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Original Research Article

In vitro antioxidant activities of *n*-butanol extract of *Helianthemum confertum*

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Abstract

Antioxidants play an important role to protect damage caused by oxidative stress. Plants having phenolic contents are reported to possess antioxidant properties. The basic aim of this research was to investigate the antioxidant properties of *n*-butanol extract of *Helianthemum confertum*, Cistaceae family.

The antioxidant activities and phenolic contents of *n*-butanol extract were evaluated in vitro using spectrophotometer methods. There antioxidant activities were determined by DPPH (1,1-diphenyl-2-picrylhydrazine) radical scavenging assay and lipid peroxidation inhibition by TBARS method. Antioxidant activities were compared to ascorbic acid. Measurement of total phenolic compounds and total flavonoids content of the n-butanol extract of Helianthemum confertum were achieved using Folin-Ciocalteau reagent and AlCl3 respectively. The results showed that this extract containing 263.33±19.85µg of gallic acid equivalents/mg extract of total phenolic and 25.35±3.15µg of quercetin equivalents/mg extract total flavonoids.

Keywords: Helianthemum confertum, antioxidants, DPPH, Lipid peroxidation, Free radical scavenging capacity.

Introduction

Interest in the search for new natural antioxidants has grown over the past years because reactive oxygen species (ROS) production and oxidative stress have been shown to be linked to diseases such as cancer, cardiovascular diseases, type 2 diabetes and obesity [1,2].

It is well known that free radicals cause cell damage through mechanisms of covalent binding and lipid peroxidation with subsequent tissue injury [3]**.** Therefore increasingly interest on the use of antioxidants, especially natural antioxidants to inhibit lipid peroxidation and to protect damage due to free radicals [4].

Medicinal plants used in the traditional medicine and healing are one of these sources of antioxidants; these plants contain several different pharmacologically active compounds that may act individually, additively or in synergy to improve health [5, 6].

Plants are richly supplied with flavonoids, phenols that are strong antioxidants, although the phenolic compounds are commonly found in both edible and non edible herbs. Scientific information on antioxidant properties of endemic plants, limited to certain regions and known only by local populations, is still rather scarce [7, 8].

In the present study we screened the *in vitro* antioxidant activity of *n*-butanol extract of *Helianthemum confertum*. The effects of this extract on l, l-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, lipid peroxidation and the total phenol and flavonoid contents were analyzed using spectrophotometer.

Materials and methods

Plant materiel

The plant material was collected from the area of Mogheul in the south-west of Algeria and authenticated by M. Mohamed Benabdelhakem, director of the nature preservation agency, Bechar [9]. A voucher specimen (HCC0512-MOG-ALG-60) has been deposited at the Herbarium of the VARENBIOMOL research unit, University of Frères Mentouri Constantine.

Extraction procedure

Air-dried aerial parts (2279 g) of *Helianthemum confertum* Dunal, non Willk. (Cistaceae) were macerated at room temperature with MeOH–H_2O (80:20, v/v) for 72 h, three times. After filtration, the filtrate was concentrated in vacuum (up to 35 C) and dissolved in distilled H_2O (900 ml) under magnetic stirring and then put at the refrigerator for one night. After filtration, the resulting solution was extracted successively with petroleum ether, $CHCl_3$, EtOAc and *n*-butanol. The organic phases were dried with Na_2SO_4, filtered using common filter paper and concentrated in vacuum (35 C) to obtain the following extracts: petroleum ether (135 mg), $CHCl_3$ (1.65 g), EtOAc (7.11 g) and *n*-butanol (35.07 g).

Total phenolic content assay

The total phenolic content was quantified using a modified version of the assay described by Singleton et al. [10] using Folin-Ciocalteu reagent. Twenty µl of *n*-butanol extract from *Helianthemum confertum* (1mg/ml) or gallic acid (standard phenolic compound) were diluted with 1580 µl of distilled water and then mixed with 100 µl of 2 N Folin-Ciocalteu reagents. The mixture was shaken and kept for 6 minutes, after 300 µl of 20% aqueous Na2CO3 solution were added and mixed properly. The mixture was incubated for 2 hours at 20 C. The absorbance was measured for all the samples at 765 nm. All tests were performed in triplicate. Gallic acid was used as a standard. The standard curve was prepared using 0, 50, 100, 150, 200, 250, 500 mg /l solutions of gallic acid in methanol: water (10:90, v/v). The concentration of total phenolic compounds was determined as µg of gallic acid equivalents per 1 mg of extract.

Determination of total flavonoid content

Total flavonoid content was estimated according to the method described by Wang et al. [11]. Briefly, to 0.5 ml of sample, 0.5 ml of 2% AlCl3 methanol solution was added. After 1 h incubation at room temperature, the absorbance was measured at 420 nm. Total flavonoid content was calculated as µg of quercetin equivalents (QE) per 1 mg of extract using the following equation obtained from a standard quercetin (R = 0,983)

Absorbance = 0, 34 Quercetin (μg) + 0.015.

DPPH radical-scavenging activity assay

The hydrogen atoms or electron donation ability of the extract was measured from the bleaching of a purple colored methanol solution of DPPH [12]. This Spectrophotometric assay used the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical as a reagent following the method described by Braca et al., (2001) [13]. Various concentrations (1, 2.5, 5, 10, 15, 20, 30, 50, 75 µg/ml) of the extract in methanol were added to 3 ml of a 0.004% methanol solution of DPPH. After an incubation period for 30min in the dark and at room temperature, the absorbance was read against a blank at 517 nm. Inhibition of free radical DPPH in percent (I %) was calculated using the following equation:

I % = (Ac- As/Ac)*100

Where Ac is the absorbance of the control reaction, and As is the absorbance of the extract. Ascorbic acid was used as standard.

Assays of lipid peroxidation using vitellose

The inhibition of lipid peroxidation was determined by quantification of MDA decomposed from the lipid peroxide, which is based on the egg vitellose reacting to thiobarbituric acid. For the *in vitro* studie, the fresh vitellose was dissected and homogenized in ice cold PSB (20 Mm, pH 7.4) to produce a 10% homogenate (v/v). The homogenate was centrifuged at 4000 rpm for 20 min. to remove precipitation, 1ml aliquots of the supernatant were incubated with the extract in the presence of 5 mM FeSO4 at 37 C for 1 h. The reaction was stopped by addition of 1 ml trichloroacetic acid (TCA, 20%, w/v) and 1.5 ml thiobarbituric acid (TBA, 1%, w/v) in succession, and the solution was then heated at 100 C for 15 min. After centrifugation at 4000 rpm for 20 min to remove precipitated protein, the color of the complex was detected at 532 nm. The control group was run in parallel without sample under similar conditions, except that 1 ml trichloroacetic acid (TCA, 20%, w/v) was added before incubation and all measurements were done in triplicate. The lipid peroxidation scavenging activity (K %) was calculated by the following equation:

K (%) =Ac-As/Ac x 100 [14].

Where Ac was the absorbance of the control, and As was the absorbance of sample.

Results and discussion

Total phenolic content

Polyphenols help in protecting cells against oxidative damage caused by free radicals due to their redox properties, which enable them to act as reducing agents, hydrogen donors and singlet oxygen quenchers [15]. This high potential of phenolic compounds to scavenge radical may be explained by their phenolic hydroxyl groups. The *n*-butanol extract of *Helianthemum confertum* exhibited higher phenolic content (263. 33±19.85µg extract of gallic acid equivalents/mg extract) (Table 1).

Table 1. The phenolic and flavonoid contents of *n*-butanol extract of *Helianthemum confertum*

Species	Extract/Fraction	µg GAE/mg extract	µg QE /mg
Helianthemum confertum	*n*-butanol	263.33± 19.85	25.35± 3.15

Total flavonoid content

Flavonoids are a class of phenolic compounds that remain of great scientific and therapeutic interest, and their antioxidant activity has attracted most attention. Their high antioxidant potential is due to their capacity to scavenge free radicals that originate from various cellular activities and lead to oxidative stress [16]. In the present study, total flavonoid content present in the extract was found to be25.35±3.15µg quercetin equivalent/mg extracts (Table 1).

DPPH radical scavenging activity

In DPPH radical scavenging assay, antioxidants react with DPPH deep violet color and convert it to yellow colored. In this study *n*-butanol extract of *Helianthemum confertum* presented concentration dependent hydrogen donating ability as shown in Figure1. DPPH scavenging was increased in a concentration dependent manner compared to ascorbic acid. The IC 50 value was found to be 26, 27±0.73 µg/ml while IC50 value for ascorbic

acid was 5µg/ml. The scavenging effect of *n*-butanol extract and vitamin C on the DPPH radical at the concentration of 25µg/ml, result the inhibition 96%, 49.84% respectively.

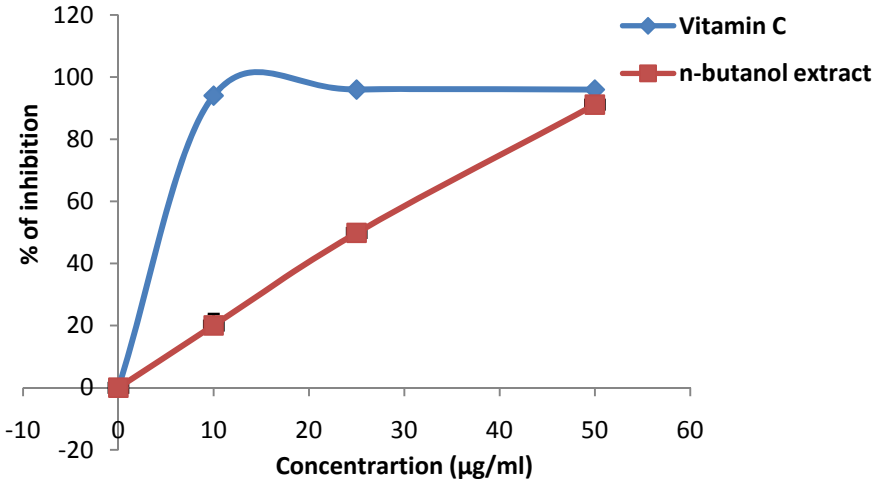

Figure1. DPPH radical scavenging activity of *Helianthemum confertum* and Vitamin C (mean±SD, n=3)

Inhibition of lipid peroxidation assay

Lipid peroxidation is very important parameter to determine the total antioxidant potential of the plant. Lipid peroxidation involves the formation and propagation of lipid radicals as malondialdehyde (MDA) with numerous deleterious effects, including destruction of membrane lipids, metabolic disorders and inflammation [15]. In this study, we measured the potential of *Helianthemum confertum* extract to inhibit lipid peroxidation in egg vitellose homogenate. As shown in Figure 2 witch demonstrated the ability of the extract to inhibit the formation of TBARS in a concentration dependent manner. The IC50 was found to be 242.15±8.25µg/ml as compared to standard ascorbic acid (20±1.25µg/ml). Plant extract showed good inhibition of lipid peroxidation. Our previous study has demostrated using other plants extracts (Genista *quadriflora* and *Chrysanthemun fantaneisii*) that these extract contributed sinficantly to the inhibition of lipid peroxidation and the scavenging effect on the DPPH radical [4,17].

The percentage inhibition of lipid peroxidation (0.3 mg/ml butanol extract of *Helianthemum confertum*) was found to be 60.88%. The ratio at 0.1 mg/ml for vitamin C was found to be 86.95% inhibition of lipid peroxidation.

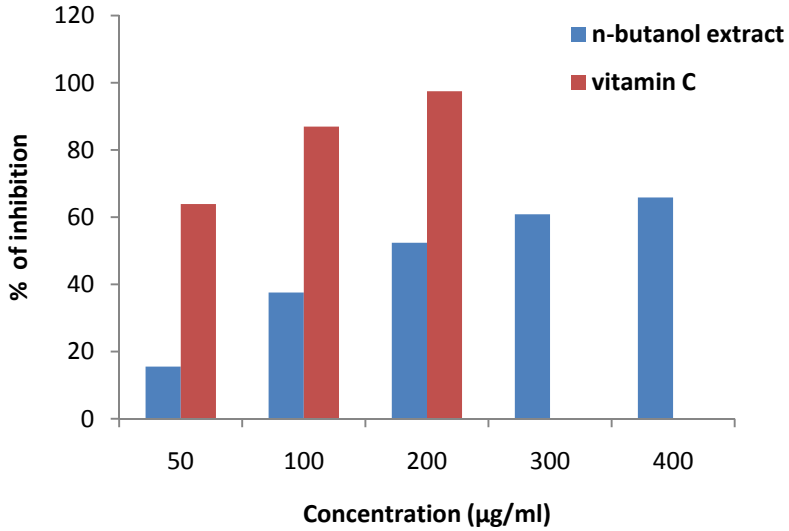

Figure 2. Inhibition of lipid peroxidation by *n*-butanol extract of *Helianthemum confertum* and Vitamin C (mean±SD, n=3)

Conclusion

This work has gathered experimental evidence that *Helianthemum confertum* extract contained substantial amount of polyphenols and flavonoids and exhibited significant antioxidant activity by effectively scavenging various free radicals. The antioxidant might be due to the synergistic actions of bioactive compounds present in them. Therefore, the plant has promising compounds to be tested as potential antioxidant drugs for treatment of diseases resulting from oxidative stress. However, these findings warrant extensive studies on chemical profiles and mechanistic action of antioxidant activities. The study will be helpful to understand this herbal medicine.

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