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ORIGINAL ARTICLE

Potential assessment of *Rumex vesicarius* L. as a source of natural antioxidants and bioactive compounds

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Abstract The present work is designed to evaluate the antioxidant activities of hydroalcoholic extract (HAE) and its fractions (viz., hexane (HF), chloroform (CF), ethyl acetate (AF), n-butanol (BF) and water (WF)) obtained from aerial part of *Rumex vesicarius* L. by using different in vitro antioxidant assays. The content in pigments (carotenoids and chlorophylls), total phenolics, flavonoids and tannins were determined using spectrophotometric methods. Qualitative analyses of major phenolics by TLC analysis were also evaluated. Experimental results obtained show that *R. vesicarius* is a rich source of β-carotene (116.83±1.60 μg/g DW), lycopene (156.40±1.59 μg/g DW) and chlorophyll a (271.45±3.46 μg/g DW). The greatest antioxidant activity was found in AF ($IC_{50.DPPH}$=0.07±0.00 mg/ml) followed by BF and CF (0.15±0.00 and 0.16±0.00 mg/ml, respectively). These fractions were also better in their effect on reducing the oxidation of β-carotene. Reducing power of crude methanol extract/fractions increased with increasing concentration of the extract. The amount of total phenolics varied from 0.37±0.01 to 43.28±0.28 mg GAE/g of dry weight, HAE had the higher content (43.28±0.28 mg GAE/g of DW). A negative correlation was found between phenolic compounds and the antioxidant efficiencies of the crude extract/fractions, suggesting that phenolic compounds are not the only contributors to the antioxidant activities of *Rumex vesicarius*. The present findings suggest that *Rumex vesicarius* L. can be used as natural antioxidant source to prevent damage associated with free radicals.

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Introduction

The use of medicinal plants for treating diseases is probably the oldest existing method that humanity has used to try to cope with illness. In fact plants represent an enormous reservoir of new, undiscovered and bioactive molecules. Over the last two decades there has been a resurgence of interest in the study and use of medicinal plants. The World Health Organization has confirmed the importance of traditional medicine to a majority of the world's population and encourages all countries to preserve and to use the safe and positive elements of traditional medicine in their national health systems (Aquino et al. 1995).

The last decade is characterized by a growing interest in natural antioxidants. As a result, an overwhelming amount of scientific information is now available and promises to keep increasing. The use of synthetic antioxidants in the food industry, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), tert-butyl hydroquinone (TBHQ), and ascorbyl palmitate (PA), is severely restricted by legislation concerning both the application and permitted concentrations (Regulation (EC) 2008). Moreover, there is some safety concerns related to the residual toxicity of chemical preservatives (EFSA 2012). Therefore, the recent worldwide tendency to avoid or at least decrease the use of synthetic additives has created a need for alternative cheap, renewable, natural and possibly safer sources of natural compounds with antioxidant activity to prevent oxidation in foods. Oxidation reactions are not only important to the food industry; antioxidants are also required to avoid deterioration of products found in the cosmetics, pharmaceutical and plastic industries (Moure et al. 2001).

A large number of scientific reports have described the properties of natural substances from plants. Of which, phenolics were one of the most notable groups. *Rumex vesicarius* L., locally called Hammeidda (Quézel and Santa 1962), is an edible green leafy plant that belongs to the family of Polygonaceae. This shrub, native to northern Africa and Asia (Pakistan and India) (Sankar et al. 2011), grows annually during the fall and spring rainy seasons. It was considered as a dietary complementary plant, since this plant is a rich source of ß-carotenes (Bélanger et al. 2010), vitamins (especially vitamin C), proteins, lipids and organic acids. It is also a good source of minerals such as; K, Na, Ca, Mg, Fe, Mn and Cu (Saleh et al. 1993; Al–Rumaih May et al. 2002; Alfawaz 2006; Filho et al. 2008). The whole plant is medicinally important and cures several diseases, the plant is stimulant, tonic, and acts as aphrodisiac agent (Gopal et al. 2008). It is also used in treatment of tumors, hepatic diseases, bad digestion, constipation, calcules, heart troubles, pains, diseases of the spleen, hiccough, flatulence, asthma, bronchitis, dyspepsia, piles, scabies, leucoderma, toothache and nausea. Finally, the plant can be used also to reduce biliary disorders and control cholesterol levels (Mostafa et al. 2011).

The previously mentioned bioactive phytochemicals, in addition to phenolic compounds and flavonoids, have a role as antioxidant and detoxifying agents. The intake of dietary antioxidant phytochemicals leads to protection against non-communicable diseases i.e. cancer, cardiovascular diseases and cataract (Rao 2003; Alberto et al. 2006; Matkowski 2008; Ghafar et al. 2010; Imran et al. 2011). The purpose of the present study was to prepare fractions from the crude extract of *Rumex vesicarius* L. that is rich in phenolic compounds and investigate their antioxidant activities, and as consequence to exploit its potential as a natural preservative.

Materials and methods

Plant materials

Aerial part of *Rumex vesicarius* L. used for this investigation was collected from Ghardaïa (South of Algeria) in March 2012, and its identity was verified by Dr. Mahboubi from Department of Ecology and Environment, University of Tlemcen, Algeria. Collected plant material was rinsed with distilled water, left at room temperature for 15 days in the dark, dried in a shaded and well-ventilated place, pulverized then kept refrigerated in glass containers before further processing.

Preparation of crude methanolic extract and fractions

Crude methanolic extract (Hydroalcoholic extract (HAE)) was obtained by refluxing of 10 g of powder in 70 % methanol (1:10, w/v) for 2 h at temperature no higher than 65 °C. The extraction was repeated three times. Extract was filtered through filter paper, evaporated under vacuum to dryness and kept at −20 °C before analysis.

The HAE was resuspended in warm water and then partitioned sequentially with hexane, chloroform, ethyl acetate, n-butanol and water. Hexane fraction (HF), chloroform fraction (CF), ethyl acetate fraction (AF), n-butanol fraction (BF) and water fraction (WF) were collected separately and concentrated using a vacuum evaporator to remove the solvent. Residues were dissolved in pure methanol and stored at −20 °C until analysis.

The yield (%) of evaporated dried extracts was calculated as 100 DW_{ext}/DW_{samp}, where DW_{ext} is the weight of extract after evaporation of solvent, and DW_{samp} is the dry weight of sample.

Determination of lipid-soluble pigments

A fine dried powder (150 mg) was vigorously shaken with 10 ml of acetone–hexane mixture (4:6) for 1 min and filtered through filter paper. The absorbance of the filtrate was measured at 453, 505, 645 and 663 nm (Barros et al. 2011). The content in pigments was calculated according to the following equations:

$$\beta\text{-carotene}(mg/100ml) = 0.216 \times A_{663} - 1.220 \times A_{645} - 0.304 \times A_{505} + 0.452 \times A_{453}$$

$$\text{Lycopene}(mg/100ml) = -0.0458 \times A_{663} + 0.204 \times A_{645} - 0.304 \times A_{505} + 0.452 \times A_{453}$$

$$\text{Chlorophyll a}\ (mg/100ml) = 0.999 \times A_{663} - 0.0989 \times A_{645}$$

$$\text{Chlorophyll b}\ (mg/100ml) = -0.328 \times A_{663} + 1.77 \times A_{645}$$

and further expressed in μg per g dry weight (DW).

Phenolic compounds analysis

Total phenolics content determination

Total phenolics were determined using Folin–Ciocalteu reagent, as described by Awah et al. (2012). Folin–Ciocalteu reagent (FCR) consists of a yellow acidic solution containing complex polymeric ions formed from phosphomolybdic and phosphotungstic heteropoly acids. Dissociation of a phenolic proton in a basic medium leads to a phenolate anion, which reduces FCR, forming a blue-coloured molybdenum oxide whose colour intensity is directly proportional to the phenolic contents (Huang et al. 2005). Briefly, crude extract and fractions (100 µl) dissolved in methanol were mixed with 750 µl of Folin–Ciocalteu reagent (diluted 10-fold in H_2O) and allowed to stand at 22 °C for 5 min; 750 µl of Na_2CO_3 (60 g/l) solution were then added to the mixture. After 90 min, the absorbance was measured at 725 nm. The total phenolic content was determined using the standard gallic acid calibration curve and expressed as mg gallic acid equivalents (GAE) per g of dry weight (DW).

Total flavonoids and flavonols content determination

The flavonoids content was determined according to the method described by Barros et al. (2011). An aliquot of the extract solution (0.5 ml) was mixed with distilled water (2 ml) and subsequently with $NaNO_2$ solution (5 %, 0.15 ml). After 6 min, $AlCl_3$ solution (10 %, 0.15 ml) was added and allowed to stand for a further 6 min; thereafter, NaOH solution (4 %, 2 ml) was added to the mixture. Immediately, distilled water was added to bring the final volume to 5 ml. Then the mixture was properly mixed and allowed to stand for 15 min. The intensity of pink colour was measured at 510 nm. Catechin was used to calculate the standard curve and the results were expressed as mg catechin equivalents (CE) per g of dry weight (DW).

The content of flavonols was also determined by the aluminium chloride method described by Awah et al. (2012). Briefly, 1 ml of each extracts was mixed with 1 ml aluminium trichloride (20 mg/ml) and 3 ml of sodium acetate (50 mg/ml). The absorbance at 440 nm was read after 2.5 h. A standard curve was prepared using quercetin in methanol under the same conditions. Results were expressed as mg quercetin equivalents (QE) per g of dry weight (DW).

Condensed tannins (proanthocyanidins) content

Determination of condensed tannins by the vanillin assay was carried out using the procedure reported by Sun et al. (1998). The method is based on the ability of condensed tannins to react with vanillin in the presence of mineral acid to produce a red color. 500 µl of extracts solutions were mixed with 3 ml of 4 % vanillin-methanol solution and 1.5 ml hydrochloric acid. The mixture was allowed to stand for 15 min. The absorbance was measured at 500 nm, while the final result was expressed as mg catechin equivalent (CE)/g dry weight.

Hydrolysable tannins (gallotannins) content

The gallotannin content of the extracts was determined with the potassium iodate assay (Saad et al. 2012). 5 ml of KIO_3 aqueous solution (2.5 % w/v) was heated for 7 min at 30 °C, and then 1 ml of the diluted sample was added. After additional 2 min of tempering at 30 °C, the absorbance was measured at 550 nm. A calibration curve was obtained using tannic acid solution (5000 mg/l) prepared by solubilization of 0.25 g of tannic acid in 50 ml of methanol (80 %). The analytical standard solutions of tannic acid were prepared by aqueous dilution. Results were expressed as mg tannic acid equivalent (TAE) per g of dry weight (DW).

In vitro evaluation of antioxidant activities

Total antioxidant capacity by phosphomolybdenum assay (P-Mo)

The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acid pH (Prieto et al. 1999). An aliquot (0.1 ml) of plant extracts was combined to 1 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated in a thermal block at 95 °C for 90 min. Then, the samples were cooled to room temperature and the absorbance was measured at 695 nm against a blank. The antioxidant capacity was expressed as mg gallic acid equivalent per gram dry weight (mg GAE/g DW). All samples were analysed in triplicate.

DPPH radical-scavenging activity

The ability of the corresponding extracts to donate hydrogen atoms or electrons was measured from the bleaching of purple coloured methanol solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH), according to the method described by Barros et al. (2011). The reaction mixture in each one of the 96 wells consisted of known concentrations of the extracts (30 µl) and aqueous methanolic solution (80:20 v/v, 270 µl) containing DPPH radicals (6×10^{-5} M). The mixture was left to stand for 60 min in the dark. The reduction of the DPPH radical was determined by measuring the absorption at 515 nm using Bio-Tek EL×800 microplate reader. The radical-scavenging activity (RSA) was calculated as a

percentage of DPPH discolouration using the equation:

$$\%\ \mathrm{RSA} = \left[\left(A_{DPPH} - A_S\right) \Big/ A_{DPPH}\right] \times 100$$

Where A_S is the absorbance of the solution when the sample extract has been added at a particular level and A_{DPPH} is the absorbance of the DPPH solution. The extract concentration providing 50 % of radical-scavenging activity (IC_{50}) was calculated from the graph of RSA percentage against extract concentration. Vitamin E was used as standard.

Iron reducing power

The reducing power of *R. vesicarius* extracts was determined through the transformation of Fe^{3+} to Fe^{2+} induced by plant extracts, according to the method of Oyaizu (1986). Samples (1 ml) at different concentrations were mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of potassium ferricyanide (1 % w/v). The tubes were incubated at 50 °C for 20 min. Afterwards, 2.5 ml of 10 % TCA were added in each tube and the mixture was centrifuged for 10 min at 3.000 g. An aliquot of the supernatant (2.5 ml) was mixed with distilled water (2.5 ml) and 0.5 ml of ferric chloride (0.1 % w/v), and the absorbance was measured at 700 nm: higher absorbance indicates higher reducing power. Vitamin E was used as authentic standard and EC_{50} value (effective concentration of the extract which corresponds to 0.5 of absorbance) was obtained from linear regression analysis.

β-Carotene bleaching test

A slightly modified Koleva et al. (2002) method was employed to estimate *R. vesicarius* extracts capacity to inhibit the β-carotene bleaching. Two milligrams of β-carotene were dissolved in 20 ml chloroform and to 4 ml of this solution, linoleic acid (40 mg) and Tween 40 (400 mg) were added. Chloroform was evaporated under vacuum at 40 °C and 100 ml of oxygenated water was added, then the fresh emulsion was vigorously shaken. An aliquot (150 µl) of the β-carotene/linoleic acid emulsion was distributed in each of the wells of 96-well microtitre plates and methanolic solutions of the test samples or authentic standards (10 µl) were added. Three replicates were prepared for each concentration. The microtitre plates were incubated at 50 °C for 120 min, and the absorbance was measured using Bio-Tek EL×800 microplate reader at 490 nm. Readings of all samples were performed immediately (t=0 min) and after 120 min of incubation. The antioxidant activity of the extracts was evaluated in terms of blanching inhibition of the β-carotene using the following formula:

$$\beta\text{-Carotene bleaching inhibition}(\%) = S - C_{120} \Big/ C_0 - C_{120} * 100$$

Where C_0 and C_{120} are the absorbance values of the control at 0 and 120 min, respectively, and S is the sample absorbance at 120 min. The results were expressed as IC_{50} values (mg ml^{-1}).

TLC screening for phytochemical analysis

Phytochemical screening for the presence of secondary metabolites was performed using thin layer chromatography (TLC) analysis. Ten microlitres of each sample (10 mg/ml in methanol) was loaded on Merck TLC 60 F_{254} silica gel sheets. Development was carried out with different eluting systems viz.: Cyclohexan: AcOEt (10: 8 (v/v)), AcOEt: $CHCl_3$: AcOH (8: 7: 0.5 (v/v/v)) and Toluene: AcOH: Formic acid (3: 5: 1 (v/v/v)) (Mamyrbekova-Bekro et al. 2013a). After drying, the plates were sprayed with (KOH, $(CH_3CO_2)_2Pb$, NH_3) to detect coumarin; $AlCl_3$ and NH_3 to detect flavonoids, Libermann-Burchard to detect sterols and polyterpens, Dragendorf's reagent to detect alcaloïdes and ferric chloride to detect tannins (Lhuillier 2007; Guy et al. 2010; Kabran et al. 2011; Rathee et al. 2012). Detection was carried out visually in visible light and under UV light (λ=366 nm).

The retention factor (*Rf*) was calculated using the following equation:

$$Rf = \frac{Distance\ \ move\ \ by\ \ the\ \ substance(cm)}{Distance\ \ move\ \ by\ \ the\ \ solvent(cm)}$$

Statistical analysis

All results were carried out in triplicate and expressed as mean ± standard deviation (SD). The concentration needed for 50 % inhibition (IC_{50}) was estimated graphically by linear regression analysis. The correlations between polyphenols and antioxidant activities were calculated using the Pearson coefficient (ρ) and linear regression analysis by Microsoft Excel program.

Results and discussion

Rumex vesicarius L. is a good source of bioactive compounds due to its content of various phytochemicals. In this study, total extracts yield, phenolic composition and antioxidant

activities of hydroalcoholic extract/fractions from aerial part of *R. vesicarius,* collected from Algeria, were determined.

Yield of crude extract and fractions

The results of using different solvents for the extraction/fractionation of phenolic compounds are given in Table 1. From this table it was evident that *R. vesicarius* contained noticeable amounts of extractable compounds. It is clear that the different solvents used for the extraction and fractionation had different abilities to extract substances from this plant. In general, the amount of total extractable compounds decreased with decreasing polarity of the solvent. The extraction yield varied from 0.25 to 14.25 % (w/w). Among all the fractions, WF obtained the highest extraction yield (8.85±4.46 %) while CF yielded the lowest (0.25±0.16 %). The yield of all the fractions is presented in the following order: WF > BF > AF > HF > CF. The low extraction yield of CF is probably due to the low solubility of major components of *R. vesicarius*.

Lipo-soluble pigments composition

The content in pigments (carotenoids and chlorophylls) of the studied medicinal plant is given in Fig. 1. Experimental results obtained show that *Rumex vesicarius* contained a highest level of β-carotene (116.83±1.60 μg/g DW), lycopene (156.40±1.59 μg/g DW), chlorophyll a (271.45±3.46 μg/g DW) and less level of Chlorophyll b (48.38±3.96 μg/g DW).

Bhaskarachary et al. (1995) and Bélanger et al. (2010) reported the quantification of β-carotene in fresh weight (26 μg/g and 45 μg/g fresh weight, respectively), but nothing is reported about content of carotenoids or chlorophyll in dry weight of the studied plant. Carotenoids are amongst nature's most widespread pigments and have also received substantial attention because of both their provitamin and antioxidant roles. Chlorophyll and its derivatives are also known to have antioxidant activity, being associated with reduced risks of diseases induced by free radicals, such as certain types of cancers (Carrapeiro et al. 2007; Barros et al. 2011). Javanmardi et al. (2003) reported that antioxidant activity of plant extracts is not limited to phenolics. The activity may also come from other antioxidant secondary metabolites, such as volatile oils, carotenoids and vitamins.

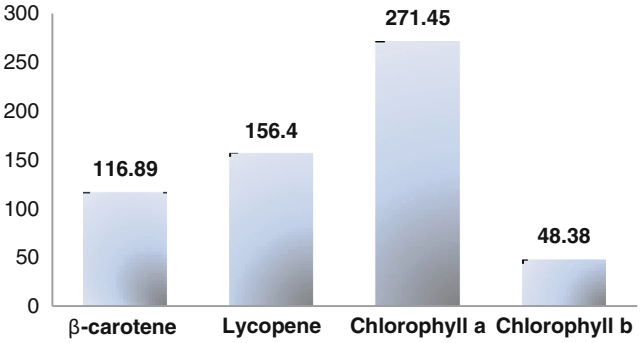

Fig. 1 Composition of *R. vesicarius* in pigments (μg/g DW)

Phenolic compounds analysis

Table 1 summarizes the total phenolics, flavonoids, flavonols and tannins content of the aerial part of *R. vesicarius*. Principal results showed that *R. vesicarius* extracts exhibited an important amount of polyphenol content followed by flavonoid as major class and low tannin fraction. The amount of total phenolics varied from 0.37±0.01 to 43.28±0.28 mg GAE/g of dry weight. HAE had the higher content (43.28±0.28 mg GAE/g of DW). This could be due to more interfering substances present in the crude extract as compared to those fractions. Moreover, total phenolic content increased in the fractions with increased the solvent polarity. The lower polarity solvents: hexane and chloroform showed much lower ability in extracting the phenolic compounds as compared to the polar solvents. Total phenolic content of fractions was found to be arranged in the following descending order: BF > AF > WF > CF > HF. This result suggests that BF fraction

Table 1 Extraction yields (%) and polyphenol contents in crude extract and fractions of *Rumex vesicarius*

Extracts/Fractions	Extraction yield	Phenolics[a]	Flavonoids[b]	Flavonols[c]	Condensed tannins[b]	Hydrolysable tannins[d]
HAE	14.25±4.35	43.28±0.28	19.72±0.37	2.68±0.02	4.34±0.19	6.21±1.28
HF	0.54±0.17	0.37±0.01	1.98±0.04	0.67±0.01	0.02±0.00	2.14±0.20
CF	0.25±0.16	2.45±0.01	1.78±0.03	0.42±0.01	0.18±0.00	2.69±0.06
AF	0.54±0.10	9.95±0.01	4.65±0.07	1.01±0.02	0.09±0.01	3.17±0.30
BF	1.61±0.30	12.40±0.02	5.60±0.04	1.09±0.00	0.01±0.00	0.41±0.16
WF	8.85±4.46	4.16±0.32	3.17±0.15	0.07±0.00	0.34±0.10	1.35±0.11

[a] Expressed as mg gallic acid equivalents/g dry weight

[b] Expressed as mg catechin equivalents/g dry weight

[c] Expressed as mg quercetin equivalents/g dry weight

[d] Expressed as mg tannic acid equivalents/g dry weight

Values are expressed as means of three replicates ± SD

might be the part that is rich in phenolic compounds and that n-butanol is suitable to extract phenolic compounds from *R. vesicarius*. The same tendency was observed for flavonoid and flavonol contents (see Table 1). Considering tannin contents, it was concluded that hydrolysable tannins content was higher than condensed tannins content with 6.21±1.28 mg TAE/g DW in HAE.

It is extremely important to point out that the most antioxidant activities from plant sources are correlated with phenolic compounds (Cai et al. 2004; Jain et al. 2008; Huang et al. 2010a, b). These compounds are known to act as antioxidants not only because of their ability to donate hydrogen or electrons but also to because they are stable radical intermediates. Our findings showed the richness of *R. vesicarius* on phenolic content. For that, the in vitro estimation of antioxidant activities was determined.

Antioxidant activity

The antioxidant capacities are influenced by many factors, which cannot be fully described with one single method. Therefore it is necessary to perform more than one type of antioxidant capacity measurement to take it in to account the various mechanisms of antioxidant action (Wong et al. 2006a; Erkan et al. 2008). In this study, the antioxidant activities of the HAE/fractions from *R.vesicarius* were evaluated by using in vitro antioxidant models, including total antioxidant activity, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, reducing power and β-Carotene bleaching test. The relation between the antioxidant activity and polyphenol contents was also studied.

Total antioxidant capacity (TAC)

This assay is a quantitative one since the antioxidant activity is expressed as numbers of equivalents of gallic acid. Table 2 showed that polar solvents have the higher antioxidant capacity, 32.97±4.41 mg ascorbic acid/g dry weight was observed in HAE of *R. vesicarius*. BF and WF exhibited higher activity (6.92±0.89 and 6.67±1.84 mg/g DW, respectively) when compared to different other solvent fractions. Published reports on the total antioxidant activity of *R. vesicarius* extracts are not available. However, a total antioxidant activity of 245–376 mg ascorbic acid/g extract has been reported in higher plant extracts (Kumaran and Karunakaran 2007).

DPPH radical scavenging activity

DPPH has been used extensively to test the ability of compounds and extracts from plants or food materials to act as free radical scavengers or hydrogen donors (Liyana-Pathirana and Shahidi 2006). As a kind of stable free radical, DPPH can accept an electron or hydrogen radical from antioxidant (A-H) to become a stable diamagnetic molecule as described in the following equation:

$$\underset{\text{(Purple)}}{(\text{DPPH}\cdot)} + (\text{A-H}) \rightarrow \underset{\text{(Yellow)}}{\text{DPPH-H}} + (\text{A}\cdot)$$

The degree of discoloration, quantified by measuring the absorbance at 515 nm, indicated the scavenging potential of the antioxidant compounds or extracts in the term of hydrogen donating ability (Mosquera et al. 2007).

Scavenging effects of *R. vesicarius* extracts on DPPH were examined at different concentrations, the IC_{50} values are given in Table 2. The ethyl acetate fraction shown the highest potent DPPH radical scavenger activity (0.07±0.00 mg/ml) followed by n-butanol and chloroform fractions (0.15±0.00 and 0.16±0.00 mg/ml, respectively). In addition, the water fraction exhibited poor scavenging of DPPH• (2.90±0.04 mg/ml). The free radical-scavenging activity of crude extract was eight times less important than those of ethyl acetate fraction, which may result from the active components through enrichment effects during the solvent–solvent partitioning processes. As it can be seen, vitamin E (α-tocopherol), well-known antioxidant compound used as the reference control in this study, had the same IC_{50} value as ethyle acetate fraction (0.07±0.01 mg/ml), this indicates potent free radical scavenging

Table 2 Antioxidant activities of hydroalcoholic extract/fractions from *R. vesicarius*

Extract/Fractions/Standard antioxidant	TAC (mg GAE/g DW)[a]	$IC_{50/DPPH}$[a]	$EC_{50/Reducing\ power}$[a]	$IC_{50/\beta\text{-Carotene}}$[a]
HAE	32.97±4.41	0.56±0.00	1.38±0.01	0.26±0.01
HF	1.62±0.00	1.01±0.03	2.87±0.03	2.57±0.38
CF	0.56±0.02	0.16±0.00	0.80±0.00	0.21±0.00
AF	3.86±0.16	0.07±0.00	0.23±0.00	0.24±0.01
BF	6.92±0.89	0.15±0.00	0.72±0.01	0.22±0.01
WF	6.67±1.84	2.90±0.04	7.13±0.07	0.63±0.01
Standard (Vit E)	–	0.07±0.01	0.24±0.01	0.24±0.01

[a] Each value is expressed as the mean ± standard deviation ($n=3$); EC_{50} = effective concentration at which the absorbance was 0.5; IC_{50} = inhibition concentration 50 %; *TAC* total antioxidant capacity

Table 3 Pearson's correlation coefficients (ρ) of phenolic compounds and antioxidant capacity

ρ	TPC	TFC	CTC	HTC	C_{TAC}	C_{DPPH}	C_R	$C_{Anti\text{-}\beta\text{-carotene bleaching}}$
TPC	1	0.996	0.949	0.787	0.976	−0.221	−0.268	−0.396
TFC		1	0.968	0.803	0.989	−0.166	−0.213	−0.328
CTC			1	0.870	0.978	−0.059	−0.101	−0.245
HTC				1	0.775	−0.244	−0.288	−0.173
C_{TAC}					1	−0.026	−0.071	−0.294
C_{DPPH}						1	0.998	0.259
C_R							1	0.295
$C_{Anti\text{-}\beta\text{-carotene bleaching}}$								1

TPC Total phenolic content, *TFC* Total flavonoid content, *CTC* Condensed tannin content, *HTC* Hydrolysable tannin content, C_{TAC} Coefficient of total antioxidant capacity, C_{DPPH} Coefficient of DPPH radical scavenging activity, C_R Coefficient of reducing power, $C_{Anti\text{-}\beta\text{-carotene bleaching}}$ Coefficient of antioxidant activity determined with β-carotene bleaching test

activity of AF of *R. vesicarius*. From the result, it revealed that *R. vesicarius* was a high potential natural antioxidant.

Reducing power

For the determination of the reductive ability, the Fe^{3+} - Fe^{2+} transformation was investigated in the presence of *R. vesicarius* extracts. Samples with different concentration were used for this assay and all of them exhibited the dose-dependent activity. As shown in Table 2, the AF revealed a good reducing power (0.23±0.00 mg/ml), while WF yielded the lowest (7.13±0.07 mg/ml). The reducing power of all fractions is presented in the following order: AF > BF > CF > HAE > HF > WF. The data of the positive control (vit E) was 0.24±0.01 mg/ml. Ethyl acetate fraction has a great ability to terminate radical chain reaction by converting free radicals and reactive oxygen species to more stable non-reactive products (Siddhuraju and Becker 2003). Our results were in accordance with those found by Yao et al. (2013).

β-Carotene/linoleic acid method

The assay measures the ability of a plant extract to prevent or minimize the coupled oxidation of β-carotene and linoleic acid in an emulsified aqueous system (Parejo et al. 2002). The IC_{50} of crude extract and its different fractions from *R. vesicarius* was revealed in Table 2. CF, BF, AF and HAE were better in their effect on reducing the oxidation of β-carotene than WF and HF. The IC_{50} values of Chloroform and butanol fractions (0.21±0.00 and 0.22±0.01 mg/ml,

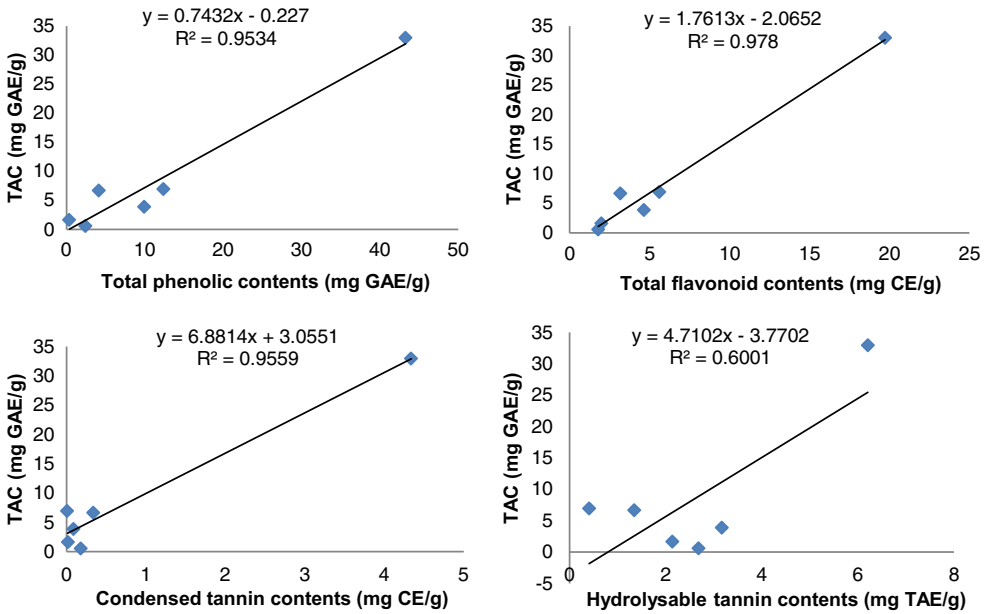

Fig. 2 Correlation coefficients (R^2) between total antioxidant capacity and phenolic compounds of *R. vesicarius* extracts

Table 4 *Rf*-values and color of spots of the chloroform fraction

Extracts	Without revelation a			With revelation														Identified compounds
				NH_3 A			Liebermann-Burchard B			$FeCl_3$ C		KOH D			$(CH_3CO_2)_2Pb$ E			
	Rf	Visible	UV/ 366 nm	Rf	Visible	UV/ 366 nm	Rf	Visible	UV/ 366 nm	Rf	Visible	Rf	Visible	UV/ 366 nm	Rf	Visible	UV/ 366 nm	
CF	0.00	Yellow	Yellow	0.00	Yellow	Yellow	0.00	Green	Yellow[g]	–	–	0.00	Brown	Yellow	0.00	Yellow	Yellow	Steroids B (Mamyrbekova-Bekro et al. 2013b; Kabran et al. 2011; Lagnika 2005)
	0.07	–	Red[l]	0.04	–	Violet	–	–	–	–	–	–	–	–	0.06	–	Violet	Anthraquinone a (Mamyrbekova-Bekro et al. 2013b)
	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.12	–	Violet[d]	Unidentified
	0.70	–	Violet[d]	–	–	–	0.89	Violet	Violet	–	–	–	–	–	0.75	–	Violet[d]	Steroidal saponin B (Mamyrbekova-Bekro et al. 2013a; Guy et al. 2010)

Mobile phase : Cyclohexan: AcOEt (10: 8 (v/v))

l Light, *d* Dark, *g* Greenish

Table 5 *Rf*-values and color of spots of the ethyl acetate fraction

Extracts	Without revelation a			With revelation										Identified compounds
				NH_3 A			KOH B			$AlCl_3$ C			$FeCl_3$ D	
	Rf	Visible	UV/ 366 nm	Rf	Visible	UV/ 366 nm	Rf	Visible	UV/ 366 nm	Rf	Visible	UV/ 366 nm	Rf	Visible	
AF	0.00	Brown	Green	0.00	Brown	Yellow	0.00	Brown	Green	0.00	Yellow	Brown	0.00	Grey	Coumarins A (Guy et al. 2010), B (Mamyrbekova-Bekro et al. 2013a, b), Flavonoids C (Kabran et al. 2011; Guy et al. 2010); Tannins D (Mamyrbekova-Bekro et al. 2013a; Kabran et al. 2011)
	0.04	–	Purple	–	–	–	0.03	Yellow	Violet	–	–	–	0.04	Grey	Tannins D (Mamyrbekova-Bekro et al. 2013a; Kabran et al. 2011)
	0.12	Yellow	Orange	0.12	–	Orange	0.14	Yellow	Orange	–	–	–	–	–	Coumarins B (Mamyrbekova-Bekro et al. 2013a; Kabran et al. 2011), Xanthone a (Ladyguina et al. 1983),
	0.16	–	Blue	0.17	Yellow	Green	–	–	–	0.16	Yellow	Blue	–	–	Phenolic acids or catechols a, C (Guy et al. 2010; Dawson et al. 1991)
	0.91	–	Purple	–	–	–	0.98	–	Purple	0.91	–	Violet	–	–	Hydroxyl flavonols a (Dawson et al. 1991)

Mobile phase : AcOEt: $CHCl_3$: AcOH (8: 7: 0.5 (v/v/v))

l Light, *d* Dark, *g* Greenish, *f* Fluorescent

respectively) were higher to that of positive control vit E (0.24 ±0.01 mg/ml). Meanwhile, the IC_{50} values of ethyl acetate fraction (0.24±0.01 mg/ml) and crude extract (0.26± 0.01 mg/ml) were close to that of positive control. According to Liyana-Pathirana and Shahidi (2006) an extract capable of retarding or inhibiting the oxidation of β-carotene may be described as a free radical scavenger and primary antioxidant.

Relationship between content in phenolic compounds and antioxidant activities

For better understanding the relationship between polyphenols and antioxidant capacities of *R. vesicarius*, all the prepared extracts were used in an analysis of the correlation using the Pearson coefficient (ρ) and linear regression analysis, the results are shown in Table 3. It is important to note that as the correlations between polyphenols and free radical scavenging ability were determined using IC_{50}, a negative ρ value (−1) is considered as perfect positive correlation (da Silva et al. 2011).

With regard to the results, a linear correlation appeared between the total antioxidant capacity and the total phenolic, flavonoid and condensed tannin contents with excellent ρ correlation coefficients (ρ=0.976, 0.989 and 0.978, respectively). Also, good ρ correlation coefficient (ρ=0.775) appeared between the total antioxidant capacity and hydrolysable tannin contents (Table 3, Fig. 2).

These results are in good accordance with previous studies which showed that high total phenolic content increases the antioxidant activity (Wong et al. 2006b; Kumaran and Karunakaran 2007). However, for other activities weak correlation was found, suggesting the involvement of non phenolic compounds such as ascorbic acid, tocopherols, saponins, carotenoids and polysaccharide (Nsimba et al. 2008; Erkan et al. 2008; Ananthi et al. 2010). Several studies established a linear correlation between the total content of phenolics and the antioxidant capacity (Cai et al. 2004; Kumaran and Karunakaran 2007) whereas, some studies reported that there is no correlation (Kähkönen et al. 1999; Yu et al. 2002; Tongpoothorn et al. 2012; Yao et al. 2013).

As shown in Table 3, the mutual correlations among the four methods were determined by Pearson correlation analysis. High correlation among DPPH and reducing power was found (ρ=0.998) whereas DPPH/β-carotene-linoleic acid bleaching and reducing power/β-carotene-linoleic acid bleaching exhibited weaker correlations. Furthermore, no correlations were obtained with TAC and other methods.

Phytochemical analysis

Due to the high activity of the ethyl acetate, n-butanol and chloroform fractions of *R. vesicarius*, they were subjected to some phytochemical and TLC analysis. The tested plant showed positive results for variable amounts of sterols/triterpenes, polyphenols, anthraquinones, coumarins, tannins and flavonoids. Alkaloids were totally absent in all the three fractions (see Tables 4, 5 and 6).

Table 4 shows the groups of secondary metabolites which are contained in chloroform fraction. The presence of steroids was confirmed by Liebermann-Burchard reagent under UV/366 nm. Two spots appear on the plate (Yellow greenish at *Rf* =0.00 and violet at *Rf*=0.89). Anthraquinones were also found in CF (red spot at *Rf*=0.07), while all other phytoconstituents were absent. These observations were made

Table 6 *Rf*-values and color of spots of the n-butanol fraction

Extracts	Without revelation a			With revelation					Identified compounds
				$AlCl_3$ A			$FeCl_3$ B		
	Rf	Visible	UV/366 nm	Rf	Visible	UV/366 nm	Rf	Visible	
BF	0.00	Brown	Yellow	–	–	–	–	–	Unidentified
	0.16	Yellow	Green	–	–	–	–	–	Unidentified
	0.19	Yellow	Yellow	0.18	Yellow	Yellow	–		Flavonols a, A (Guy et al. 2010; N'gaman Kohué et al. 2009)
	0.39	Yellow	Green	0.42	Yellow	Yellow	0.39	Grey	Flavonoids A (N'gaman Kohué et al. 2009), Tannins B (Mamyrbekova-Bekro et al. 2013a; Kabran et al. 2011)
	0.47	Yellow	Green	0.51	Yellow	Yellow	–	–	Flavonoids A (N'gaman Kohué et al. 2009)
	0.6	Yellow	Yellow	0.63	Yellow	Yellow	0.6	Brown	Flavonols a, A (Guy et al. 2010; N'gaman Kohué et al. 2009), Tannins B (Kabran et al. 2011)
	0.85	–	Green	0.89	–	Pink	–	–	Unidentified
	0.87	–	Orange	0.91	–	Pink	–	–	Xanthone a (Mamyrbekova-Bekro et al. 2013a; Ladyguina et al. 1983)

Mobile phase: Toluene: AcOH: Formic acid (3: 5: 1 (v/v/v))

y Yellowish, *l* Light, *f* Fluorescent

by Lagnika (2005), Guy et al. (2010), Kabran et al. (2011) and Mamyrbekova-Bekro et al. (2013a, b) in their work on other plants. TLC studies on ethyl acetate fraction showed the presence of flavonoids, tannins and coumarins (Table 5). Presence of coumarins was confirmed after spraying of KOH reagent (*Rf*= 0.14, yellow in the visible and orange fluorescent under UV) (Mamyrbekova-Bekro et al. 2013a; Kabran et al. 2011). Flavonoids have variable coloring spots under UV. Indeed, xanthones (Rf=0.12) appear in orange (Ladyguina et al. 1983), methylated flavones and hydroxyl flavonols are blue and purple (Dawson et al. 1991). This is the case of the *Rf*=0.16 and 0.91. Presence of flavonoids was confirmed after spraying of $AlCl_3$ and NH_3 reagents. $AlCl_3$ reveals yellow spots in visible and in UV colorings from blue to brown (Lagnika 2005) while NH3 reveals them as fluorescent yellow, green and blue spots under UV (Dawson et al. 1991; Ladyguina et al. 1983). The presence of tannins was further confirmed by $FeCl_3$ reagent. The grey color was obtained after spraying of the reagent (*Rf*=0.00 and *Rf*=0.04) (Mamyrbekova-Bekro et al. 2013a; Kabran et al. 2011).

In butanolic fraction, we have tried to identify polyphenols and saponins. For this purpose, specific revelators were chosen. Table 6 summarizes the groups of phenolic compounds which are contained in BF.

Using the methodological approach (color of spot before and after spraying of reagents) used previously for the detection and identification of phenolic compounds; we showed the richness of BF in flavonoids. Thus, traces of tannins were detected.

Previous studies of the genus have led to the isolation of anthraquinone derivatives (Midiwo and Rukunga 1985; Demirezer 1994), particularly in the roots, which showed various pharmacological properties, such as antitumor, antimutagenicity and antioxidant activities (Demirezer et al. 2001). Flavonoids reported in Rumex species were either flavonols or C-glycosides. Thus *R. vesicarius* contained vitexin, isovitexin, orientin and isoorientin (Halim et al. 1989). The presence of 8-C-glucosyl-apigenin, 8-C-glucosyl-luteolin, 6-C-hexosyl-quercetin, 3-O-rutinosyl-quercetin, 7-O-rhamno-hexosyl-diosmetin, 7-O-rhamno-acetylhexosyl-diosmetin, catechin, epicatechin, ferulohexoside, 6-C-glucosyl-naringenin, epicatechin gallate, 6-C-glucosyl-catechin, and epigallocatechin gallate has also been reported in *R. vesicarius* (El-hawary et al. 2011).

Conclusion

This study was designed to evaluate the antioxidant activities of hydroalcoholic extract and its fractions obtained from aerial part of *Rumex vesicarius* (Polygonaceae) by using different in vitro antioxidant assays. Results indicate that ethyl acetate fraction (AF) possessed significant antioxidant activities approaching the activity of α-tocopherol examined by the same tests. Interestingly, a negative correlation was found between phenolic compounds and the antioxidant efficiencies of the crude extract/fractions, suggesting that phenolic compounds are not the only contributors to the antioxidant activities of *Rumex vesicarius*. In contrast, DPPH radical-scavenging activity and reducing power are compatible due to their similar mechanism of radical scavenging. Qualitative TLC analysis showed the presence of some phenolic compounds such as flavonoids, tannins, coumarins and steroids that could provide scientific evidence for some folk uses in the treatment of diseases related to the production of reactive oxygen species and oxidative stress. Further work is still needed to identify and characterize the inherent phytocompounds from AF and other fractions and to investigate the in vivo antioxidant efficacy of *R. vesicarius*.

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