Research Article

Anti-Inflammatory and Antioxidant Activities of Rhubarb Roots Extract

Eman A. Ibrahim[1], Doha H. Abou Baker[2], Farouk K. El-Baz[1]*

[1]Plant Biochemistry Department, National Research Centre (NRC), 33 EL Bohouth St. (Former EL Tahrir st.), Dokki, Giza, Egypt.

[2]Medicinal and Aromatic Plant Department, National Research Centre (NRC), 33 El Bohouth St. (former El Tahrir st.), Dokki, Giza, Egypt.

***Corresponding author's E-mail:** fa_elbaz@hotmail.com

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ABSTRACT

Rhubarb (*Rheum rhaponticum* L.) is considered one of the commonly used edible and medicinal plant. This work was carried out to investigate the anti-inflammatory and antioxidant activities of Rhubarb roots extract. Total tannin, total phenolic and total flavonoid contents of ethanolic and water extracts as well as phenolic and flavonoid compounds were also determined in Rhubarb roots. The highest content of total tannin, phenolic and flavonoid were found in ethanolic extract. The major phenolic acids were benzoic and ferulic acids followed by vanillic acid, while the major flavonoid was narengine. The results indicated that the highest antioxidant and anti-inflammatory activities were found in ethanolic extract and highly correlated with phenolic content. The results of the current work indicated that ethanolic extract has the potential to be used as a natural anti- inflammatory and antioxidant.

Keywords: Rhubarb roots, anti-inflammatory, antioxidant.

INTRODUCTION

Rhubarb is a perennial plant belonging to Polygonaceae. It also, considers a common vegetable known as sibiric Rhubarb.[1] This herbis a storehouse of a large number of important crude drugs.[2] Phytochemical investigation on Rhubarb has proved major bioactive ingredients arephenolic compounds in six skeletal type including anthraquinones (physcion, chrysophanol, emodin, aloe-emodin and rhein and their glucosides), anthocyanins (cyanidin 3-rutinoside and cyanidin 3-glucoside), flavonoids (catechin, quercetin 3-O-rhamnoside, quercetin3-O-galactoside, and quercetin 3-O-rutinoside), stilbene (*trans*-rhapontigenin and desoxyrhapontigenin (*cis*-rhapontigenin, resveratrol and piceatannol).[3]

Rhubarb is well-known for its strong biological activities such ascathartic, anticancer, hepatoprotective, anti-inflammatory, anti-diabetic, analgesic, antiplatelet, antibacterial, anti-oxidative, and antimutagenic effects.[4-11]

Rhubarb roots are used as oriental laxative medicine and an antipsoriatic drug, also used against diarrhea, as well as stomachic, antiemetic, hemorrhoids, measles, smallpox and cholagogue.[12,13] Rhubarb also showed the protective effect against liver injury and fatty liver.[14-15]

The purpose of the current work was to investigate the anti-inflammatory and antioxidant activities of Rhubarb roots extract.

MATERIALS AND METHODS

Preparation of Plant Extracts

Rhubarb was purchased from local market in Egypt. The Rhubarb roots were extracted, briefly, 10 g of the dried powder were soaked with 100 ml water and 80% ethanol and shacked for 24 h at room temperature. The extraction was repeated twice and then filtered. The resulting of different extracts was used for the determination of total tannin, phenolic, flavonoid, as well as their anti-inflammatory and antioxidant activities.

Quantitative Analysis

Total Tannin Content

Total tannin content was determined using the Folin-Ciocalteu reagent assay according to Tambe and Bhambar.[16] About 0.1 ml of Rhubarb roots extract or standard solutions of (tannic 20-120 mg/l) was added to 7.5 ml distilled water then 0.5 ml of Folin reagent and 1 ml of 35% sodium carbonate solution were added. The volume was made up for 10 ml with distilled water and the absorbance was measured against blank at 725 nm by using spectrophotometer.

Total Phenolic Content

Total phenolic in Rhubarb roots ethanolic and water extracts were determined by using Folin-Ciocalteu's reagent.[17] The reaction mixture was prepared from mixing 0.5 ml of Rhubarb ethanol extract, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml 7.5% $NaHCO_3$. The mixtures were incubated in a thermostat at 45°C for 45 min. The absorbance was spectrophotometrically recorded at 760 nm. Standard series concentrations of Gallic acid were prepared in ranges 2-12 µg/ml and treated as the samples.

Total Flavonoid Content

Total flavonoid content of Rhubarb roots extract was determined by the aluminum chloride method using quercetin as a standard.[18] 0.2 ml of the extracts or standard solutions (quercetin, 20–120 mg/l) was mixed with 0.3 ml 5 % $NaNO_2$. After 5 min, 0.3 mL of 10% $AlCl_3$

was added then incubated for 6 min and 2 mL of 1 mol/L NaOH was subsequently added. The solutions were mixed well and the absorbance was measured against prepared reagent blank at 510 nm by using spectrophotometer.

Identification of Phenolic and Flavonoid Compounds

The phenolic and flavonoid compounds of Rhubarb roots were extracted according to the method described by Hakkinen[19] and Mattila.[20]

Ten grams sample were extracted using 10 ml of aqueous and 80% ethanol by homogenization for 2 min then centrifuged at 25,000 g for 10 min. The supernatant was decanted into a round-bottom flask. The pellet was re-suspended in aqueous and 80% ethanol, centrifuged, and the supernatants were combined, evaporated by rotary at 40 °C until dryness and resolved in methanol-HPLC. Extracts were filtrated through 0.20µm millipore membrane filter and set up to a known volume (10 ml). Three milliliters were collected in a vial for subsequent HPLC separation. HPLC instrument (Hewlett Packard, series 1050, country) equipped with column C18 hypersil BDS with particle size 5µm. Injection volume was 75 µl carried out with autosamplling injector. The column temperature was maintained at 35°C. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate 1.0 ml/min. Elutes were monitored using UV detector set at 280 nm for phenolic acid and 330 nm for flavonoid. Chromatographic peaks were identified by comparing the retention times with the respective retention times of known standard reference material. Retention time and peak area were used to calculation of phenolic acid and flavonoid compounds concentration by the data analysis of Hewlett Packard software. Phenolic acid and flavonoid compounds were expressed as µg/100g sample on dry weight basis.

In-vitro Anti-inflammatory Activity

Anti-inflammatory activity of different extracts from Rhubarb roots extract was carried out using the method of Rahman.[21] The different concentrations of Rhubarb roots extract or standard drug diclofenac sodium (50, 100, 150,200 ug/ml) were mixed with 0.45ml bovine albumin serum. The mixtures were incubated at 37°C for 20 min and then heated to 57°C for 3 min, after cooling 2.5ml phosphate buffer PH 6.4 were added to the samples. The absorbance was measured at 255nm using UV visible spectrophotometer.

Antioxidant Activity

DPPH Free Radical Scavenging Assay

The ability of different extracts from Rhubarb roots to scavenge DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical was determined according to the method of Ye.[22] Briefly, a 0.1 mM of ethanolic DPPH solution was prepared, to give the initial absorbance value of 0.993 at 517 nm. The different concentrations of Rhubarb extracts (in 0.1 ml) were added to 3.0 ml of ethanolic DPPH solution. After incubation for 30 min in the dark, the absorbance was measured at 517nm.

The percentage of DPPH scavenging activity was calculated using the following formula:

$$Scavenging\ Activity\ \% = \left[1 - \left(A_{sample} - \frac{A_{blank}}{A_{control}}\right)\right] \times 100$$

ABTS Free Radical Scavenging Assay

The ABTS free radical scavenging activity assays were carried out according to Arnao.[23]

Potassium persulfate (2.6 mM) was added to 7.4 mM of ABTS+ and kept for 12–16 h at room temperature in dark.

The ABTS+ solution (1mL) was diluted with 60ml methanol to an absorbance of 1.1 ± 0.02 at 734 nm before analysis.

ABTS+ solution (2.80 mL) was added to sample fractions (0.150 mL, 50–150 µg/mL).

The After incubation for 2h in the dark, the absorbance was measured at 734nm.

Trolox was used as the positive control. The ABTS free radical-scavenging activity (%) was calculated using the following equation:

$$Scavenging\ Activity\ \% = \left[1 - \left(\frac{A_1 - A_2}{A_0}\right)\right] \times 100$$

Where, A_0 was the absorbance of the control (without sample), A_1 was the absorbance in the presence of the sample, and A_2 was the absorbance without ABTS+).

Total Antioxidant Activity

Total antioxidant activity assay was carried out according to Prieto.[24]

One ml of different extracts from Rhubarb roots ethanolic and water extracts (100 to 400 µg/mL) were mixed with 3 ml of reagent solution (0.6 M sulfuric acid, 28mM sodium phosphate, and 4 mM ammonium molybdate).

The tubes were capped and incubated at 95°C for 90 min.

After cooling, the absorbance was measured at 695 nm. Standard series concentrations of ascorbic acid were treated as the sample.

Statistical Analysis

All results are expressed as mean value of three replicate.

Data were statistically analyzed through analysis of variance (Anova) and Duncans test at P≥0.01 using CoStat Statistics Software.

RESULTS AND DISCUSSION

Quantitative Analysis

Total Tannin Content of Rhubarb Roots Extract

The results presented in Table 1 showed that, the Rhubarb roots extract contain total tannin ranged from 559.81mg/100g DW (water extract) to 1248mg/100g DW

(ethanolic extract). On the other hand Kemper[25] found that Rhubarb roots contain 5 - 10% tannins.

Tannins are astringent kind of phenolic compounds found in many herbs. The author considered that, plants that contain more than 10% tannins have potential adverse effects including upset stomach, renal damage, hepatic necrosis, and increased risk of esophageal and nasal cancer.[25]

Table 1: Total Tannin Content

Extracts	Tannin Content (mg/100g DW)
Ethanolic	1248.33±14.47[a]
Water	559.81±36.46[b]
LSD	104.29

- Data are represented as mean ± S.D.
- Statistical analysis is carried out by one way analysis of variance using Co-stat program.
- Unshared letters are significant value between groups at P ≥ 0.01.

Total Phenolic of Rhubarb Roots Extract

Phenolic compounds are known as powerful chain breaking antioxidants.[26-27]

As shown in Table 2 ethanolic extract had a high phenolic content 1115.04 mg/100g d.w than water extract 655.47mg/100g d.w.

Table 2: Total Phenolic Content of Rhubarb Roots Extract

Extracts	Phenolic content (mg/100g d.w)
Ethanolic	1115.04±5.14[a]
Water	655.47±10.28[b]
LSD	30.56

- Data are represented as mean ± S.D.
- Statistical analysis is carried out by one way analysis of variance using Co-stat program.
- Unshared letters are significant value between groups at P ≥ 0.01.

In previous studies, Zhou and Yu[28] studied the phenolic content of some vegetables and found that, kale had the highest total phenolic content (1630-1880 mg) followed by Rhubarb (1320 mg), spinach (930–1300 mg) and broccoli (940-1060 mg).

Moreover, Takeoka[29] found that, the phenolic content of various Rhubarb (Rheum spp.) varieties, varied from 673 ± 41 (Loher Blut) to 4173 ± 23 mg Gallic Acid Equivalent/100 g DW (Plum Hutt) and had a low correlation (r = 0.663) with antioxidant activity.

Some of the varieties had higher total phenol than kale, a vegetable rich in phenol. These results are consistent with the current results.

Total Flavonoid of Rhubarb Roots Extract

In various studies, antioxidant activity of the plant extracts is correlated with flavonoids content.[30]

In the present study the most flavonoid rich extract was found in ethanolic extract, which showed 687mg/100g d.w compared with 149.01mg/100g in water extract (table 3). Flavonoids have been found to possess anti inflammatory, antimicrobial and antioxidants in various studies.[31-33]

Table 3: Total Flavonoid Content of Rhubarb Roots Extract

Extracts	Flavonoid Content (mg/100g d.w)
Ethanolic	687 ± 4.58[a]
Water	149.01 ± 8.47[b]
LSD	25.62

- Data are represented as mean ± S.D.
- Statistical analysis is carried out by one way analysis of variance using Co-stat program.
- Unshared letters are significant value between groups at P ≥ 0.01.

Identification of Rhubarb Roots Phenolic Compounds Using HPLC

The phenolic compounds in ethanolic and water extracts are given in Table 4.

On the basis of the results of quantitative analysis, the major phenolic compunds were vanillic acid (4189.93 mg/100g d.w) benzoic (1652.91 mg/100g d.w) and ferulic acids (1622.59 mg/100g d.w) followed by catechol (785.44 mg/100g d.w).

In another study, Ye[34] studied the chemical composition of Rhubarbs roots comprehensively and they found that, 107 phenolic compounds were identified. These compounds include sennosides, anthraquinones, stilbenes, glucose gallates, naphthalenes, and catechins.

As shown in Table 5 ethanol extract of Rhubarb roots showed high content of flavonoids compared to water extract. In addition, narengine (471.05mg/100 g extract) was the major flavonoid in Rhubarb roots ethanolic extract followed by quercetrin, rutin, and hisperdin (171.30, 154.66 and 149.50 mg/100 g DW, respectively). Krafczyk[35] investigated the phenolic content of Rhubarb by HPLC, and found that about 14 compounds were detected as follows: two stilbenes (trans-rhapontigenin, trans-desoxyrhapontigenin), Five stilbene glycosides (trans-rhaponticin, cis- and transdesoxyrhaponticin, trans-resveratrol-4-O-D-glucopyranoside, trans-piceatannol-3-O-D-glucopyranoside) and seven flavonoids [rutin, quercetin-3-O-glucuronide, isovitexin, 6,8-di-C-D-glucosylapigenin, schaftoside, isoschaftoside, (+)-catechin].

Table 4: Phenolic Compound of Rhubarb Roots Extract

Phenolic Compounds	Phenolic Content (µg/100g d.w)	
	Ethanolic	Water
Gallic	761.46	400.66
Pyrogallol	1282.78	791.97
3-Hydroxy tyrosol	471.58	31.05
4-amino benzoic	42.42	5.13
Protocatechuic	397.79	62.83
Chlorogenic	401.72	11.45
Catechol	785.44	40.94
Epicatechein	420.45	44.09
Catechein	-	60.59
Caffeine	174.39	12.77
P-hydroxy benzoic	308.46	54.12
Caffeic	108.08	26.35
Vanillic	234.47	7.00
Ferulic	1622.59	26.91
Iso-ferulic	89.51	175.38
e-vanillic	4189.93	261.31
Reversetrol	221.27	78.8
Ellagic	536.51	55.08
α-coumaric	298.23	16.20
Benzoic	1652.91	422.10
3,4,5-methoxy cinnamic	308.88	102.59
Coumarin	108.37	19.57
Saiycilic	-	70.13
p-coumaric	37.47	5.25
Cinnamic	262.46	52.33

Table 5: Flavonoid Compounds of Rhubarb Roots Extract

Flavonoid Compounds	Flavonoid content (µg/100g d.w)	
	Ethanolic	Water
Narengin	471.05	62.71
Rutin	154.66	7.53
Hisperdin	149.50	120.71
Rosmarinic	108.66	15.99
Quercetrin	171.30	132.37
Quercetin	49.07	10.03
Narengenin	10.98	2.65
Kampferol	61.097	3.71
Hespertin	69.49	13.48
Apegenin	36.57	11.74
7-hydroxyflavone	7.86	2.32

Anti-inflammatory Activity of Rhubarb Roots Extract

Oxidative stress is considered the major factor responsible for inflammatory diseases which have recently treated with antioxidant plants due to it is high content of phenolic compounds.[36]

Inflammation is the reaction of living tissues to injury, infection or irritation, which occurs changes, such as: the increase of vascular permeability, membrane alteration and increase of protein denaturation.[37]

Ethanolic and water extracts of Rhubarb were investigated for anti-inflammatory activity and compared to positive control. The anti-inflammatory effect of ethanolic extract was significantly higher than water one (Table 6).

Based on these results, the ethanol extract has the potential to be used as a natural anti-inflammatory.

Table 6: Anti-inflammatory Activity of Rhubarb Roots Extract

Extracts	Inhibition %		
	Sample Concentration (µg/ml)		
	100	150	200
Ethanolic	42.45 ± 1.45^{f}	57.14 ± 0.50 ^{e}	76.85 ± 7.8 ^{c}
Water	45.26 ± 1.7^{f}	53.11 ± 1.1^{e}	66.46 ± 0.50^{d}
Diclofenac sodium	81.74 ± 0.63b^{c}	85.5 ± 0.20^{ab}	88.48 ± 0.30^{a}
LSD 0.01	6.5		

- Data are represented as mean ± S.D.
- Statistical analysis is carried out by one way analysis of variance using Co-stat program.
- Unshared letters are significant value between groups at P ≥ 0.01.

Table 7: DPPH Free Radical Scavenging Activity of Rhubarb Roots Extract

Extracts	Inhibition %		
	Sample concentration (µg/ml)		
	50	100	150
Ethanolic	64.75 ± 4.06^{d}	88.4 ± 3.53^{c}	148.8±6.84^{a}
Water	18.28 ±1.11^{g}	31.39 ± 0.97^{f}	53.85 ±3.75^{de}
Ascorbic acid	45.86 ± 4.64^{e}	84.2 ±4.39^{c}	113.8±10.83^{b}
LSD 0.01	12.39		

- Data are represented as mean ± S.D.
- Statistical analysis is carried out by one way analysis of variance using Co-stat program.
- Unshared letters are significant value between groups at P ≥ 0.01.

Table 8: ABTS Radical Scavenging Activity of Rhubarb Roots Extract

Extracts	Inhibition %		
	Sample concentration (µg/ml)		
	50	100	150
Ethanolic	91.62 ± 0.62^{b}	93.73 ± 0.59^{b}	124.89 ± 4.18^{a}
Water	11.89 ± 1.22^{g}	28.83 ± 3.00^{f}	47.14 ± 2.28^{d}
Ascorbic acid	40.76 ± 0.37^{e}	81.53 ± 0.75^{c}	122.3 ± 1.13^{a}
LSD 0.01	4.71		

- Data are represented as mean ± S.D.
- Statistical analysis is carried out by one way analysis of variance using Co-stat program.
- Unshared letters are significant value between groups at P ≥ 0.01.

Table 9: Total Antioxidant Activity of Rhubarb Roots Extract

Extracts	Inhibition %			
	Sample concentration (µg/ml)			
	100	200	300	400
Ethanolic	288.88 ± 9.66^{e}	452.62 ± 29.5^{c}	701.75 ± 26.54^{b}	832.16 ± 3.64^{a}
Water	145.40 ± 5.27^{h}	181.20 ± 2.71^{g}	256.41 ± 6.55^{f}	340.34 ± 3.5^{d}
Ascorbic acid	127.97 ±.93^{h}	147.37 ± 12.15^{h}	230.16 ± 6.2^{f}	243.46 ± 3.5^{f}
LSD 0.01	29.85			

- Data are represented as mean ± S.D.
- Statistical analysis is carried out by one way analysis of variance using Co-stat program.
- Unshared letters are significant value between groups at P ≥ 0.01.

Antioxidant Activity of Rhubarb Roots Extract

The antioxidant potential of both roots extracts were evaluated using different antioxidant tests, namely DPPH radical scavenging, ABTS and total antioxidant activity.

DPPH

DPPH radical scavenging extracts exhibited stronger activity than known standard, and these results are in harmony with Celep[38] who concluded that, phenolic compounds are a major class of bioactive components, which have been demonstrated to be better antioxidants *in vitro* than ascorbic acid.

The scavenging effects of Rhubarb roots extract were examined at different concentrations.

As shown in Table 7, both extracts exhibited a significant activity.

In particular ethanolic extract showed the higher activity than water extract one.

Ethanolic and water extracts showed dose-dependent activity relationships.

Rhubarb has been considered as top source of antioxidant phenols, the antioxidant activity of phenolic compounds were found to vary widely with their chemical structure.[39-40]

These results are in accordance with the results of the previous studies, indicating high antioxidant potential of Rhubarb than ascorbic acid.[41]

Ozturk[42] studied the antioxidant potential of Rhubarb roots and stems.

They found that, all the extracts exhibited stronger activity than known standards, roots showed the highest inhibition activity (93.1 and 84.1%) for chloroform and methanol extracts, while 82.2 and 82.0% inhibitions was detected by stem extracts of the same solvents, respectively.

Antioxidant activities and the total phenolic and flavonoid contents of the plant extracts showed a positive linear correlation.

The plant extracts with high phenolic and flavonoid contents are considered a good source for antioxidant activity because they have high reactivity as hydrogen or electron donors beside, they are capable of chelating metal ions owing to their hydroxyl group at various positions.[43-44]

ABTS Scavenging Activity

The results presented in Table 8 indicated that ethanol extract showed higher ABTS scavenging activity, than ascorbic acid.

Takeoka[45] determined antioxidant activity using ABTS in twenty-nine Rhubarb varieties and found that, antioxidant activity ranged from 463±50 to 1242±2 mol Trolox per g d.w.

Total Antioxidant Activity of Rhubarb Roots Extract

The antioxidant activity of ethanolic extract of Rhubarb roots exhibited stronger activity than ascorbic acid (table 9).

The results are in agreement with those obtained by Ozturk[46], as they found that the total antioxidant activity increased with increasing the amount of methanol and chloroform Rhubarb root extracts.

CONCLUSION

Rhubarb roots ethanolic extract could be considered a rich source of antioxidant compounds.

Due to its abundance in polyphenolic activity, this extract exhibit effective radical scavenging activities more than ascorbic acid.

There was a good correlation between the tannin, phenolic, flavonoid compounds and with the anti-inflammatory activities.

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