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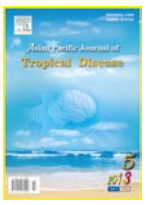

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Antioxidant and anti-acetylcholinesterase activities of extracts from *Rapistrum rugosum* in Tunisia

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PEER REVIEW

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Comments

The authors studied the levels of antioxidant substances measured by different methodologies and statistically compared the correlation of the results. They concluded that phenolic compounds are one of the main components responsible for the antioxidant behavior of the extracts and fractions. They also demonstrated the anti-acetylcholinesterase activity in the extracts.
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ABSTRACT

Objective: To investigate the antioxidant potential and anti-acetylcholinesterase activity of *Rapistrum rugosum* extracts.
Methods: The crude, ethyl acetate, butanol and water extracts prepared from flowers, roots, stems and leaves of *Rapistrum rugosum* were tested at 1 mg/mL to determine their total polyphenol content, total flavonoid content and total condensed tannin content. Their antioxidant activity was assessed at different concentrations (0.0312, 0.0625, 0.1250, 0.25, 0.50 and 1.00 mg/mL) by using DPPH, ABTS, reducing power and β-carotene bleAChIng inhibition activity. Anti-acetylcholinesterase activity was also determined.
Results: The extract of leaves and stems had the highest total phenolic content [(110.45±0.03) mg gallic acid equivalent/g dry weight]. The ethyl acetate extract of flowers had the highest total flavonoid content [(24.62±0.13) mg quercetin equivalent/g dry weight]. The butanolic fraction of flowers had the highest total condensed tannin content [(317.85±0.01) mg catechin equivalent/g dry weight]. The crude extracts of flowers exhibited an interesting antioxidant activity for DPPH assay (93.00±0.01)% at 1 mg/mL. The greatest acetylcholinesterase inhibitory activity (IC_{50}=1.60 mg/mL) was exhibited by the crude extracts from the flowers.
Conclusions: The results demonstrated that *Rapistrum rugosum* contains active constituents which possess antioxidant and anti-acetylcholinesterase activities.

KEYWORDS

Phenols, DPPH, ABTS, Reducing power, β-carotene, Alzheimer's disease

1. Introduction

Rapistrum rugosum L. (*R. rugosum*) (Brassicaceae), locally named as ''lebsen'', is a wild plant common to Libya, Algeria, Tunisia and Mediterranean regions[1].

Le Floc'h does not indicate any traditional use of this plant in Tunisia[2]. The results of the recent researches clearly indicate the importance of Brassicaceae vegetables which contain several bioactive compounds and which not only act as antioxidants, but also have other health-promoting properties[3-5].

Reactive oxygen species (ROS) are generated by many redox processes that normally occur in the metabolism of aerobic cells. If not eliminated, ROS can attack important

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biological molecules, such as lipids, proteins, enzymes, DNA and RNA. Although the human body possesses many defense mechanisms against oxidative stress, including antioxidant enzymes and non-enzymatic compounds, an excess of free radicals can go out of control, the organism being unable to scavenge all ROS. Their excess has been implicated in the development of chronic diseases, such as cancer, arteriosclerosis, nephritis, diabetes mellitus, rheumatism, ischemic and cardiovascular diseases and also in the ageing process. Oxidative stress can also play an important role in the development of neurodegenerative disorders, such as Alzheimers and Parkinsons diseases[6,7].

There is convincing epidemiological evidence that consumption of fruits and vegetables is, in general, beneficial for health, thanks to the protection provided by the antioxidant phytonutrients contained in them[8-10].

Brassicaceae vegetables are reported to reduce the risks of some cancers, especially due to their contents of glucosinolates and derived products[11]. Flavonoids and other phenolics are also considered contributing to this capacity[3,12,13]. The cruciferous plant family, another name used for Brassicaceae, contains 338 genera and 3 350 species that are distributed worldwide[14]. To the best of our knowledge, the antioxidant and anti-acetylcholinesterase activities of extracts of *R. rugosum* (L.) (Brassicaceae) have not been studied hitherto. Therefore, the aim of this study is to evaluate the *in vitro* antioxidant and anti-acetylcholinesterase properties of some extracts of *R. rugosum*

In addition, the total phenolic, tannin and flavonoid contents of the extracts were determined.

2. Materials and methods

2.1. Plant material

The whole parts (leaves+stems, roots and flowers) of *R. rugosum* were collected in March 2010 from the region of Kasserine, Tunisia. The plant material was identified by Dr. Fethia Harzallah Skhiri, High Institute of Biotechnology of Monastir, Tunisia. A voucher specimen (RR-10) was deposited at the herbarium in the Faculty of Science, University of Monastir, Tunisia.

2.2. Chemicals

2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), butlylated hydroxytoluene (BHT), Folin-Ciocalteu reagent, catechin, gallic acid, quercetin, acetylcholinesterase (AChE) type VI-S, from electric eel 349 U/mg solid, 411 U/mg protein, 5,5′-dithiobis[2-nitrobenzoic acid] (DTNB), acetylthiocholine iodide (AChI), tris hydroxymethyl aminomethane (tris-HCl buffer), dimethylsulfoxide, ferric chloride ($FeCl_3$), trichloroacetic acid, potassium ferricyanide [$K_3Fe(CN)_6$], eserine, $AlCl_3$, vanillin and H_2SO_4 were purchased from Sigma. All other chemicals were of analytical grade purity.

2.3. Plant extraction

The plant samples (100 g) were air-dried for several weeks. Powdered plant tissues (leaves+stems, roots and flowers) were extracted by maceration with 80% methanol for three times.

The resultant extracts were concentrated under reduced pressure. The crude extracts were extracted successively with equal volumes of two organic solvents, ethyl acetate and butanol, with increasing polarity to give ethyl acetate, butanol and the remaining aqueous extract. Each fraction was dried under vacuum and stored at 4 °C.

2.4. Total phenolic, flavonoid and tannin contents

2.4.1. Determination of total phenolic content

The amount of total phenolic was determined according to the method of Kumar[15], using Folin-Ciocalteu reagent. Tested extracts were prepared at a concentration of 1 mg/mL. A volume of 100 μL of extract was transferred into a test tube and 0.75 mL of Folin-Ciocalteu reagent (previously 10-fold diluted with deionized water) was added and mixed. The mixture was allowed to stand at a temperature of 25 °C for 5 min. A total of 0.75 mL of saturated sodium carbonate solution was added to the mixture and then mixed gently. After standing at 25 °C for 90 min, the absorbance was read at 725 nm using an UV-Vis spectrophotometer.

The standard calibration (0.01-0.05 mg/mL) curve was plotted using gallic acid. The total phenolic content was expressed as gallic acid equivalents in micrograms per milligram of vegetable extract (μg of gallic acid/mg dry weight).

2.4.2. Total flavonoid content

The $AlCl_3$ method was used to determine the total flavonoid content of the sample extracts[16]. A total of 1.5 mL (1 mg/mL) of extracts were added to equal volumes of a solution of 2% $AlCl_3$-$6H_2O$ (2 g in 100 mL methanol). The mixture was vigorously shaken, and absorbance at 367 nm was read after 10 min of incubation.

The total flavonoid content was expressed as μg quercetin/g dry weight, through the calibration curve of quercetin.

The calibration curve range was 0-50 μg/mL (R^2=0.99). All samples were analyzed in three replications.

2.4.3. Total tannin content

Condensed tannins were measured using the modified vanillin assay described by Tamilselvi[17]. Three millilitres of 4% methanol vanillin solution and 1.5 mL of concentrated H_2SO_4 were added to 50 μL of the sample (1 mg/mL). The mixture was allowed to stand for 15 min, and the absorbance was measured at 500 nm against methanol as a blank. The amount of total condensed tannins is expressed as μg (+)-catechin/g dry weight. The calibration curve range was 0-400 μg/mL (R^2=0.99). All samples were analyzed in three replications.

2.5. Antioxidant activities

2.5.1. DPPH· scavenging capacity

The DPPH· radical scavenging capacity of the studied extracts was measured from the bleaching of purple colored ethanol solution of DPPH· (0.002%) described by Chakraborty[18]. A volume of 0.5 mL of each sample extracts was mixed with the same volume of DPPH· ethanolic solution. After incubation of 30 min in darkness and at a temperature of 25 °C, absorbance was read at 520 nm wavelength.

A mixture of 0.5 mL of DPPH· solution and 0.5 mL of ethanol was taken as a blank. Decrease in absorption induced by the tested samples was compared to that of the positive control BHT. Calculated IC_{50} values denote the concentration required to scavenge 50% of DPPH· radicals. All measurements were performed in triplicate. Results were expressed in inhibition percentage at different sample concentrations (mg/mL) after 30 min.

Inhibition of free radical DPPH in percent (I%) was calculated as follows:

$$I\% = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagent except the test compound), and A_{sample} is the absorbance of the test compound. The sample concentrations providing 50% inhibition (IC_{50}) was calculated by plotting inhibition percentages against concentrations of the sample. All tests were carried out in triplicate and IC_{50} values were reported as means±SD of triplicates.

2.5.2. ABTS radical scavenging activity assay

Antiradical activity was done by using the ABTS·+ free radical decolorization assay developed by Chakraborty with some modifications[18]. Briefly, the preformed radical monocation of ABTS was generated by reacting ABTS solution (7 mmol/L) with 2.45 mmol/L $K_2S_2O_8$. The mixture was allowed to stand for 15 h in the dark at room temperature. The solution was diluted with ethanol to obtain the absorbance of (0.7±0.2) units at 734 nm. Samples were separately dissolved in ethanol to yield the following concentrations (0.0312, 0.0625, 0.125, 0.25, 0.50 and 1.00 mg/mL). In order to measure the antioxidant activity of samples, 10 μL of each extract at various concentrations was added to 990 μL of diluted ABTS·+.

The absorbance was measured spectrophotometrically at 734 nm after 20 min. All measurements were performed in triplicate. The decrease percentage of absorbance at 734 nm was calculated for each point and the antioxidant capacity of the test samples was expressed as percent inhibition (%). The percentage scavenging of ABTS·+ was calculated by the following formula:

Scavenging activity (%)=(Ao-Ax)/Ao×100

Ax and Ao were the absorbance at 734 nm of samples with and without extract, respectively.

2.5.3. Reducing power

Reducing power of extracts of *R. rugosum* was measured by the method of Zouari[19]. According to this method, the reduction of Fe^{3+} to Fe^{2+} was determined by measuring absorbance of the Perl's Prussian blue complex. This method is based on the reduction of (Fe^{3+}) ferricyanide in stoichiometric excess relative to the antioxidants.

For this purpose, different concentrations (0.0312, 0.0625, 0.125, 0.25, 0.50 and 1.00 mg/mL) of extracts of *R. rugosum* in distilled water were mixed with 1 mL of 0.2 mol/L sodium phosphate buffer (pH 6.6) and 1 mL (1%) of potassium ferricyanide [$K_3Fe(CN)_6$].

The mixture was incubated at 50 °C for 20 min, and then acidified with 1 mL of trichloroacetic acid (10%). Finally, 0.25 mL of $FeCl_3$ (0.1%) was added to this solution. Distilled water was used as blank and for control. Absorbance of this mixture was measured at 700 nm using a UV spectrophotometer. Increased absorbance indicated ferric reducing power capability of sample.

2.5.4. β-carotene/linoleic acid method

β-carotene bleaching inhibition of *R. rugosum* extracts was determined according to the method of Zaouali with slight modifications[20]. Briefly, 2 mL of β-carotene solution (1.5 mg β-carotene/2.5 mL chloroform) was added to 20 μL of linoleic acid and 200 μL of Tween-20. The chloroform was removed at 40 °C under vacuum using a rotary evaporator.

Immediately, 50 mL of distilled water were added to the dried mixture to form a β-carotene-linoleic acid emulsion. In order to determine the β-carotene bleaching activity of the extract, 5 mL of emulsion were added to 500 μL of extracts (1 mg/mL). The mixtures were incubated in a water bath at 50 °C for 120 min and the absorbance of the reaction mixtures was read at 470 nm. Antioxidant activity of extracts

was calculated by using the following equation:

AA%=(β-carotene content after 2 h assay/initial β-carotene content)×100

Tests were carried out in triplicate. Extract concentration providing 50% inhibition (IC_{50}) was obtained by plotting inhibition percentage versus extract concentrations.

2.6. Acetylcholinesterase inhibition assay

Inhibition of AChE by plant extracts was evaluated as described by Moyo[21]. The assay is based on the spectrophotometric measurement of the increase in yellow color produced from thiocholine when it reacts with the dithiobisnitrobenzoate ion. Eserine was used as a positive control and water served as a negative control. The increase in absorbance value due to the spontaneous hydrolysis of the substrate was corrected by subtracting the ratio of the reaction before adding the enzyme from the rate after the enzyme addition. Percentage inhibition by extracts and eserine were calculated using the equation below.

Inhibition (%)=(1-Sample reaction rate/Blank reaction rate)×100

2.7. Statistical analysis

The results were given as mean±SD for at least three replicates for each sample.

The EC_{50} (reducing power) and IC_{50} (DPPH, ABTS and AChE) values were calculated by linear regression analysis.

3. Results

3.1. Total phenolic, flavonoid, tannin content and antioxidant activity

3.1.1. Total phenolic content

The total phenolic content of the different extracts of *R. rugosum* was evaluated, using the Folin-Ciocalteu method (Table 1).

The total phenolic content of the water extract from leaves and stems of *R. rugosum*, as well as that of the butanolic extract from leaves and stems, ethyl acetate extract from roots, and crude extract from leaves and stems) were (110.45±0.03), (104.86±0.02), (104.09±0.07) and (97.90±0.01) μg of gallic acid/mg dry weight, respectively.

On the other hand, ethyl acetate extract from flowers present a moderate total phenolic content (89.27±0.05 μg of gallic acid/mg dry weight) followed by the water extract from roots (85.5±0.02 μg of gallic acid/mg dry weight) and flowers butanolic extract (81.36±0.01 μg of gallic acid/mg dry weight).

Table 1
Total polyphenol, total flavonoid and tannin contents of *R. rugosum* extracts.

Extracts		Total polyphenols content[a]	Flavonoid Content[b]	Tannin Content[c]
Leaves + Stems	Crude extract	97.90±0.01	22.18±0.20	247.38±0.01
	EtOAc	66.40±0.01	22.23±0.07	187.38±0.01
	Water fraction	110.45±0.03	21.08±0.16	217.38±0.07
	Bu-OH	104.86±0.02	18.19±0.02	215.95±0.03
Flowers	Crude extract	50.18±0.04	22.13±0.12	237.85±0.12
	EtOAc	89.27±0.05	24.62±0.13	260.47±0.05
	Water fraction	45.77±0.01	22.26±0.12	300.95±0.05
	Bu-OH	81.36±0.01	23.22±0.09	317.85±0.01
Roots	Crude extract	64.36±0.01	12.51±0.08	231.90±0.08
	EtOAc	104.09±0.07	20.33±0.11	185.71±0.07
	Water fraction	85.5±0.02	21.13±0.09	267.61±0.11
	Bu-OH	39.05±0.05	19.23±0.18	227.85±0.05

[a]: μg gallic acid/mg dry weight; [b]: μg quercetin/mg dry weight; [c]: μg catechin/mg dry weigh. Bu-OH: butanolic extract; EtOAc: ethyl acetate extract.

3.1.2. Total flavonoid and total tannins content

The total flavonoid contents of the crude plant extracts are reported as quercetin equivalents (Table 1). The content of flavonoid varied from (12.51±0.08) to (24.62±0.13) μg quercetin/mg dry weight. Among the extracts, ethyl acetate and butanolic extracts from flowers were found to contain the highest amount of flavonoid compounds [(23.22±0.09) and (24.62±0.13) μg quercetin/mg dry weight, respectively] (Table 1).

The total tannins content of extracts ranged from (185.71±0.07) to (317.85±0.01) μg catechin/mg dry weight extract (Table 1). This study showed that the butanol extract of flowers exhibited the highest tannins content with (317.85±0.01) μg catechin/mg dry weight extract, followed by the flowers and roots water extracts of (300.95±0.05) and (267.61±0.11) μg catechin/mg dry weight extract, respectively.

3.2. Antioxidant activities

3.2.1. DPPH scavenging activity

DPPH is a free radical, being stable at room temperature and producing a purple solution in ethanol. In presence of antioxidant compounds, the DPPH is reduced, producing a colorless ethanol solution. We have found that the crude and the ethyl acetate extracts from flowers showed important antioxidant properties, with an inhibition reactivity of (93.00±0.01)% and (91.00±0.01)% at 1 mg/mL, respectively. The ethyl acetate extract had the highest antioxidant activity similar to that of BHT and gallic acid with a maximal inhibition percentage of (94.70±0.01)% and (96.70±0.01)% at the same concentration (0.125 mg/mL) (Table 2).

Compared with BHT, other extracts from leaves+stems,

Table 2

Antioxidant activities of *R. rugosum* extracts on DPPH, ABTS, reducing power and â-carotene/linoleic acid test.

Extracts		DPPH IC_{50} (mg/mL)	PI of DPPH scavenging (1 mg/mL)	Reducing power EC_{50} (mg/mL)	PI of ABTS scavenging (1 mg/mL)	β-carotene/linoleic acid test IC_{50} (mg/mL)
Roots	Crude extract	0.027±0.001	83.00±0.01	0.117±0.020	64.31±0.09	0.338±0.020
	EtOAc	0.027±0.001	84.00±0.01	0.272±0.020	68.38±0.01	0.338±0.010
	Water fraction	0.032±0.001	86.00±0.01	0.351±0.092	47.53±0.19	0.957±0.020
	Bu-OH	0.030±0.001	72.00±0.02	0.101±0.010	57.92±0.19	0.806±0.040
Leaves+stems	Crude extract	0.024±0.001	77.23±0.01	0.282±0.020	56.76±0.20	0.290±0.020
	EtOAc	0.023±0.001	87.00±0.01	0.184±0.020	50.90±0.01	0.840±0.090
	Water fraction	0.023±0.001	86.00±0.01	0.555±0.010	44.25±0.20	0.106±0.010
	Bu-OH	0.020±0.001	87.00±0.01	0. 420±0.010	64.01±0.01	0.290±0.030
Flowers	Crude extract	0.019±0.001	93.00±0.01	0.153±0.092	43.97±0.01	–
	EtOAc	0.016±0.001	91.00±0.01	0.770±0.010	57.18±0.01	0.203±0.040
	Water fraction	0.020±0.001	84.00±0.01	0.279±0.012	45.69±0.01	0.157±0.010
	Bu-OH	0.023±0.001	88.41±0.01	0.470±0.050	59.13±0.01	0.150±0.010
	BHT	0.018±0.006	94.70±0.01[d]	0.020±0.006	95.20±0.60	0.100±0.040
	Gallic acid	0.044±0.006[a]	96.70±0.01[d]	7.900±0.200[a]	94.46±0.20	0.090±0.010

a:IC_{50} (μg/mL): the concentration at which 50% is inhibited; d: Scavenging activity of BHT at 0.125 mg/mL.
PI: percentage inhibition; IC_{50} (mg/mL): the concentration at which 50% is inhibited; EC_{50} (mg/mL): effective concentration at which the absorbance is 0.5 nm. Bu-OH: butanolic extract; EtOAc: ethyl acetate extract.

roots and flowers presented a moderate activity with an inhibition percentage ranging from (72.00±0.02)% to (88.41±0.01)% (Table 2).

The values of IC_{50} calculated for *R. rugosum* extracts from leaves+stems, roots and flowers confirmed the reactivity of these samples against DPPH· free radicals.

The best results were obtained with the ethyl acetate [IC_{50}=(0.016±0.001) mg/mL] and the crude extracts from flowers [IC_{50}=(0.019±0.001) mg/mL].

The ethyl acetate extract from flowers had the highest antioxidant activity. This good activity may be due to the higher content of phenolic compounds. Falleh pointed out the correlation coefficient between phenolic compounds and IC_{50} values of the DPPH[22], indicating that polyphenolics may play an important role in free-radical scavenging. In general, the antiradical and antioxidant activities of the plant extracts are ascribed to the phenolic contents[23].

3.2.2. Radical cation $ABTS^{\cdot+}$ scavenging activity

The ABTS method gives a measure of the antioxidant activity of extract by determining the reduction of the radical cation as the percentage of inhibition (PI) of absorbance at 734 nm. Chakraborty reported that the decolorization of the $ABTS^{\cdot+}$ cation reflects the capacity of an antioxidant to donate electrons or hydrogen atoms in order to inactivate this radical species[18].

Table 2 shows the antioxidant activity of all extracts from *R. rugosum*.

A significant PI of $ABTS^{\cdot+}$ was observed due to the scavenging ability of the ethyl acetate and crude extracts from roots and butanolic extract from leaves+stems. A (68.38±0.01)% radical-scavenging activity was observed by roots ethyl acetate extract at 1 mg/mL, followed by the crude and butanolic extracts (64.310±0.092)% and (64.010±0.100)%, respectively. The antioxidant activity is average compared to that of BHT and gallic acid with a maximal inhibition percentage of (95.20±0.60)% and (94.46±0.20)% at the same concentration (0.125 mg/mL)

At a concentration of 1 mg/mL, the butanolic extract from flowers exhibited an inhibition percentage of (59.13±0.01)%.

3.2.3. β-carotene bleaching inhibition activity

In the β-carotene/linoleic acid test, the oxidation of linoleic acid generates peroxyl free radicals which will then oxidise the highly unsaturated β-carotene. The presence of antioxidants will minimize the oxidation of β-carotene. The β-carotene bleaching inhibition effect of BHT, gallic acid and the extracts are shown in Table 2. BHT and gallic acid have a strong antioxidant activity in the test [IC_{50} of (0.10±0.04)mg/mL and (0.09±0.01) mg/mL]. Water extract from leaves+stems, butanolic and water extracts from flowers had similar strong activities inhibiting β-carotene bleaching, whose IC_{50} values were (0.106±0.010), (0.150±0.010) and (0.157±0.010) mg/mL, respectively.

3.2.4. Reducing power

The antioxidant can donate an electron to free radicals leading to their neutralization.

The reducing power was measured by the direct electron donation in the reduction of Fe^{3+}(CN-)6-Fe^{2+}(CN-)$_6$[19]. The extract was visualized by forming the intense Prussian blue color complex and then measured at 700 nm. As shown in Figure 1, a higher absorbance value indicates a stronger

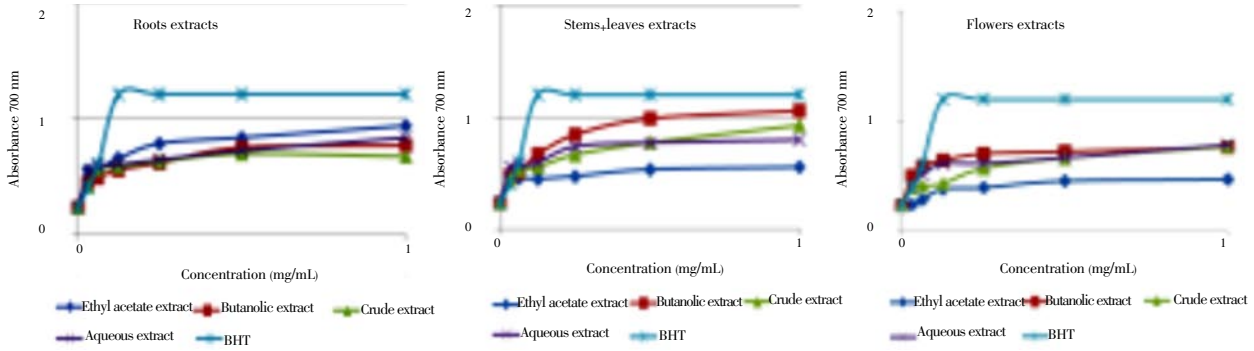

Figure 1. Antioxidant activity of various extracts of *R. rugosum* determined using reducing power assay.

reducing power of the samples.

The strong reducing power of *R. rugosum* extracts at 1 mg/mL concentration were in the following decreasing order (optic density value): ethyl acetate extract from leaves+stems > ethyl acetate extract from roots > butanolic extract from leaves+stems > aqueous extract from roots.

The reducing power of ethyl acetate extracts from leaves+stems and roots was significantly higher than that of butanolic extract from leaves+stems and water extract from roots at concentrations ranging from 0.032 to 1.00 mg/mL (Figure 1).

The maximum and minimum reducing powers were noted in the ethyl acetate extract from leaves+stems and ethyl acetate extract from flowers, respectively in *R. rugosum*.

A low EC_{50} value is indicative of a good reducing power (Table 2). A butanolic extracts from roots (EC_{50}=0.101 mg/mL) and crude extract from roots (EC_{50}=0.117 mg/mL) of *R. rugosum* had the lowest EC_{50} value, indicating that they contained the best reducing power compared to BHT and gallic acid [EC_{50}=0.02 mg/mL and (0.09±0.04) mg/mL].

These results indicate that flavonoids, tannins and polyphenols may play a more important role in reducing power.

3.3. Determination of AChE inhibitory activity

Inhibition of AChE, the key enzyme in the breakdown of acetylcholine, is considered as one of the treatment strategies against several neurological disorders such as Alzheimer's disease, senile dementia, ataxia, and myasthenia gravis[24]. Plants have traditionally been used to enhance a cognitive function and to alleviate other symptoms associated with Alzheimer's disease nowadays[25]. The AChE inhibition was determined using an adaptation of the method described in the literature[26].

Table 3 shows the AChE inhibitory activity (%) and IC_{50} values of extracts. Generally, the extracts exhibited a dose-dependent AChE inhibition activity (%). All the flowers extracts had a better AChE inhibition value ranging from (62.74±0.01)% to (97.43±0.01)% than similar extracts of stems+leaves and roots.

Table 3

AChE inhibition capacity of extracts from *R. rugosum*, represented by IC_{50} (mg/mL) and inhibitory activity (%).

Samples		AChE (IC_{50}, mg/mL)	AChE inhibition (%) (10 mg/mL)
Flowers	EtOAc	7.66±0.01	62.74 ±0.01
	Bu-OH	6.89±0.50	67.52±0.01
	Water fraction	1.68±0.01	97.43±0.01
	Crude extract	2.51±0.01	95.00±0.01
Leaves+stems	EtOAc	5.84±0.01	66.99±0.01
	Bu-OH	1.60±0.01	85.71±0.01
	Water fraction	8.22±0.01	53.05±0.01
	Crude extract	8.50±0.01	43.04±0.01
Roots	EtOAc	3.22±0.01	59.62±0.01
	Bu-OH	8.91±0.01	50.70±0.01
	Water fraction	3.46±0.01	67.52±0.01
	Crude extract	1.73±0.01	80.04±0.01
Eserine		0.0029±0.0100 μg/mL	

Mean±SE (n=3) for either percentage inhibition at various concentrations or IC_{50}. Bu-OH: Butanolic extracts; EtOAc: Ethyl acetate extracts.

A positive linear correlation between the antioxidant activity, determined by the IC_{50} values of DPPH radical-scavenging activity method and total phenolic, total flavonoid and total tannin content of *R. rugosum* extracts was observed (Table 4).

Table 4

Linear correlation coefficients, r^2, for relationships between the assays for the different extracts of *R. rugosum* parts.

Chemical contents	DPPH (IC_{50})			Reducing power (EC_{50})			β-carotene (IC_{50})		
	roots	Leaves+stems	flowers	roots	Leaves+stems	flowers	roots	Leaves+stems	flowers
Total phenolic content	0.400	0.066	0.024	0.644	0.134	0.217	0.318	0.974	0.284
Total flavonoid content	0.343	0.919	0.237	0.452	0.215	0.591	0.198	0.143	0.214
Total condensed tannin	0.629	0.069	0.536	0.045	0.041	0.136	0.769	0.482	0.754

4. Discussion

R. rugosum extracts contained high levels of total phenolic compounds, tannins and flavonoids. All the sample extracts from this species also exhibited high antioxidant and free radical scavenging activities and some even showed a higher potency than the standard synthetic antioxidants in some instances; for example, the aqueous extract of flowers had a higher activity in the DPPH assay than the BHT. The results of the present study suggest that the plant extracts provide a substantial source of secondary metabolites, which act as natural antioxidants and acetylcholinesterase inhibitors.

The relatively high correlation coefficient from leaves+stems extracts between IC_{50} values of DPPH test and total flavonoid content (r^2=0.919) indicated that flavonoids were one of the main components responsible for the antioxidant behavior of leaves+stems extracts. A significant correlation (r^2=0.629 and r^2=0.536) between IC_{50} values of DPPH radical-scavenging activity and the total tannin content was noted for roots and flowers extracts of *R. rugosum*, respectively.

Roots extracts showed that the reducing power exhibited a significant correlation with the total phenolic content (r^2=0.644), a moderate correlation with the total flavonoid content (r^2=0.452), but a weak correlation with the total condensed tannin (r^2=0.045).

The antioxidant activity in the reducing power assay of flowers showed a moderate correlation with the total flavonoid content (r^2=0.591).

The relatively high correlation coefficient from leaves+stems extracts between IC_{50} values of β-carotene/linoleic acid and total phenolic content (r^2=0.974) indicated that phenolic compounds were one of the main components responsible for the antioxidant behavior of leaves+stems extracts.

Positive linear correlations were observed between the total tannin content and the antioxidant activity of the extracts from roots (r^2=0.769) and flowers (r^2=0.754) in the β-carotene/linoleic acid test.

These results indicate that the activity of the other extracts may be caused by other metabolites such as vitamins, namely vitamins E and C, carotenoids or enzymes involved in the antioxidant mechanisms.

All the plant extracts contained some level of inhibitory activity against AChE. A low IC_{50} value is indicative of a good inhibition of the enzyme. Butanolic extracts from leaves+stems, water extract from flowers and water extracts from roots of *R. rugosum* had the lowest IC_{50} value, indicating that they contained the best inhibition of the enzyme. The observed activities might be due to the presence of flavonoids, polyphenols and tannins in the different parts of *R. rugosum*.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

The study of bioactive metabolites with antioxidant and anti-acetylcholinesterase is important for the possibility of their application for health care. The introduction is clear and situates the reader in the thematic.

Research frontiers

The selection of methodology was very good and adequate with the objectives of the investigation. The interpretation of the results was according to the purpose of the investigation.

Related reports

The related reports used in the discussion showed good relationship with the objectives of the research.

Innovations & breakthroughs

The selection of the material under study is important because crucifers are a group of vegetables that are included in the normal diet and have bioactive substances improving health.

Applications

The knowledge of bioactive substances, the scientific demonstration of their activity and the security of their consumption is essential for the development of biotechnological applications or the use of their structure as model for the synthesis of new substances with incremented activity and use security.

Peer review

The authors studied the levels of antioxidant substances

measured by different methodologies and statistically compared the correlation of the results. They concluded that phenolic compounds are one of the main components responsible for the antioxidant behavior of the extracts and fractions. They also demonstrated the anti-acetylcholinesterase activity in the extracts.

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