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Advances in Bioresearch

ORIGINAL ARTICLE

In Vitro Antioxidant, Anti-Alzheimer and Antidiabetic Activities, Phenolic and Flavonoid Contents of Different Polarity Extracts from *Serratula cichoracea* (DC.) (Asteraceae) Aerial Parts

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ABSTRACT

The present work was conducted to evaluate the quantitative analysis of aerial parts of Serratula cichoracea (DC.) and to evaluate their potential antioxidant, anti-alzheimer and antidiabetic properties in vitro. The chloroform, ethyl acetate and n-butanol extracts of aerial parts of Serratula cichoracea (DC.) were subjected to quantitative determination of polyphenolic and flavonoid contents. The antioxidant activity for the three extracts was performed by four methods: DPPH, ABTS, CUPRAC and reducing power assays. Anti-cholinesterase activity was performed against acetylcholinesterase (AChE) and Butyrylcholinesterase (BuChE) enzymes using the Ellman method. The antidiabetic activity was evaluated by using alpha-glucosidase inhibition assay. The ethyl acetate extract exhibited the best antioxidant activity in all tests (IC_{50} value 12.17±0.23 µg/mL in DPPH assay, IC_{50} value 6.33±0.27 µg/mL in ABTS assay, $A_{0.50}$ value 13.66±3.35 µg/mL in CUPRAC assay and $A_{0.50}$ value 58.40±5.17µg/mL in reducing power assay) and showed the highest amount of total pheneloc content (TPC) and flavonoid content (TFC). This extract showed moderate inhibition against AChE and BuChE (IC_{50} value > 200 and 104.24±0.34 µg/mL, respectively) compared to galantamine (IC_{50} values 6.27±1.15 µg/mL and 34.75±1.99 µg/mL, respectively) and the good α-glucosidase inhibitory activity (IC_{50} value 78.23±0.53µg/mL). These results showed that S. cichoracea (DC.) especially ethyl acetate extract can be a potential source of natural antioxidants and can be used in pharmaceutical industries as natural moderate anti-alzheimer and antidiabetic agents.

Keywords: *Serratula cichoracea (DC.), Antidiabetic, Anti-Alzheimer, Antioxidant, total phenolic, flavonoid contents.*

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INTRODUCTION

The Asteraceae family is one of the largest flowering plant families in the world, comprising 1600 genera and more than 23000 species. Several members of this family are grown as ornamental plants for their flowers such as, *chrysanthemum*, *dahlia*, *zinnia*, *gerbera*, and others [1]. Moreover, they are used for some industrial purposes as French amaranth (*Tagetes patula*) is common in commercial poultry feed and its oil is extracted for use in the cola and cigarette industry. Although, many Asteraceae species possess medicinal properties and are used in traditional medicine [2]. Because the sesquiterpene lactones and flavonoids contained within it have therapeutic properties. They are known as antioxidant, antibacterial, antimicrobial, antiproliferative, antifungal, anti-inflammatory, anti-tumor and anti-fatigue [3-11]. The genus *Serratula* is one of the best known genera of this family, their species are important for their uses in chemical and pharmaceutical purposes, traditional and folk medicines [12,13]. They are also

known as antioxidant, anticholinesterase, antimicrobial, antibacterial and antitumor [14-16]. In Algeria there are three genera of *Serratula*. Among them *Serratula cichoracea* ssp. *mucronata* (Desf.) Lacaita [17], which was subjected to two previous studies of the extract of ethyl acetate for flowers, and a group of flavonoids and phytoecdysteroid compounds were separated from it, including 3-methylquercetin, apigenin , acacetin, luteolin, genkwanin, ajugasterone C and 22-*epi*-ajugasterone C . Although, this extract and 3-methylquercetin were subjected for *in vitro* antioxidant properties and both showed significant antioxidant activity [18,19]. It should be noted that the flowers of this plant are a natural source of 3-methyl quercetin, 1 gram/15 grams of flowers ethyl acetate extract. A gram of 3-methylquercetin is estimated at 5740 USD. This compound has been shown in several studies to have multiple pharmacological uses, as an antioxidant, anti-allergic, and antimicrobial, and it has shown potent antiviral activity [20].

All previous results encouraged us to continue studying this plant, as this research was devoted to studying the aerial parts (flowers and leaves)(Figure1) of the three extracts in terms of the total polyphenol and flavonoid contents, and in order to seek new natural bioactive sources, we have tested the three extracts *in vitro* for their antioxidant activity and their inhibitory capacity against certain enzymes involved in several diseases like acetylcholinesterase, butyrylcholinesterase known by their relation with Alzheimer's disease and the digestive enzyme α-glucosidase linked with diabetes type 2.

MATERIAL AND METHODS

Experimental

Plant Material

Aerial parts of *Serratula cichoracea* (DC.) (Asteraceae) (Figure 1), synonym homotypic : *Centaurea* L. (1753) (L.), Synonyms Heterotypic *Acosta Adans*. (1763), *Aegialophila* Boiss. & Heldr. (1849), *Calcitrapa* Heist. & Fabr. (1759), *Colymbada* Hill (1762), *Melanoloma* Cass. (1823), *Stephanochilus* Coss. & Durieu ex Maire, Jacea Mill., *Klasea flavescens* subsp. *cichoracea* (L.) Greuter & Wagenitz [21] were collected in the flowering stage during the month of May from the area of El Kala, (GPS coordinates: 36° 53' 25.644" N 8° 26' 42.275" E) and authenticated by Dr. D. Sarri (University of M'Sila) on the basis of Quezel and Santa (1963) [17]. A sample has been deposited in the Herbarium of the Research Unit: Valorisation des Ressources Naturelles, Molecules Bioactives et Analyses Physicochimiques et Biologiques (VARENBIOMOL), Université Frères Mentouri Constantine1.

Figure1: Leaves and flowers of *Serratula cichoracea* (DC.) collected from El Kala

Extraction of the plant material

Air-dried and powdered aerial parts (flowers and leaves) (Figure1), from *S. cichoracea* (DC.) (2700 g) were macerated and are subjected to mechanical extraction with EtOH-H_2O (80:20 v/v) for 24 hours three times. The crude extract was concentrated under reduced pressure up to 35°C and diluted with 1000 mL distilled water under magnetic stirring. After filtration, the remaining aqueous solution was extracted successively with solvents of increasing polarities, starting with petroleum ether, chloroform ($CHCl_3$), ethyl acetate (EtOAc) and lastly *n*-butanol (*n*-BuOH). The organic layers were dried with Na_2SO_4 giving, after removal of solvents under reduced pressure, petroleum ether (0.48 g), $CHCl_3$ (8 g), EtOAc (23 g), and *n*-BuOH (32 g) extracts.

DETERMINATION OF TOTAL BIOACTIVE COMPOUNDS

Determination of Total Phenolic Content (TPC)

The Total Phenolic Contents (TPC) of the three extracts from *S. cichoracea* (DC.) were determined by Folin-Ciocalteu reagent method [22]. The absorbance of the sample was read at the wavelength 765 nm. Standard solutions of gallic acid with concentrations 25 to 200 µg/mL were used to obtain the standard curve. The results

were determined from the standard curve and expressed as mg gallic acid equivalents per gram of dry extract (mg GAE/g dry extract). Analyses were done in triplicate for each extract.

Determination of Total Flavonoid Content (TFC)

The Total Flavonoid Contents (TFC) of the three extracts were determined spectrophotometrically as previously reported [23]. The absorbance was read at the wavelength 415 nm. Standard solutions of quercetin with concentration 25 to 200 µg/mL were used to obtain the standard curve, and the results were expressed as mg quercetin equivalents per gram of dry extract (mg QE/g extract). All the experiments were carried out in triplates.

BIOLOGICAL ACTIVITIES (*IN VITRO*) OF THE THREE EXTRACTS

Antioxidant Activity *In vitro* by four tests

The antioxidant activity of extracts of *S. cichoracea* (DC.) was determined using four complementary tests: DPPH free radical scavenging, $ABTS^{\bullet+}$ scavenging, cupric reducing antioxidant capacity and reducing power assay.

DPPH Free Radical Scavenging Assay

The free radical scavenging activity of the extracts and standards (butylhydroxytoluene (BHT), butylhydroxyanisole (BHA) and α- tocopherol) was determined spectrophotometrically by the DPPH assay described by Blois [24]. 0.1 mM methanolic solution of DPPH was prepared and 160 µL of this solution was added to 40 µL of sample solutions in methanol at different concentrations (1.5625-100 µg/mL). Thirty minutes later, the absorbance was measured at 517 nm. The following equation was used to calculate the scavenging of DPPH radical:

Inhibition(%)=$[(A_{control}-A_{sample})/A_{control}]$ x100. Where $A_{Control}$ is the absorbance of the initial concentration of the DPPH and A_{Sample} is the absorbance of the remaining concentration of DPPH in the presence of the extract.

The curve of the percentage of the scavenging activity against the concentration of sample was prepared by the MS Excel-based program to obtain the IC_{50}. All the tests were conducted in triplicate. The experimental data were expressed as mean±SD.

ABTS Cation Radical Decolorization Assay

The spectrophotometric analysis of ABTS scavenging activity was determined according to the method of Re *et al*. [25], with slight modifications. The $ABTS^{\bullet+}$ was produced by the reaction between 7 mM ABTS in distilled water and 2.45 mM potassium persulfate and leaving the mixture to stand at room temperature for 12 h in the dark. The $ABTS^{\bullet+}$ solution was diluted to get an absorbance of 0.700 ± 0.020 at 734 nm with ethanol. 40 µL of various concentrations (1.5625-100 µg/mL) was added to 160 µL of $ABTS^{\bullet+}$ solution, after 10 min the absorbance was measured at 734 nm using a 96-well microplate reader. BHT and BHA were used as antioxidant standards for comparison of the activity. The scavenging capability of $ABTS^{\bullet+}$ was calculated using the following equation:

$ABTS^{\bullet+}$ scavenging activity (%) = $[(A_{control}-A_{sample})/A_{control}]$ x100. Where $A_{control}$ is the initial concentration of the $ABTS^{\bullet+}$ and A_{sample} is the absorbance of the remaining concentration of $ABTS^{\bullet+}$ in the presence of the extract. The extract concentration providing 50% radical scavenging activity (IC_{50}) was calculated from the graph of $ABTS^{\bullet+}$ scavenging effect percentage against extract concentration.

Cupric Reducing Antioxidant Capacity (CUPRAC) Assay

Cupric reducing antioxidant capacity (CUPRAC) of the extracts was determined according to the method described by Apak *et al* [26] with slight modifications. 40 µL of various concentrations (1.5625-100 µg/mL) was added to 60 µL of ammonium acetate buffer (1 M, PH 7.0) solution, 50 µL of copper (II) chloride solution (10 mM prepared from $CuCl_2$, $2H_2O$) and 50 µL of neocaproine solution (7.5 mM). The mixture was incubated for 60 min at room temperature. After 1 h, the absorbance at 450 nm was recorded against a blank reagent by using a 96-well microplate reader. The reducing capacity of the extracts was compared with those of BHA and BHT. The results were given as $A_{0.5}$ (µg/mL) corresponding to the concentration indicating 0.50 absorbance intensity.

Reducing power assay

The reducing power of the extracts was determined according to the method of Oyaizu [27]. 10 µL of sample solutions at different concentrations were mixed with 40 µL of 0.2 M phosphate buffer (pH 6.6) and 50 µL of potassium ferricyanide (1%). The mixture was incubated at 50°C for 20 min. After that 50 µL of tri-chloro acetic acid (TCA) (10%) was added and mixed with distilled water (40 µL) and 10 µL of ferric chloride (0.1%), the absorbance was read spectrophotometrically at 700 nm against water blank. Higher absorbance of the reaction mixture indicates greater reducing power.

Determination of Anti-Alzheimer Activity

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities were measured by slightly modifying the spectrophotometric method of Ellman *et al*. [28]. 150 µL of sodium phosphate

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buffer (100 mM, pH 8.0), 10 µL of the sample solution dissolved in ethanol at different concentrations (3.125-200 µg/mL) and 20 µL AChE or BChE solution in buffer were mixed and incubated for 15 min at 25 °C, and 10 µL of DTNB (0.5 mM) was added. The reaction was then initiated by the addition of 10 µL of acetylthiocholine iodide (0.5 mM) or 10 µL of butyrylthiocholine chloride (0.2 mM). The absorbance of the solution was measured at 412 nm by the use of 96-well microplate reader. The percentage inhibition of AChE or BChE enzymes was calculated by comparison of reaction rates of samples relative to blank sample using the following formula:

Inhibition of AChE or (BChE) (%) = [(E-S)/E] ×100. Where E is the activity of enzyme without test sample, and S is the activity of enzyme with test sample. The experiments were carried out in triplicate. The galantamine was used as the reference compound. The results were given as IC_{50} value (µg/mL) corresponding the concentration of 50% inhibition.

Antidiabetic activity

The inhibitory action of α-glucosidase has been carried out respecting Palanisamy *et al*. [29] method with few modifications. 50 µL of various concentration of the solution to test (15.625-1000/mL) was mixed with 50 µL of 4-Nitrophenyl α-D-glucopyranoside (5 mM) and 100 µL of the enzyme (0.1U/mL), the mixture was incubated for 15 minutes at 37 °C. A blank was made for each sample. Absorption was read at 405 nm (0 min and 15 min). Acarbose was used as a positive control at varying concentrations.

α-glucosidase's inhibitory function was calculated using the following equation:

$$\% \text{ Inhibition} = [(\text{Absorbance}_{\text{extract}} - \text{Absorbance}_{\text{blank}}) / \text{Absorbance}_{\text{control}}] \times 100.$$

Control: Enzyme + Substrate + Solvent of the extract

Statistical Analysis

All data for the antioxidant, anticholinesterase and alpha-glucosidase inhibition activity tests were the average of triplicate analyses. The results were given as a mean±standard deviation (SD) of three parallel measurements. Statistical analysis was performed using Variance Analysis (ANOVA). The results are considered to be significant when $P< 0.05$.

RESULTS

Determination of Total Bioactive Compounds

The TPC in various extracts were reported in terms of gallic acid equivalents using the standard curve equation y =0.002x + 0.045, R^2 = 0.997. TPC in various extracts of *Serratula cichoracea* (DC.) showed different results ranged from 520.23± 3.60 to 26.35 ± 2.24 mg GAE/g of extract. The ethyl acetate extract had the highest phenolic content (520.23±3.60 mg GAE/g) followed by *n*-butanol extract (Table 1, Figure 2). TFC in various extracts were reported in terms of quercetin equivalents using the standard curve equation y = 0.006x +0.017, R^2 = 0.976. TFC in different extracts showed different results ranged from 190.25±2.87 to 7.20 ± 0.42 mgQE/g of extract (Table 1, Figure 2). The ethyl acetate had the highest TFC (190.25±2.87 mgQE/g), the lowest (7.20 ± 0.42 mgQE/g) was given by the chloroform extract.

Table 1: Total phenolic and flavonoid contents of the three extracts of *S. cichoracea* (DC.)

Extracts	Total Phenolic Content (mgGAE/g extract)	Total Flavonoid Content (mgQE/g extract)
Chloroform extract	26.35±2.24	7.20±0.42
Ethyl acetate extract	520.23±3.60	190.25±2.87
n-Butanol extract	251.35±4.17	64.28±1.87

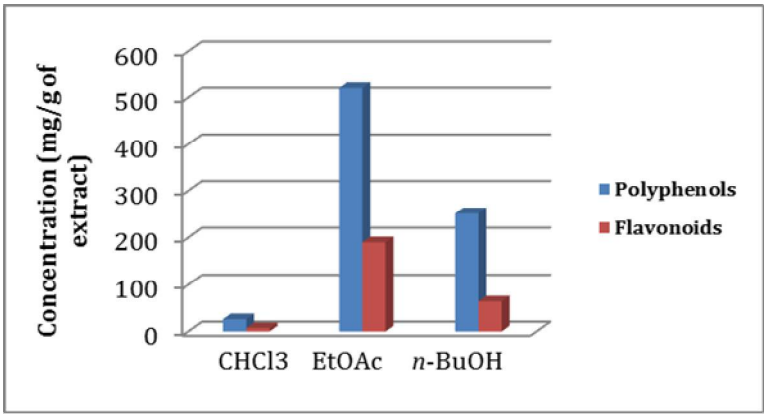

Figure 2: Total phenolic and total flavonoid contents in the three extracts of *S. cichoracea* (DC.)

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RESULTS OF THE BIOLOGICAL ACTIVITIES OF THE THREE EXTRACTS

Antioxidant activity

There was no previous study regarding antioxidant activity of the three different polarity extracts (chloroform, ethyl acetate and *n*-butanol) of aerials parts from *S. cichoracea* (DC.)Four methods were selected to determine the antioxidant capacity of the extracts. The free radical scavenging activity of extracts and standards (BHT, BHA and α-tocopherol) are documented in Figure 3 and Table 2. The results reveal that the DPPH• radical scavenging activity increased linearly with increasing of concentration. In this study the ethyl acetate extract exhibited the highest activity (IC_{50} = 12.17±0.23 µg/mL) in comparison with the other extracts and almost equal activity to that of BHT (IC_{50} = 12.12±0.41 µg/mL), α-tocopherol (IC_{50} = 12.15±1.82 µg/mL), and further to the BHA (IC_{50} = 5.73±0.41 µg/mL), followed by *n*-BuOH extract (IC_{50} = 18.52±3.04 µg/mL), which also exhibited a good activity but less than those of antioxidant standards, however chloroform extract showed weak activity (IC_{50}>200 µg/mL).

Table 2: Antioxidant activity by the DPPH assay[a].

Concentrations Extracts (µg/mL)	% Inhibition in DPPH Assay							
	1.5625	3.125	6.25	12.5	25	50	100	IC_{50} (µg/mL)
$CHCl_3$ extract	NA	NA	NA	NA	1.24±17.18	5.08±2.49	12.35±3.15	>200
EtOAc extract	2.25±2.17	4.39±2.86	8.79±2.99	54.52±1.17	66.43±3.77	70.81±0.94	74.36±0.72	12.17±0.23
n-BuOH extract	NA	3.25±5.35	7.88±16.76	51.13±3.42	60.97±1.00	65. 23±17.8	68.33±4.99	18.52±3.04
BHA[b]	22.21±1.24	31.73±1.22	54.89±3.60	67. 0±1.13	76.77±0.54	78.67±1.31	79.01±0.89	5.73±0.41
BHT[b]	15.28±0.76	25.37±2.06	53.35±0.89	62.85±0.76	74.22±2.06	82.34±0.89	85.45±0.31	12.12±0.41
α-Tocopherol[b]	14.25±1.82	24.12±1.51	46.25±0.12	61.46±1.82	78.34±1.51	81.25±0.12	81. 25±0.19	12.15±1.82

[a]Values expressed as means ± S.D of three parallel measurements. (p <0.05).
[b]Reference compounds.
NA: no absorbance

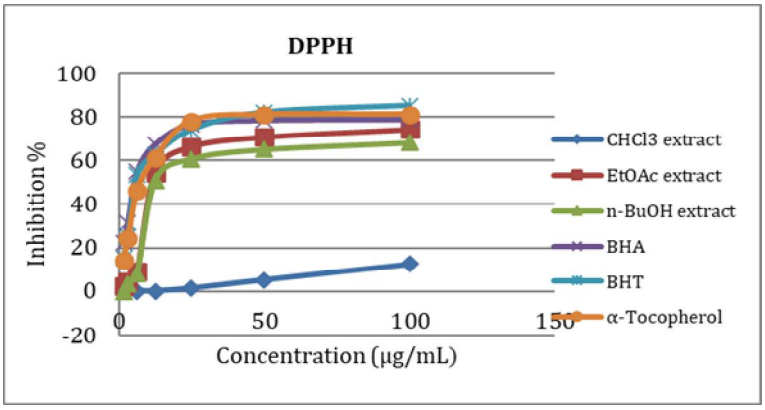

Figure 3: DPPH radical scavenging activity of the three extracts, BHA, BHT and α-Tocopherol at different concentrations. (mean ±SD, n=3).

The results of ABTS assay of the extracts are also compared with those of BHT and BHA (Figure 4 and Table 3). The best ABTS scavenging activity was found in ethyl acetate extract (IC_{50} = 6.33 ± 0.27 µg/mL), followed by *n*-butanol extract (IC_{50} =10.84±1.08 µg/mL), whereas the lowest activity was recorded for chloroform extract (IC_{50} = 59.41±1.55 µg/mL). In comparison to IC_{50} values of BHT and BHA, ethyl acetate (EtOAc) extract exhibited the strongest capacity for neutralization of ABTS radicals.

Table 3: Antioxidant activity by the ABTS assay[a].

Concentrations Extracts (µg/mL)	% Inhibition in ABTS Assay							
	1.5625	3.125	6.25	12.5	25	50	100	IC_{50} (µg/mL)
$CHCl_3$ extract	NA	NA	NA	NA	1.98±4.03	1.98±1.82	3.43±1.40	59.41±1.55
EtOAc extract	5.48 ±3.85	31.73±3.94	54.58±6.60	77.53±7.53	83.47±4.45	85.27±1.86	87.91±0.65	6.33±0.27
n-BuOH extract	NA	2.36±1.31	23.80±2.52	59.58±6.62	81.21±0.91	83.76±7.29	87.72±0.65	10.84±1.08
BHA[b]	49.22±0.75	59.22±0.59	78.55±3.43	90.36±0.00	92.18±1.27	93.37±0.86	94.87±0.87	1.59±0.03
BHT[b]	83.42±4.09	93.52±0.09	93.58±0.09	93.63±0.16	93.63±0.95	94.20±0.90	95.39±2.62	1.03±0.06

[a]Values expressed as means ± S.D of three parallel measurements. (p <0.05). [b]Reference compounds. NA: no absorbance

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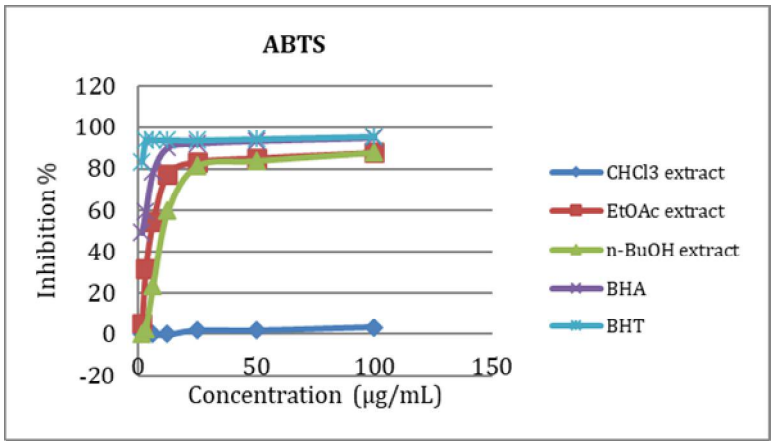

Figure 4: ABTS activity of the three extracts, BHA, BHT at different concentrations. (mean ±SD, n=3).

The results of CUPRAC of the extracts are compared with those of BHA and BHT (Figure 5 and Table 4), the activity (absorbance) increased linearly with the increasing amount of extracts. The ethyl acetate (EtOAc) extract exhibited the highest activity ($A_{0.50}$ = 13.66 ± 3.35 µg/mL) among the extracts, followed by *n*-butanol (*n*-BuOH) extract ($A_{0.50}$ = 32.53±0.28 µg/mL) and chloroform ($CHCl_3$) extract ($A_{0.50}$ > 200 µg/mL). However, none of the extracts exhibited higher activity than those of the antioxidant standards.

Table 4: Antioxidant activity by the CUPRAC assay[a].

Concentrations Extracts (µg/mL)	% Inhibition in CUPRAC Assay							
	1.5625	3.125	6.25	12.5	25	50	100	$A_{0.50}$ (µg/mL)
$CHCl_3$ extract	0.09±0.03	0.11±0.05	0.09±0.01	0.11±0.03	0.11±0.02	0.13±0.00	0.17±0.01	>200
EtOAc extract	0.15±0.00	0.23±0.01	0.34±0.01	0.45±0.09	0.83±0.16	1.15±0.08	1.64±0.14	13.66±3.35
n-BuOH extract	0.10±0.00	0.13±0.00	0.18±0.00	0.26±0.00	0.41±0.01	0.71±0.01	1.16±0.04	32.53±0.28
BHA[b]	0.11±0.04	0.19±0.01	0.33±0.04	0.66±0.07	1.03±0.07	1.48±0.09	2.04±0,14	9.62±0.87
BHT[b]	0.23±0.07	0.46±0.00	0.78±0.01	1.34±0.08	2.36±0.17	3.45±0.02	3.76±0.03	3.64±0.19

[a]Values expressed as means ± S.D of three parallel measurements. (p <0.05).
[b]Reference compounds.

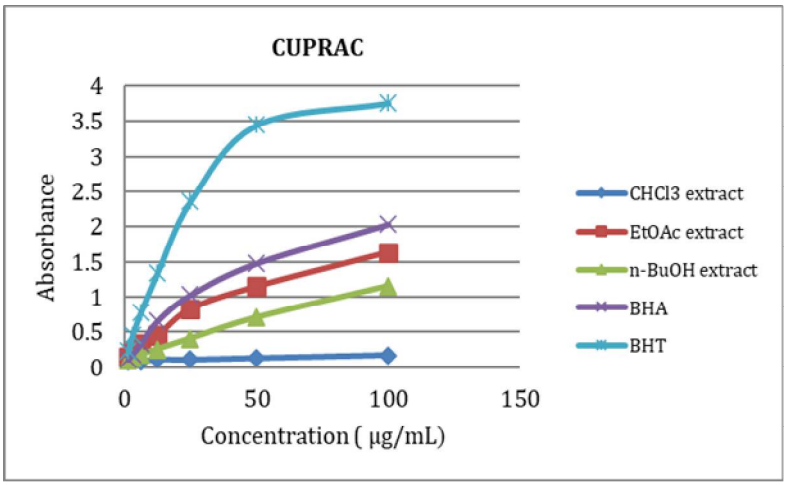

Figure 5: CUPRAC activity of the three extracts, BHA, BHT at different concentrations. (mean ±SD, n=3).

The results of reducing power assay of the three extracts are compared with those of ascorbic acid and α-tocopherol (Figure 6 and Table 5). In this assay, the results obtained are also identical to those obtained in previous experiments, where the ethyl acetate (EtOAc) showed the highest efficacy ($A_{0.50}$ = 58.40±5.17 µg/mL), followed by *n*-butanol (*n*-BuOH) extract and chloroform ($CHCl_3$) extract respectively ($A_{0.50}$ = 108.90±89.49 µg/mL and 285.68±93.35 µg/mL, respectively).

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Table 5: Antioxidant activity by the Reducing power assay[a].

Concentration Extracts (µg/mL)	% Inhibition in Reducing power Assay							
	3.125	6.25	12.5	25	50	100	200	$A_{0,5}$ (µg/mL)
$CHCl_3$ extract	0.06±0.02	0.07±0.01	0.08±0.01	0.09±0.00	0.09±0.02	0.09±0.01	0.11±0.05	285.68±93.35
EtOAc extract	0.25±0.04	0.35±0.06	0.39±0.12	0.48±0.06	0.52±0.03	0.50±0.03	0.54±0.09	58.40±5.17
n-BuOH extract	0.20±0.04	0.27±0.06	0.38±0.05	0.43±0.04	0.50±0.02	0.40±0.04	0.48±0.05	108.90±89.49
Ascorbic acid [b]	0.35±0.05	0.46±0.03	0.84±0.12	0.93±0.30	1.18±0.34	1.37±0.20	1.44±0.21	6.77±1.15
α-Tocopherol [b]	0.11±0.00	0.16±0.00	0.21±0.03	0.35±0.03	0.73±0.03	1.37±0.08	1.81±0.09	34.93±2,38

[a]Values expressed as means ± S.D of three parallel measurements. (p <0.05).
[b]Reference compounds.

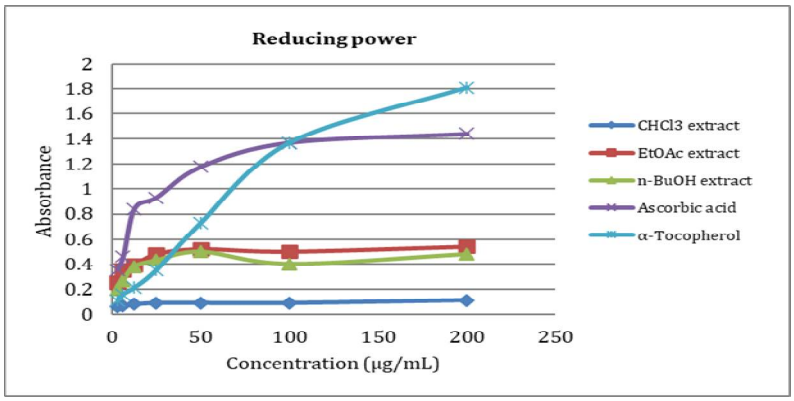

Figure 6: Reducing power activity of the three extracts, Ascorbic acid and α-Tocopherol at different concentrations. (mean ±SD, n=3).

Anti-Alzheimer activity

Table 6 shows the inhibitory activities of AChE (Figure7) and BChE (Figure 8) of the three extracts, compared to those of galantamine. The ethyl acetate extract showed weak inhibitory activity against BChE and AChE (104.24±0.34 µg/ML and IC_{50} >200 µg/mL respectively), but this inhibitory activity of the two enzymes was lower than that of galantamine standard (IC_{50} = 34.75±1.99 µg/mL and IC_{50} = 6,27±1,15 µg/mL).The *n*-butanol extract showed mild inhibitory activity of BChE , while it showed no activity against AChE enzyme. As for chloroform extract, no inhibitory activity was shown with both enzymes.

Table 6: Acetylcholinesterase and butyrylcholinesterase inhibitory activities[a] of the three extracts of *S. cichoracea* (DC.)

Extracts	AChE assay IC_{50} (µg/mL)	BChE assay IC_{50} (µg/mL)
$CHCl_3$ extract	NA	NA
EtOAc extract	>200	104.24±0.34
n-BuOH extract	NA	>200
galantamine[b]	6,27±1,15	34.75±1.99

[a]Values expressed as means ± S.D of three parallel measurements. (p <0.05).
[b]Reference compound.
NA: not active.

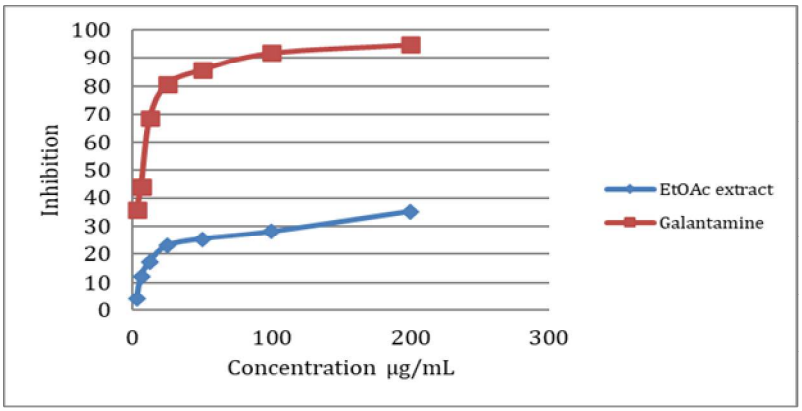

Figure 7: Acetylcholinesterase inhibitory activity of ethyl acetate extract (EtOAc) of *S. cichoracea* (DC.)

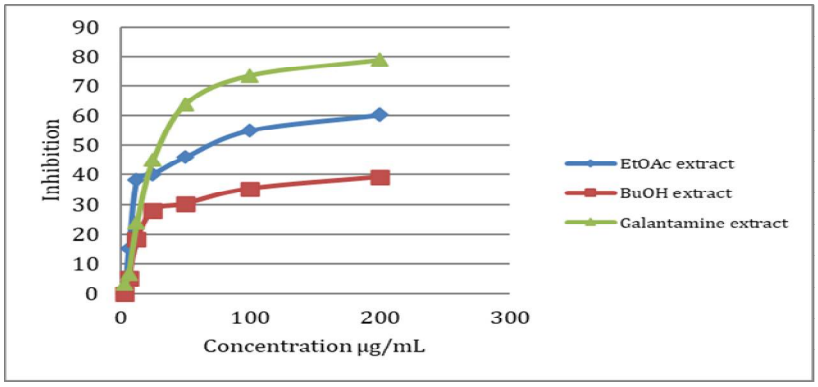

Figure 8: Butyrylcholinesterase inhibitory activity of ethyl acetate (EtOAc) and *n*-butanol extracts of *S. cichoracea* (DC.)

Antidiabetic activity

Table 7 shows the inhibitory activity of α-Glucosidase for the three extracts, compared to that of Acarbose, wherein ethyl acetate extract (IC_{50} = 78.23±0.53 µg/mL) showed the highest inhibitory activity of α-glucosidase compared to the rest of the extracts and compared to Acarbose used as a positive control (IC_{50} =275.43 ±1.59 µg/mL), followed by *n*-butanol extract which showed slight inhibitory activity of α-Glucosidase (IC_{50} > 800 µg/mL). While chloroform extract showed no inhibitory activity for α-Glucosidase at all concentrations.

Table 7: α-Glucosidase Inhibitory activity of of the three extracts of *S. cichoracea* (DC.)

% α-Glucosidase Inhibitory activity								
Extracts	15,625 µg/mL	31,25 µg/mL	62,5 µg/mL	125 µg/mL	250 µg/mL	500 µg/mL	1000 µg/mL	IC_{50} (µg/mL)
$CHCl_3$ extract	NA	NA	NA	NA	NA	NA	NA	No activity
EtOAc extract	10.40±0.23	16.32±1.15	39.81±1.21	66.18±0.63	121.67±1.01	120.56±0.68	NT	78.23±0.53
n-BuOH extract	NA	NA	NA	6.35±0.12	20.78±0.84	34.75±0.54	57.86±0.41	IC_{50} > 800
Acarbose[b]	78.,125	156. 25µg	312.5µg	625 µg	1250 µg	2500µg	5000µg	IC_{50}µg/mL
	27.43±2.18	38.91±3.20	54.86±1.799	67.29±2.63	80.19±1.66	85.54±0.45	91.05±0.72	275.43±1.59

NA: not active.
NT: Not tested.
Values expressed are means ± S.Dof three measurements $p \leq 0.05$.
[b]Reference compound

DISCUSSION

It is well known that the natural phenolic compounds have many biological activities. Recently, several studies have led to belief that the antioxidant effects of the natural polyphenols play an important role in prevention and management of numerous degenerative diseases (such as Alzheimer and cancers) by reducing the oxidative stresses generated by free radicals and oxidants [30-32]. The extracts of *S. cichoracea* (DC.) (leaves and flowers) (Figure 1) have been identified as a new source of natural phenols and therefor they have been studied for their antioxidants and free radical scavenging activities.

In our research, the total phenolic contents (TPC) showed wide variation from 26.35±2.24 mg/g GAE to 520.23± 3.60 mg/g GAE according to the UV/Vis spectrophotometry method; the greatest phenolic content was observed with ethyl acetate extract followed by *n*-butanol extract, while the least was recorded for chloroform extract (Table 1, Figure 2). The greatest flavonoid content was also observed with ethyl acetate extract (190.25±2.87 mgQE/g), while the smallest content was recorded for chloroform extract (7.2 ± 0.42 mgQE/g). This explains the results obtained in the antioxidant activity tests, where the ethyl acetate extract has shown the highest activity in all tests including DPPH, ABTS, CUPRAC, Reducing power. The IC_{50} value of this extract in the DPPH test was estimated at 12.17±0.23 µg/mL, which is equal to the value obtained using the controls BHT and α-tocopherol (IC_{50} value 12.12±0.41 µg/mL and 12.15±1.82 µg/mL, respectively), followed by *n*-butanol extract which showed a good activity (IC_{50} value 18.52±3.04 µg/mL). The best ABTS scavenging activity was also found in ethyl acetate extract (IC_{50} value 6.33 ± 0.27 µg/mL)(Table 3, Figure 4), This latter extract exhibited also the highest reducing power tested by CUPRAC and reducing power assays ($A_{0.50}$ value 13.66±3.35 µg/mL and 58.40±5.17 µg/mL,

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respectively), this is followed by *n*-butanol extract and then the chloroform extract . The total antioxidant capacity values have been shown to follow the same order of phenolic content in the extracts respectively and the differences in values were found to be significant and consistent with their phenolic content. In addition, it has been shown that the use of antioxidants may reduce the progression of Alzheimer's disease and reduce neuronal degeneration [33]. In fact, it has been proposed that the decrease of the amount of acetylcholine, a neuromodulator, may lead to the development of this disease [34]. Thus, acetylcholinesterase inhibitor medications have been used to treat Alzheimer's disease (such as donepezil and galantamine). In order to find natural inhibitor, the three extracts were tested for their acetyl- and butyrylcholinesterase inhibitory activities. According to our results (Table 6, Figure 7 and 8), among the extracts, there was no significant activity against the acetylcholinesterase enzyme. The anticholinesterase activity and the results of the antioxidant activity were different in terms of their inability to support each other, as reported in the literature [15, 35, 36]. The best antioxidant extract (EtOAc) indicated moderate butyrylcholinesterase inhibitory activity (IC_{50} value 104.24±0.34 µg/mL). Since the extract is a mixture of many compounds, synergistic or anti-synergistic effects may play a vital role in exhibiting the anticholinesterase activity.

On other side, some researchers have indicated a positive correlation between the total polyphenol and flavonoid content and the ability to inhibit α-Glucosidase [37]. *In vitro* and *in vivo* studies also have showed that flavonoids possess a high inhibition capacity towards α-Glucosidas [38]. α-Glucosidase inhibitors which inhibit this enzyme in the intestine are effective in delaying glucose absorption and preventing the increase of glucose level in blood after eating. Thus, the α-glucosidase inhibitors play an important role in treatment of diabetes type 2 [39]. In our paper, we investigated the inhibitory activities of the *S. cichoracea* (DC.) extracts against α-glucosidase. In our results (Table7) the ethyl acetate extract had the highest α-glucosidase inhibitory activity (IC_{50} value 78.23±0.53 µg/mL), even higher than Acarbose (IC_{50} value 275.43±1.59 µg/mL)), followed by *n*-butanol (IC_{50} >800 µg/mL). The high activity of the EtOAc extract toward α-glucosidase in our study may be resulted from the synergy of the various phenolic and flavonoids compounds present in the extract.

CONCLUSION

In this study, antioxidant, anti-Alzheimer and antidiabetic activities of the aerial parts extracts of *serratula cichoracea* (DC.) were determined. The ethyl acetate extract exhibited the highest antioxidant activities in all assays which might be due to their high phenolic content. This extract also indicated the moderate BChE inhibitory activity and the good α-glucosidase inhibitory activity. Our results which showed a strong correlation between TPC from side and antioxidant activity and α-glucosidase inhibitory activity from the other side are in good agreement with literature data.

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