In Vitro Antioxidant Activity, Phenolic and Flavonoid Contents of Different Polarity Extracts from *Chrysanthemum segetum* L. Growing in Algeria

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Available Online: 10th September, 2016

ABSTRACT

In the present study, we report the evaluation of antioxidant properties and phenolic and flavonoid contents of *Chrysanthemum segetum* L. growing in Algeria. The chloroform (CHCl₃), ethyl acetate (AcOEt) and n-butanol (n-BuOH) extracts prepared from the aqueous EtOH extract of the flowers, were tested using two methods of antioxidant assays: 2, 2'- diphenyl-1-picrylhydrazyl (DPPH) and cupric ion reducing antioxidant capacity (CUPRAC). The results showed that EtOAc extract which had the highest level of polyphenol and flavonoid contents (216.18±12.97 mgGAE/g and 126.64±11.35 mgQE/g respectively), exhibited the most potent antioxidant capacity in each assays, showing the highest IC₅₀ of DPPH scavenging activity (23.58 µg/mL) and the highest A₅₀ of CUPRAC capacity (14.85 µg/mL) compared to the n-BuOH extract and CHCl₃ which was the weakest extract. This study suggested that the differences of the potency of the antioxidant activity may be explained by the differences in the polyphenol and flavonoid levels.

Keywords: Antioxidant activity, DPPH, CUPRAC, Polyphenolic, flavonoids, *Chrysanthemum segetum*.

INTRODUCTION

Antioxidants play an important role to protect against damage caused by oxidative stress. Plants having phenolic contents are reported to possess antioxidant properties. The genus *Chrysanthemum*, also known as golden flower, comprises about 300 species¹. All species are distributed in two main centers, one in the East Asia, the other in the Mediterranean area, particularly in the Canary Islands and Algeria²³. In Algeria this genus includes twenty species with eight endemic⁴. The species of the genus *Chrysanthemum* are rich source of secondary metabolites with a variety of biological activities⁵⁻¹⁰ such as antibacterial and antiviral properties¹¹⁻¹². *Chrysanthemum segetum* is an archaephyte, which origins from the Mediterranean area¹³, it is widely distributed in the Tell of Algeria⁴. To the best of our knowledge, antioxidant activities of *C. segetum* have never been reported. Only some studies have been down about isolation and characterization of flavonoids¹⁴, coumarins¹⁵ and polyacetylenes¹⁶⁻¹⁸.

MATERIALS AND METHODS

Plant material

The aerial parts of *Chrysanthemum segetum*, were collected on June 2014 from the area of El Kala in the provinces. The basic aim of this work was to investigate the antioxidant potential of three extracts (chloroform, ethyl acetate and n-butanol extracts) of *Chrysanthemum segetum* of Asteraceae family. East of Algeria and authenticated by Professor M. Kaabeche (Biologie Department, University of Setif, Algeria). A voucher specimen has been deposited in the Herbarium of the VARENBIOMOL research unit, Université des Frères Mentouri Constantine.

Extraction and isolation

Air-dried flowers (1500 g) of *Chrysanthemum segetum* were macerated at room temperature with EtOH/H₂O (80:20 v/v) for 24 h, three times. After filtration, the filtrates were combined, concentrated in vacuum (up to 35°C) and dissolved in distilled H₂O (600 ml) under magnetic stirring and then put at the refrigerator for one night. After filtration, the resulting solution was successively extracted several times with CHCl₃, EtOAc and n-BuOH. The organic phases were dried with Na₂SO₄, filtered using filter paper and concentrated in vacuum (35°C) to obtain the following extracts: chloroform (3.27 g), EtOAc (11.97 g) and n-Butanol (29.90 g).

Determination of total bioactive compounds

Determination of total phenolic content (TPC)
TPC of the extracts of *C. segetum* were determined spectrophotometrically following the Folin–Ciocalteu method\(^{19}\). The absorbance was read at wavelength 765 nm. Analysis was done in triplicate for each extract.

Standard solutions of gallic acid with concentration 25-200 µg/mL were used to obtain a standard curve. The TPC was reported as a percentage of total gallic acid equivalents per 100 g of extract (mg gallic acid...
Determination of total flavonoid content (TFC)
TFC of the extracts of *C. segetum* were determined spectrophotometrically as previously reported\(^5\). The absorbance was read at wavelength 415 nm. Analysis was done in triplicate for each extract. Standard solutions of quercetin with concentration 25-200 µg/mL were used to obtain a standard curve. The TFC was reported as a percentage of total quercetin equivalents per 100 g of extract (mg QE/g).

**Antioxidant capacity of C. segetum**

**DPH scavenging assay**
The DPPH scavenging activity was determined spectrophotometrically by the method described by Blois\(^1\). α-Tocopherol, BHT, BHA, were used as antioxidant standards for comparison of the activity. The results were given as 50% inhibition concentration (IC\(_{50}\)).

**Cupric reducing antioxidant capacity (CUPRAC)**
The cupric reducing antioxidant capacity was determined according to the method of CUPRAC assay. Parallel to the results of CUPRAC, DPPH radicals were found for EtOAc extract which neutralized 50% of free radicals at the concentration of 23.58 µg/mL. A moderate activity was found for chloroform extract. In comparison to IC\(_{50}\) values of BHT and α-Tocopherol, EtOAc extract exhibited the strongest capacity for neutralization of DPPH radicals. The results of CUPRAC assays of the extracts, compared with those of BHT and BHA (Table 2 and Figure 2), showed that activity (absorbance) increased linearly with the increasing amount of extracts. The ethyl acetate extract manifested the highest activity (A\(_{50}\) value: 14.85±2.45), followed by n-butanol (A\(_{50}\) value: 24.57±2.80) and chloroform extract (A\(_{50}\) value: 42.97±5.71). However, none of the extracts exhibited higher activity than those of antioxidant standards.

**RESULTS AND DISCUSSION**

**Determination of total bioactive compounds**
TPC in various extracts were demonstrated in term of gallic acid equivalent using the standard curve equation y = 0.002x + 0.045, R\(^2\) = 0.997. TPC in various extracts of *C. segetum* showed different result ranged from 42.70 to 216.18 mg GAE/g. Ethyl acetate had the highest phenolic content (216.18 mg GAE/g) (Table 1 and Figure 1). TFC in the various extracts were demonstrated in term of quercetin equivalent using the standard curve equation y = 0.006x + 0.017, R\(^2\) = 0.976. TFC in the different extracts showed result ranged from 28.42 to 126.64 mg QE/g (Table 1 and Figure 1). Ethyl acetate extract had the highest TFC (126.64 mg QE/g) and the lowest (28.42 mg QE/g) was given by chloroform extract.

**Antioxidant activity**
There was no previous study regarding antioxidant activity of the three different polarity extracts (chloroform, ethyl acetate and n-butanol) from *C. segetum*. Two methods were selected to evaluate the antioxidant properties of the extracts, DPPH free radical scavenging activity which measures the ability of electron transfer to the media and the CUPRAC method which also measures electron transferring of the antioxidant. In
ACKNOWLEDGEMENTS
The authors thank the Center of Biotechnology Research, Division of Health, Laboratory of Biochemistry, Constantine, Algeria.

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