Indian Journal of Novel Drug Delivery

IJNDD

An Official Publication of Karnataka Education and Scientific Society

Research Article

Free Radical Scavenging and Antioxidant Properties of *Helichrysum arenarium* L. Extracts

MOUNIRA MERGHEM*

Laboratory of Phytotherapy Applied to Chronic Diseases, Faculty of Natur and Life Sciences, Ferhat Abbas University, Sétif-1, Algeria.

ARTICLE DETAILS

Article history:
Received on 28 February 2022
Modified on 11 March 2022
Accepted on 16 March 2022

Keywords:
Helichrysum arenarium,
Antioxidant Activity,
Polyphenols,
DPPH Scavenging,
β-carotene.

ABSTRACT

Helichrysum is a well-known species in the Astéracées family. It contains many species, including Helichrysum arenarium L, which is used in traditional medicine because of its active compounds. The aim of this study is to evaluate the antioxidant activity of the aqueous and ethanolic extracts of Helichrysum arenarium L, the results showed that the total polyphenolic content of the ethanolic extract was 224.39±7.71 µg GAE/mg extract and flavonoids was 28.4±2.343 µg QE/mg extract. The total polyphenolic content of the aqueous extract was 211.81±20.43 µg GAE/mg extract and flavonoids were 26.64±0.36 µg QE/mg extract. The antioxidant activity was determined *in vitro* using two methodes: DPPH free radical scavenging and ß-Caroténe linoleic acid. In the DPPH assay, ethanolic extract showed the highest scavenging capacity (IC_{50} = 0.036 ± 0.000 mg/ml), followed by aqueous extract with IC_{50} of 0.065± 0.000 mg/ml. While the ß-Caroténe /linoleic acid test showed that the aqueous extract had a greater activity compared to the ethanolic extract (85.584 ± 13.48%, 67.049 ± 8.37%, respectively).

INTRODUCTION

Oxidative stress can cause a wide range of diseases including Alzheimer's and Parkinson's, diabetes, cardiovascular disease, rheumatoid arthritis, cancer and other diseases [1]. The condition is brought by excessive generation of free oxygen and nitrogen species or their inefficient quenching in the cell. Free oxygen and nitrogen species are unstable molecules that are present in the environment (exogenous) and generated in the body (endogenous) during the normal aerobic metabolic processes in the body [2].

For many years, traditional medicinal plants have been shown be an alternative source of antioxidants potentially for treating diseases in tropical and developing countries [3, 4]. A variety of phytochemicals from the plant extracts, including terpenoids, phenolic acids, lignans, tannins flavonoids, quinones, coumarins or alkaloids, has been reported for antioxidant activity [5, 6].

Author for Correspondence:
Email: mouniramerghem@yahoo.fr

Currently, antioxidants can be obtained synthetically and naturally. The synthetic antioxidants have restriction for use, as they are suspected to be carcinogenic. Therefore, the importance of searching for and exploiting natural antioxidants has increased greatly in present years [7].

The genus *Helichrysum* (family Asteraceae) commonly used in folk medicine [8]. *Helichrysum arenarium* L. is one of the most widely consumed plants among different species of the genus *Helichrysum* [9]. *Helichrysum arenarium* has long been used in traditional medicine as an herbal tea for the treatment of various health-related problems such as fever and nervousness, as well as gall-bladder and urinary-tract diseases. Recently, the World Health Organization (WHO) and European Medicines Agency (EMA) have officially approved the use of *Helichrysum arenarium* infusions and tinctures for the treatment of digestive disorders [9, 10].

MATERIALS AND METHODS

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).

Plant Material

Helichrysum arenarium was collected from Bougaa région, Wilaya of Sétif Northeast of Algeria.

Preparation of Plant Extract

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 *Helichrysum arenarium* powder in distilled water for 15 minutes, the resulting mixture was filtered using Whatman Filter Paper and then evaporated in rotary vacuum evaporator at 45°C.

Ethanolic Extract

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

Determination of Total Polyphenol Content

Total phenolic content was determined using Folin-Ciocalteu method, according to Li et al [11], 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.

Determination of Total Flavonoids Contents

The total flavonoids in plant extracts were determined using the aluminum trichloride ($AlCl_3$) method [12]. Briefly, 1 ml of 2% $AlCl_3$ in methanol was mixed with 1 ml of the extract. After 10 min of incubation in dark at room temperature the absorbance of the reaction mixture was measured at 430 nm. Quercetin (1-40 mg/l) was used as standard for calibration curve and the total flavonoids content were expressed as micrograms quercetin equivalents (QE) per milligram of extract.

Evaluation of Antioxidant Activity

DPPH Free Radical-Scavenging Assay

The free radical scavenging activity of the extracts was measured by 2,2- diphenyl-1-picrylhydrazyl (DPPH) assay [13]. 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:

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

Where,

A_{blank}: Absorbance of the control.

A_{sample}: Absorbance of extract.

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. [14]. 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 and methanol as a negative control. The antioxidant activity of extracts was calculated using the following equation:

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

Where,

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

Total Polyphenols and Flavonoids Contents

Total Polyphenols were estimated using Folin-Ciocalteau method. Gallic acid was used as the standard and the polyphenols content is expressed in micrograms of gallic acid equivalents per milligrams of extract (µg GAE/mgE). Total flavonoids were carried out according to the method of aluminum trichloride (AlCl3) using quercetin as standard, the flavonoid content is expressed in micrograms of quercetin equivalent per milligrams of extract (µg QE/mgE). The results are recorded in the Table 1. The content of total polyphenols 224.39±7.71 µg GAE/mg extract and flavonoids 28.4±2.343 µg QE/mg extract of the ethanolic extract is higher than the aqueous extract 211.81±20.43 and 26.64±0.36 respectively. This is due to the different type of solvent which affected the yield of total phenols and flavonoids.

Table 1: Total polyphenols and flavonoids content of *Helichrysum arenarium* extracts.

Extract	Polyphenols	Flavonoids
	µg GAE/mg extract	µg QE/mg extract
AqE	211.81±20.43	26.64±0.36
EE	224.39±7.71	28.4±2.343

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

Antioxidant Activity Evaluation

DPPH Radical Scavenging Activity

IC_{50} was calculated for the extracts of *Helichrysum arenarium* L. which is the concentration of substrate that causes 50% loss of DPPH activity (color) [15], the lower IC_{50} value indicates strong ability of the extract to act as DPPH scavenger. The results are presented in Table 2, the ethanolic extract exhibited the highest anti-free radical activity with IC_{50} values of 0.036±0.000 mg/ml followed by aqueous extract with (0.065±0.000 mg/ml). This is due to that the extracts possess many effective compounds, such as phenolic compounds, which include the most prominent antioxidants as flavonoids**.**

These results are similar to those obtained by Bektas et al [16], which showed that DPPH radical-scavenging activity exhibited by methanol extract of *Helichrysum arenarium* was (IC_{50} = 0.047 mg/mL).

Table 2: DPPH scavenging activity of *Helichrysum arenarium* extracts and standards

Extracts	IC_{50}(mg/mL)
AqE	0.065± 0.000
EE	0.036 ± 0.000
Gallic acid	0.001 ± 0.000#
BHT	0.043 ± 0.003#

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

β-carotene/Linoleic Acid Bleaching Assay

The presence of antioxidants could neutralize free radicals derived from linoleic acid and thus prevent the oxidation and bleaching of β -carotene. The results of β-carotene bleaching at 24 hours in the presence of the extract of *Helichrysum arenarium* and the standard antioxidant are shown in Table 3. According to these results, it is obvious that the standard and the extracts tested inhibit the coupled oxidation of the acid linoleic acid and β-carotene compared to the negative control, the highest inhibition value was for BHT, which used as a control, followed by the aqueous extract with an antioxidant activity (85.584±13.48%), and ethanolic extract (67.049±8.37).

When compared with the results obtained by Bektas et al [16], for a methanolic extract of *Helichrysum arenarium* L. which chowed that the inhibition of ß-carotène linoleic acid oxidation was estimated at 57%. There is a slight difference with ethanolic extract of this study.

Table 3: Antioxidant activities of *Helichrysum arenarium* extracts at 24 hours of incubation measured by β-carotene bleaching method

Extracts	Inhibition %
AqE	85.584 ± 13.48
EE	67.049 ± 8.37
BHT	100 ± 3.972
H2O	30.91 ± 3.864

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

Increased production of reactive oxygen/nitrogen species and decreased capacity of antioxidant defenses in the body leads to oxidative stress [17, 18]. The body has several mechanisms to counteract this oxidative stress

by producing antioxidants, either naturally generated in situ (endogenous antioxidants), or externally supplied through foods (exogenous antioxidants). The roles of antioxidants are to neutralize the excess of free radicals, to protect the cells against their toxic effects and to contribute to disease prevention [19]. Moreover, medicinal plants have been shown to have antioxidant activity by virtue of the presence of phenolic diterpenes, flavonoids, tannins and phenolic acids [20]. Literature surveys indicate that the plant phenolics constitute one of the major groups of compounds acting as primary antioxidants [21].

According to study of Czinner et al [8], *Helichrysum arenarium* L. plant contains the flavonoids, Apigenin, naringenin, kaempferol, luteolin , quercetin, caffeic acid aglycones, and naringenin-5-*O*-glucoside (helichrysin), apigenin-7-*O*-glucoside, kaempferol-3-*O*-glucoside (trace), luteolin- 7-*O*-glucoside, quercetin-3-*O*-glucoside.

CONCLUSION

This work showed a richness of *Helichrysum arenarium* L. plant in phenolic compounds and flavonoids. Moreover, the results obtained showed that the extract gives good antioxidant activity with free radical scavenging ability and excellent inhibitory effect of lipid peroxidation, these results suggests that this plant can be used as an easily accessible source of natural antioxidants.

REFERENCES

[1] Behnaz H, Azizah AH, Roheeyati AM, Norihan MS, Ahmad SA, Alfi K and Sabu MC. Antioxidant activity of different extracts from leaves of *Pereskia bleo* (Cactaceae). Journal of Medicinal Plants Research. 2012; 6(15): 2932-2937.

[2] Bhat AH, Dar KB, Anees S. Oxidative stress, mitochondrial dysfunction and neurodegenerative diseases; a mechanistic insight. *Biomedicine & Pharmacotherapy*. 2015; 74: 101–110.

[3] Kuete V, Kamga J, Sandjo L, Ngameni B, Poumale H, Ambassa P and Ngadjui B. Antimicrobial activities of the methanol extract, fractions and compounds from *Ficus polita* Vahl. (Moraceae). *BMC Complementary and Alternative Medicine*. 2011; *11*: 1-6.

[4] Saeed A, Sultana B, Anwar F, Mushtaq M, Alkharfy KM and Gilani AH. Antioxidant and antimutagenic potential of seeds and pods of green cardamom (*Elettaria cardamomum*). *International Journal of Pharmacology*. 2014; *10:* 461-469.

[5] Bakkali F, Averbeck S, Averbeck D and Idaomar M. Biological effects of essential oils -A review. *Food and Chemical Toxicology*. 2008; *46*: 446-475.

[6] Tagne RS, Telefo BP, Nyemb JN, Yemele DM, Njina SN, Goka SMC. Anticancer and antioxidant activities of methanol extracts and fractions of some Cameroonian medicinal plants. *Asian Pacific Journal of Tropical Medicine*. 2014; 7(1): 442-447.

[7] Mervat MM, El Far and Hanan AA ,Taie. Antioxidant activities, total anthrocynins, phenolics and flavonoids contents of some sweet potato genotypes under stress of different concentrations of sucrose and sorbitol. Aust. J. Basic Appl. Sci. 2009; 3:3609-3616.

[8] Czinner E, Hagymasi K, Blazovics A, Kery A, Szoke E, Lemberkovics E. *In vitro* antioxidant properties of *Helichrysum arenarium* (L.) Moench. Journal of Ethnopharmacology. 2000; 73: 437–443.

[9] Akaberi M, Sahebkar A, Azizi N, Emami SA. Everlasting flowers: phytochemistry and pharmacology of the genus Helichrysum. Ind. Crops Prod. 2019; 138- 111471.

[10] Les F, Venditti A, Casedas G, Frezza C, Guiso M, Sciubba F, Serafini M, Bianco A, Valero MS, Lopez V. Everlasting flower (Helichrysum stoechas Moench) as a potential source of bioactive molecules with antiproliferative, antioxidant, antidiabetic and neuroprotective properties. Ind. Crop. Prod. 2017. 108: 295–302.

[11] Li HB, Cheng KW, Wong CC, Fan KW, Chen F, Jiang Y. Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. Food Chemistry. 2007; 102:771-776.

[12] Bahorun T, Gressier B, Trotin F, Brunete C, Dine T, Vasseur J, Gazin JC, Pinkas M, Luycky M, Gazin M. Oxygen species scavenging activity of phenolic extract from Hawthorn fresh plant organs and pharmaceutical preparation. Drug Research. 1996; 16.

[13] Burits M, Bucar F. Antioxidant activity of *Nigella sativa* essential oil. *Phytotherapy Researc.* 2000; 14:323-328.

[14] Kartal N, Sokmen M, Tepe B, Daferera D, Polissiou M, Sokmen A. Investigation of the

antioxidant properties of *Ferula orientalis* L. using a suitable extraction procedure. *Food chemistry.* 2007; 100:584–589.

[15] Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J Sci Technol. 2004*;* 26 (2): 211-219.

[16] Bektas T, Munevver S, Askin AH, Atalay S. *In vitro* antioxidant activities of the methanol extracts of four Helichrysum species from Turkey. Food Chemistry. 2005; 90: 685–689.

[17] Fidrianny I, Ramadhani S, and Komar R. *In vitro* antioxidant capacities of three organs of bitter gourd (Momordica charantia L.) form west Java-Indonesia using DPPH and FRAP assays. *International Journal of Pharmacognosy and Phytochemical Research*. 2015; 7(5): 1034–1041.

[18] Ferry M and Roussel AM. Micronutrient status and cognitive decline in ageing. *European Geriatric Medicine*. 2011; 2(1): 15–21.

[19] Pham-Huy LA, He H, Pham-Huyc C. Free Radicals, Antioxidants in Disease and Health. J. Biomed Sci. 2008; 4(2): 89-96.

[20] Dawidowicz AL, Wianowska D, Baraniak B. The antioxidant properties of alcoholic extracts from *Sambucus nigra* L. (antioxidant properties of extracts). Lebensmittel-Wissenschaft und Technologic. 2006; 39: 308–315.

[21] Pietta PG. Flavonoids as antioxidants. Journal of Natural Products. 2000; 63: 1035–1042.