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Research Article

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

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ABSTRACT
In the present study, we reporte the evaluation of antioxidant properties and phenolic and favonoid contents of *Chrysanthemum segetum* L. growing in Algeria. The chloroform ($CHCl_3$), 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 IC_{50} of DPPH scavenging activity (23.58 µg/mL) and the highest A_{050} of CUPRAC capacity (14.85 µg/mL) compared to the *n*-BuOH extract and $CHCl_3$ 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
The genus *Chrysanthemum*, also known as golden flower, comprises about 300 species[1]. 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 Algeria[2,3]. In Algeria this genus includes twenty species with eight endemic[4]. The species of the genus *Chrysanthemum* are rich source of secondary metabolites with a variety of biological activities[5-10] such as antibacterial and antiviral properties[11-12]. *Chrysanthemum segetum* is an archaeophyte, which origins from the Mediterranean area[13], it is widely distributed in the Tell of Algeria[4]. 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 flavonoids[14], coumarins[15] and polyacetylenes[16-18].

MATERIALS AND METHODS

Plant material

The aerial parts of *Chrysanthemum segetum*, were collected on June 2014 from the area of El Kala in the properties. 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 (Biology Department, University of Setif, Algeria). A voucher specimen has been deposited in the Herbarium of the VARENBIOMOL research unit, Universite des Frères Mentouri Constantine.

Extraction and isolation

Air-dried flowers (1500 g) of *Chrysanthemum segetum* were macerated at room temperature with EtOH/H_2O (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 H_2O (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 $CHCl_3$, EtOAc and *n*-BuOH. The organic phases were dried with Na_2SO_4, 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)

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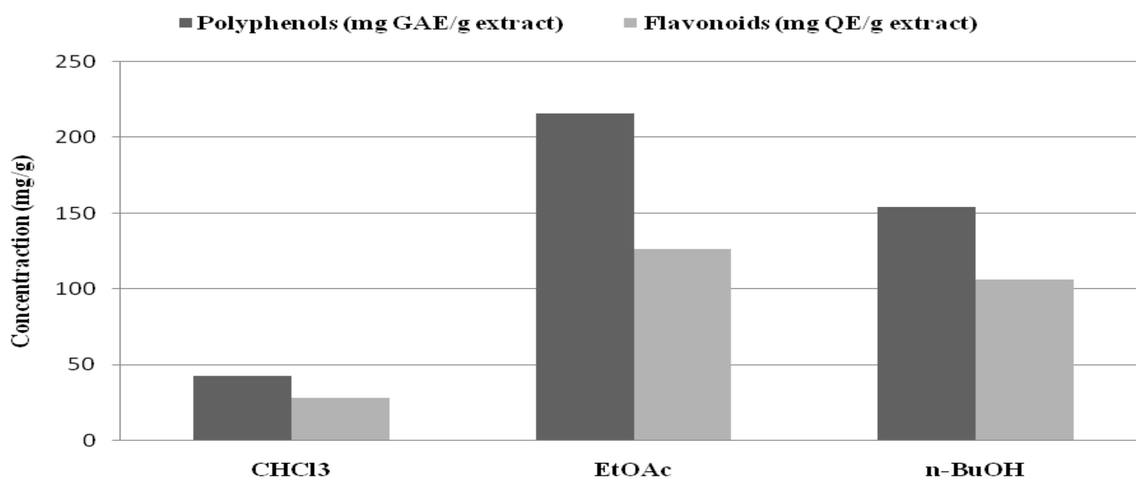

Figure 1: Total phenolic and total flavonoid contents in the three extracts of *C. segetum*

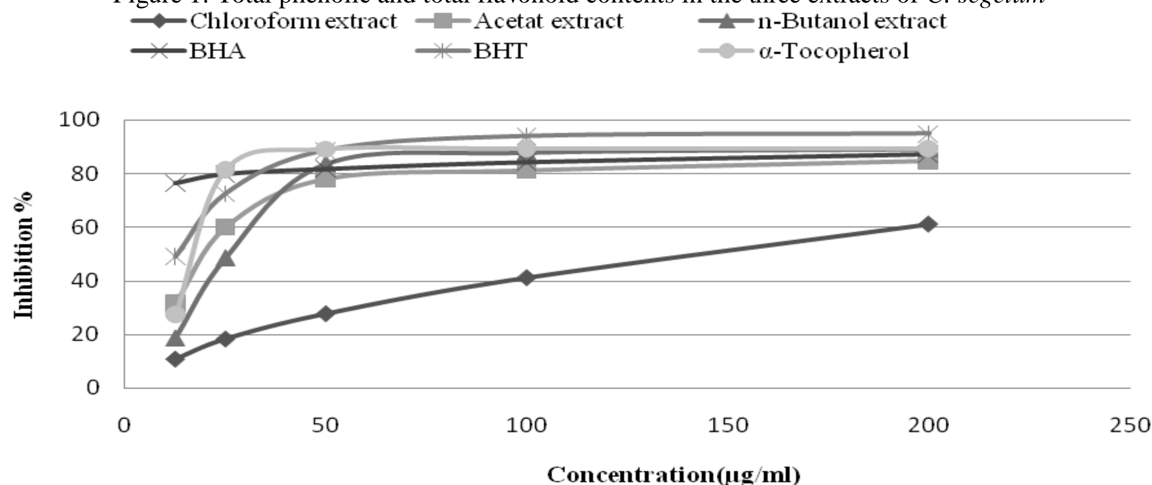

Figure 2: DPPH radical scavenging activity of the three extracts, BHA, BHT and α-Tocopherol at different concentrations (mean±SD, n=3).

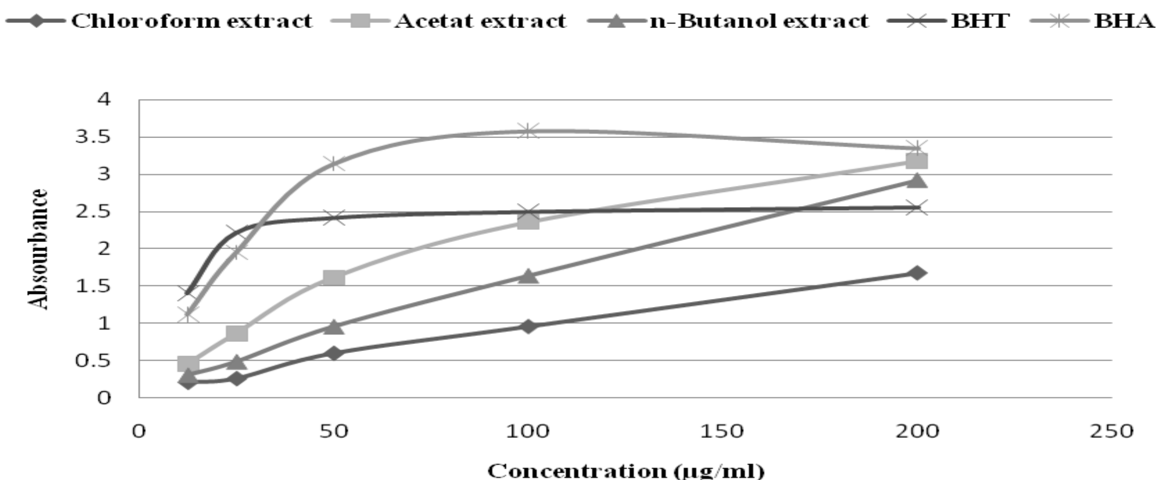

Figure 3: CUPRAC activity of the three extracts, BHA and BHT at different concentrations (mean±SD, n=3).

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

Table 1: Total phenolic and flavonoid contents of the three extracts.

Extracts	Total phenolic content (mg GAE/g)	Total flavonoid content (mg QE/g)
Chloroform	42.70±2.31	28.42±2.03
Ethyl acetate	216.18±12.97	126.64±11.35
n-butanol	154.51±2.92	106.64±11.27

Table 2: Antioxydants activity of extracts of *C. segetum* by DPPH• and CUPRAC assays.

Extracts	DPPH assayIC50(µg /mL)	CUPRAC assay A_{050}(µg /mL)
$CHCl_3$ extract	146.95±6.59	42.97±5.71
EtOAc extract	23.58±1.71	14.85±2.45
n-BuOH extract	28.68±3.55	24.57±2.80
BHA[b]	6.14±0.41	5.35±0,71
BHT[b]	12.99±0.41	8.97±3.94
α-Tocopherol[b]	13.02±5,17	NT

Values are expressed means±S.D. of three parallel measurements. ($p<0.05$

[b]Reference compounds.

BHA: Butylatedhydroxyanisole, BHT: Butylatedhydroxyltoluene, NT: Not tested

equivalent [GAE]/ g).

Determination of total flavonoid content (TFC)

TFC of the extracts of *C. segetum* were determined spectrophotometrically as previously reported[20]. 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).

Determination of antioxidant activity

DPPH scavenging assay

The DPPH scavenging activity was determined spectrophotometrically by the method described by Blois[21]. α-Tocopherol, BHT, BHA, were used as antioxidant standards for comparison of the activity. The results were given as 50% inhibition concentration (IC_{50})

$$I\% = [(Ac- As)/Ac] \times 100$$

Cupric reducing antioxidant capacity (CUPRAC)

The cupric reducing antioxidant capacity was determined according to the method of Apak et al.[22]. α-Tocopherol, BHT, BHA, were used as antioxidant standards for comparison of the activity. The results were given as A_{050} (µg/mL), which corresponds to the concentration producing 0.500 absorbance.

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 case of bulky compounds, CUPRAC gives better and accurate results. The free radical scavenging activity of the three different extracts from the *C. segetum* is expressed in terms of percentage of inhibition (%) and IC_{50} values (µg/mL) (Table 2 and Figure 2). Parallel to examination of the antioxidant activity of these plant extracts, the values of three standard compounds were obtained and compared to the values of the antioxidant activity. The compounds were BHA, BHT and α-Tocopherol. The examination of antioxidant activities of the extracts from *C. segetum* showed different values which varied from 61.22% to 89.25%. The largest capacity to neutralize DPPH radicals was 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 3) showed that activity (absorbance) increased linearly with the increasing amount of extracts. The ethyl acetate extract manifested the highest activity (A_{050} value: 14.85±2.45), followed by *n*-butanol (A_{050} value: 24.57±2.80) and chloroform extract (A_{050} value: 42.97±5.71). However, none of the extracts exhibited higher activity than those of antioxidant standards.

CONCLUSION

Antioxidant capacity of *Chrysanthemum segetum* was performed using two methods in parallel because different methods could give different results. The ethyl acetate extract exhibited the highest activity in the two assays followed by *n*-butanol and chloroform extracts. Our results which showed a strong correlation between TPC and antioxidant activity are in good agreement with literature data. The difference between the extracts and the control was statistically significant in the used antioxidant assays ($p<0.05$).

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