or protecting the antioxidant defense mechanisms [13]. Phenolic compounds of plants fall into several categories, chief among these are the flavonoids which have potent antioxidant activities [14]. Therefore, the phenolic compounds have been used in many antioxidant activity assays before biological system. Many methods have been used to determine the antioxidant activity, in which DPPH radical scavenging systems [15-16]. There is a correlation between total polyphenols and antioxidant activity and they are one of the most effective antioxidant constituents of the plant and effective donors of hydrogen to the DPPH radical, because of their ideal structural chemistry [17]. Indeed, not only phenolic compounds which are antioxidant substances par excellence but other non-phenolic substances which can be more effective and powerful antioxidants [18]. The basis of β-carotene/linoleic acid assay is discoloration of β-carotene in reaction with linoleic acid free radical. That radical is formed at elevated temperature upon removal of hydrogen atom located between two double bonds of linoleic acid. The pentadienyl free radical so formed then attacks highly unsaturated b-carotene molecules in an effort to reacquire a hydrogen atom. As the b-carotene molecules lose their conjugation, the carotenoids lose their characteristic orange colour. Fortunately, this process can be monitored spectrophotometrically. The presence of a phenolic antioxidant can hinder the extent of b-carotene destruction by ‘‘neutralizing’’ the linoleate free radical (i.e. utilizing its redox potential) and any other free radicals formed within the system. Hence, this forms the basis by which plant extracts can be screened for their antioxidant potential [19].

The ethanolic and aqueous extracts of *Bellis sylvestris* exhibited antioxidant activity may be due to the presence of flavonoids, Tannins, Phenol, Saponins, and Terpenoids.

CONCLUSION

The result of the present study showed that the extract of *Bellis sylvestris*, which contain highest amount of flavonoid and phenolic compounds, exhibited the greatest antioxidant activity.

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