



## ***In vitro* antioxidant, anti-inflammatory and anticholinesterase activities of *Rumex vesicarius* L**

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Received on:17-08-2015; Revised on: 21-09-2015; Accepted on: 27-10-2015

### ABSTRACT

**Objective:** To investigate phenolic content, flavonoid content, the antioxidant, anti-inflammatory, and anticholinesterase activities of ethyl acetate extracts of *Rumex vesicarius* L from leaf, stem, flower and seed. **Materials and Methods:** The assessment of the antioxidant potential of crude leaf, stem, flower and seed extracts, using the ferric reducing antioxidant power (FRAP) assays and 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and  $\beta$ -Carotene radical scavenging capacity assays was carried out. Furthermore, the 15-lipoxygenase inhibitory activity was evaluated by the ferrous oxidation-xylenol orange (FOX) assay method. Moreover, Cholinesterase inhibitory activities were examined using Ellman's colorimetric method. The total phenolic content measured by Folin-ciocalteu method was as well conducted. **Results:** The raw seed, flower, stem and leaf extracts of *Rumex vesicarius* L was found to contain a high content of total phenolic content (36.44 to 54.15 mg GAE/g DW) and flavonoids (12.36 to 21.97 mg CE/g DW). All extracts organs exhibited a higher antioxidant activity and inhibitory effect of radicals scavenging activity against FRAP, ABTS and  $\beta$ -Carotene. For the anti-inflammatory activity, the extracts tested had significant 15-lipoxygenase inhibitory ranging from 33.82 to 62.45% and had a noticeable inhibition towards AChE and BChE. **Conclusion:** the results suggest that the seed, flower, stem and leaf of *Rumex vesicarius* L exhibited high antioxidant and anti-inflammatory, this property may be related with high quantity of phenolic and flavonoid in extracts, this plant can be considered as a good source of natural antioxidant and anti-inflammation drugs as well as potent anticholinesterase effect.

**KEYWORDS:** *Rumex vesicarius* L, antioxidant, anticholinesterase, anti-inflammatory, phenolic content.

### INTRODUCTION

*Rumex vesicarius* L is an annual, glabrous herb of the Polygonaceae, It spreads throughout desert and semi-desert areas of North Africa, Asia and Australia<sup>[1]</sup>, and is distributed widely in the tropics as an ornamental. It is also known as bladder dock, rosy dock, blister sorrel or country sorrel and is mostly cultivated as a leafy vegetable. In South Algeria, *Rumex vesicarius* L is widely used as food, as a medicinal herb<sup>[2]</sup>, it is used in treatment of liver diseases, digestive problems, toothache, nausea, pain, anti-inflammatory, antitumor as well as antischistosomal, and antimicrobial activities<sup>[3,4]</sup>. It was also found to have aphrodisiac effect<sup>[5,6]</sup>, diseases of the spleen, hiccuph, flatulence, asthma, bronchitis, dyspepsia, piles, scabies, leucoderma, toothache and nausea. The plant also used as cooling, laxative, stomachic, tonic, analgesic, appetizer, diuretic, astringent, purgative, antispasmodic and antibacterial agents<sup>[7]</sup>. The roasted seeds were eaten for cure of dysentery. Finally, the plant can be used also to reduce biliary

disorders and control cholesterol levels<sup>[8,9]</sup>. Despite its importance, only few studies have been conducted on *Rumex vesicarius* L. Reactive oxygen species (ROS) have the damaging effects on cells<sup>[10,11]</sup>. Antioxidant compounds present in many plants can protect cells against this damaging caused by ROS. Cancer, cardiovascular diseases, diabetes, inflammation, degenerative diseases, anemia, ageing, and ischemia are common complicated illnesses in human directly or indirectly affected by ROS<sup>[12]</sup>, natural compounds either in the form of raw extracts or their chemical constituents are very effective to prevent the destructive processes caused by oxidative stress<sup>[13]</sup>. Phenolic compounds are plant secondary metabolites, which are important determinants in the sensory and nutritional quality of fruits, vegetables and other plants<sup>[14]</sup>. Phenolic compounds have a variety of physiological activity, such as antioxidant, antimutagenic, anti-allergenic, anti-inflammatory antimicrobial effects<sup>[15]</sup>, anticarcinogenic, antiatherogenic<sup>[16,17,18]</sup> and thus are now widely used in the fields of biology, medicine and food. Thus, the aim of the present work was to quantify phenolic content, flavonoids of seed, flower, stem and leaf extracts of *Rumex vesicarius* L, and to evaluate the antioxidant capacity of these extracts and cell based assay as well as to screen anti-inflammatory and anticholinesterase activities.

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Figure 1: *Rumex vesicarius* L, A: Seed, B: leaf, C: Flower, D: Stem

## MATERIALS AND METHODS

### Plant material and extraction

*The Rumex vesicarius* L were collected from southeast of Algeria, state of El Oued on December 2013. The leaves, stems flower and seed then separated from each other, washed and dried at room temperature. All these organs were ground to a powder with a basic electric grinder and stored in the dark at room temperature before use. Then the powder was put in a hot air oven at 60 °C until complete drying. Depending on the physical characteristics of the samples, the time ranged from 18 at 30 h. The bioactive compounds were extracted according to the method described by Jonathan et al<sup>[19]</sup>. 50 g of leaf, stems flower and seed were extracted with 400 ml of ethyl acetate for 5 h in Soxhlet. The extracts were filtered and evaporated under vacuum at 45°C before being dried and lyophilized for 10 h, the raw extract was stored at -40 °C.

### Total phenolic content

The total phenolic contents in all organs were determined by the

folin-Ciocalteu method<sup>[20]</sup>. Briefly, 100 µL of both the sample and the standard (gallic acid) of known concentrations were made up to 2.5 mL with water and mixed with 0.25 mL of 1N Folin-ciocalteu reagent. After 5 min, 2.5 ml of sodium carbonate aqueous solution (2%, w/v) was added to the mixture and was completed the reaction for 30 minutes in darkness at room temperature. The absorbance was read at 765 nm using a UV-visible spectrophotometer (Shimadzu UV-1800, Japan). For the blank the same protocol was used but the extract was replaced by solvent. The concentration of total polyphenols in the extracts was expressed as mg gallic acid equivalent (GAE) per g of dry weight using UV-Visible (Shimadzu UV-1800, Japan) and the equation of calibration curve:  $Y = 0.00778x$ ,  $R^2 = 0.991$ ,  $x$  was the absorbance and  $Y$  was the gallic acid equivalent. All results presented are means ( $\pm$ SEM) and were analyzed in three replications.

### Total flavonoids

The determination of flavonoids was performed according to the colorimetric assay<sup>[21]</sup>. Distilled water (4 mL) was added to 1 mL of leaf, stem, flower and seed extract. Then, 5% sodium nitrite solution (0.3

ml) was added, followed by 10% aluminum chloride solution (0.3 mL). Test tubes were incubated at ambient temperature for 5 min, and then 2 ml of 1 M NaOH were added to the mixture. Immediately, the volume of reaction mixture was made to 10 ml with distilled water. The mixture was thoroughly vortexed and the absorbance of the pink colour developed was determined at 510 nm. A calibration curve was prepared with catechin and the results were expressed as mg catechin equivalents (CE)/g of dry weight.

#### Measurement of ferric reducing power (FRAP assay)

The reducing power was determined by using FRAP assay<sup>[22]</sup>. Briefly, the FRAP reagent contained 2.5 mL of 10 mM tripyridyltriazine (TPTZ) in 40 mM HCl, 2.5 mL of 20 mM FeCl<sub>3</sub> and 25 mL of 0.3M acetate buffer (pH 3.6), was freshly prepared. A volume 0.2 mL, of ethanolic extract (various concentrations) or standard was mixed with 1.8 mL of freshly prepared FRAP reagent. The absorbance of each sample solution was subsequently measured at 595 nm. For the calibration curve, FeSO<sub>4</sub> was prepared in same solvent extraction in the range of 100–700 μM and Quercetin was used as positive controls. The results were expressed as mg/ml of Fe(II), using the equation obtained from the calibration curve of FeSO<sub>4</sub>:  $Y = 6.908x$ ,  $R^2 = 0.998$ .

#### ABTS assay (2,20-azinobis[3-ethylbenzothiazoline-6-sulfonate])

The ABTS scavenging assay was carried out in triplicate, ABTS reagent was prepared by 10 mL of 7 mM ABTS solution and 178 μL of 140 mM potassium persulfate aqueous, the mixture was incubated at room temperature in darkness for 13 h before use. 2 μL ethyl acetate extracts or standard was added to 1.588 μL diluted ABTS solution to react in the dark at room temperature for 10 min<sup>[23]</sup>, and the absorbance was measured at 732 nm. The percentage inhibition of ABTS radical by the extract and BHT as calculated and compared following the equation:

$$\text{ABTS radical scavenging activity} = \left[ \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{control}}} \right] \times 100$$

Where,

$\text{Abs}_{\text{control}}$ : Is the absorbance of ABTS radical + ethyl acetate

$\text{Abs}_{\text{sample}}$ : Is the absorbance of ABTS radical + ethyl acetate extract or standard.

#### β-Carotene linoleic acid bleaching assay

The antioxidant capacity is determined by measuring the inhibition of the production of volatile organic compounds and the formation of conjugated diene hydroperoxides arising from linoleic acid oxidation<sup>[24]</sup>. Firstly the β-Carotene was prepared by dissolving 2 mg of this reagent in 10 mL of chloroform. In the round-bottom flask are added 2 mL of β-Carotene solution, 40 mg of linoleic acid and 400 mg of Tween 80, after this preparation, the chloroform is removed at 40 °C using the rotary evaporator). The resulted mixture was added to 100 mL of distilled water (aerated) under vigorous shaking and protection from the light. A 4.8 mL of the last solution was transferred into different tube containing 0.2 mL of each extracts in ethyl acetate with different concentrations. A control sample was prepared of 0.2

mL ethyl acetate and 4.8 mL of β-Carotene reagent. The tubes were incubated at 50 °C for 2 h. the absorbance at 470 was measured, using UV-Visible spectrophotometer<sup>[26,27]</sup>. The essay was carried out in triplicate and the results were provided as 50 % inhibition (EC<sub>50</sub> μg/ml). The antioxidant activity was calculated using the following equation:

$$\% \text{ antioxidant activity} = \left( \frac{(A_0 - A_t)}{(A_0^\circ - A_t^\circ)} \right) \times 100$$

Where,  $A_0$  and  $A_t$  are respectively the absorbance calculated at zero time of incubation for simple extracts and control.  $A_0^\circ$  and  $A_t^\circ$  are the absorbance measured after 2 h respectively for simple extract and control. All measurements were made in triplicate and averaged.

#### Anti-inflammatory activity (Soybean lipoxygenase inhibition assay)

The assay is based on measuring the formation of the complex Fe3p/xylenol orange in a spectrophotometer at 560 nm<sup>[25]</sup>. 15-Lipoxygenase (15-LOX) was incubated with extracts or standard inhibitor at 25 °C for 5 min. Then linoleic acid (final concentration, 140 mM) in Tris-HCl buffer (50 mM, pH 7.4) was added and the mixture was incubated at 25 °C for 20 min. The assay was terminated by the addition of 100 mL of FOX reagent consisting of sulfuric acid (30 mM), xylenol orange (100 mM), iron (II) sulfate (100 mM) in methanol/water (9/1). For the control, only LOX solution and buffer were pipetted into the wells. Blanks (background) contained the enzyme LOX during incubation, but the substrate (linoleic acid) was added after the FOX reagent. The lipoxygenase inhibitory activity was evaluated by calculating the percentage of the inhibition of hydroperoxide production from the changes in absorbance values at 560 nm after 30 min at 25 °C.

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

Where,  $A_{\text{control}}$  is the absorbance of control well,  $A_{\text{blank}}$  is the absorbance of blank well and  $A_{\text{sample}}$  is the absorbance of sample well.

#### Determination of anticholinesterase activity

Acetylcholinesterase and butyrylcholinesterase inhibitory activities were measured by slightly modifying the spectrophotometric method<sup>[26]</sup>, AChE from electric eel and BChE from horse serum were used, while acetylthiocholine iodide and butyrylthio choline chloride were employed as substrates of the reaction. DTNB (5,50-Dithio-bis(2-nitrobenzoic)acid was used for the measurement of the cholinesterase activity. Briefly, 150 μL of 100 mM sodium phosphate buffer (pH 8), 10 μL of sample solution dissolved in ethanol at different concentrations and 20 μL AChE (5.32 x10<sup>-3</sup> U) or BChE (6.85 x10<sup>-3</sup> U) solution were mixed and incubated for 15 min at 25 °C, and then 10 μL of DTNB (0.5 mM) was added. The reaction was then initiated by the addition of 10 μL of acetylthiocholine iodide (0.71 mM) or 10 μL of butyrylthiocholine chloride (0.2 mM). The hydrolysis of these substrates was monitored spectrophotometrically by the formation of yellow 5-thio-2-nitrobenzoate anion as the result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of acetylthio- choline iodide or butyrylthiocholine chloride, respectively, at a wavelength of 412 nm utilizing a 96-well microplate reader. Percentage of inhibition of AChE or BChE enzymes was determined by

comparison of reaction rates of samples relative to blank sample (ethanol in phosphate buffer pH 8) using the formula  $(E-S)/E \times 100$ , 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. Galantamine was used as a reference compound.

### Statistical Analysis

Experimental values are given as means  $\pm$  standard error (SEM) of three replicates. Statistical significance was determined by one way variance analysis (ANOVA). Statistical calculations were carried out by OriginPro Version 8.0 software (OriginLab Corporation). Values of  $p < 0.05$  were regarded as significant and values of  $p < 0.01$  were regarded as very significant.

## RESULTS

### Total phenolic contents and flavonoids

Ethyl acetate extracts of leaf, stem, flower and seed were found to be rich in total phenolics and flavonoids contents. The total phenolic content is given in figure 2. SE was found to have the highest value  $54.15 \pm 1.5$  mg GAE/g DW, FE  $42.65 \pm 1.2$  mg GAE/g DW, STE  $36.44 \pm 1.1$  mg GAE/g DW and the lowest value in LE  $30.45 \pm 0.9$  mg GAE/g DW). The content of total flavonoids was also found to vary significantly ( $p < 0.05$ ) and content ranged from  $12.36 \pm 0.4$  mg CE/g DW to  $21.97 \pm 0.65$  mg CE/g DW. The Total flavonoids in increasing order was: SE > FE > STE > LE.

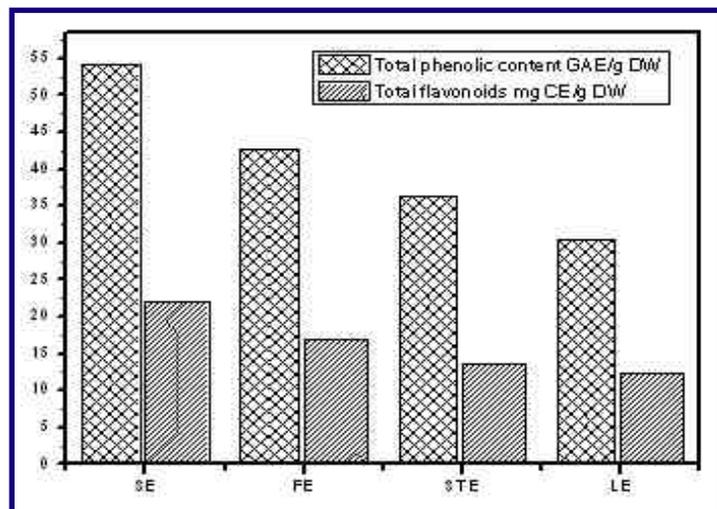


Figure 2: Total phenolic content (mg GAE/g DW) and total flavonoids (mg CE/g DW) of SE, FE, STE, LE of *Rumex vesicarius* L. FRAP assay

The antioxidant properties of a given compound depends not only on its chemical structure but also on the type of the generated radical it can neutralize. For this reason, we tested the antioxidant potential of leaf, stem, flower and seed extracts of *Rumex vesicarius* L against more than one radical type. The percentage inhibition of scavenging activities of the ethyl acetate extracts for ferric reducing were shown in Figure 3. The  $IC_{50}$  values of leaf extract (LE), stem extract (STE), flower (FE) and seed extract (SE) and standard were found to be

$184.09 \pm 7.11$   $\mu$ g/ml,  $173.55 \pm 7.27$   $\mu$ g/ml,  $155.97 \pm 6.23$   $\mu$ g/ml,  $130.67 \pm 4.95$   $\mu$ g/ml and  $68.82 \pm 2.41$   $\mu$ g/ml respectively.

### ABTS scavenging activity

The extracts from four organs were also measured and compared for their free radical scavenging activity against the ABTS radical cation. Figure 4 shows that all extracts used in this study had significant ABTS radical scavenging activities. The  $IC_{50}$  values ABTS radical scavenging activities of LE, STE, FE and SE were in the range of  $340 \pm 8.5 \pm \mu$ g/ml at  $469 \pm 12.02$   $\mu$ g/ml, the highest ABTS radical scavenging activities was found in SE  $IC_{50} = 340 \pm 8.5 \pm \mu$ g/ml, the medium in FE  $384.94 \pm 10.34$   $\mu$ g/ml, STE  $428.21 \pm 9.50$   $\mu$ g/ml and the lowest value in LE  $IC_{50} = 27.04 \pm 0.65 \pm \mu$ g/ml.

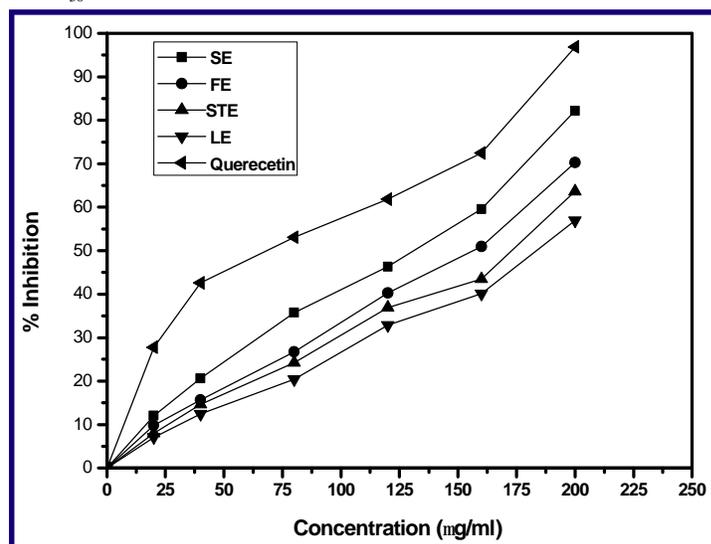


Figure 3: The ferric reducing activity (FRAP assay) of SE, FE, STE, LE and Quercetin of *Rumex vesicarius* L.

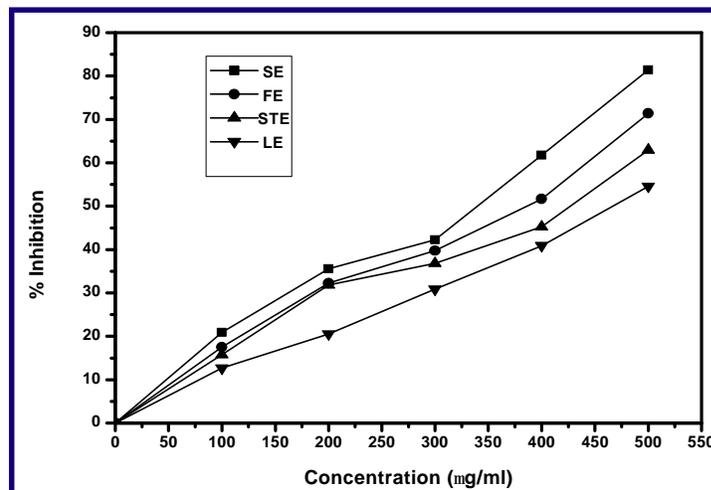


Figure 4: The ABTS radical scavenging activity of SE, FE, STE and LE of *Rumex vesicarius* L.

### $\beta$ -Carotene

In  $\beta$ -Carotene linoleate, model system free radical arises from oxidation of linoleic acid, attacked by the highly unsaturated  $\beta$ -Carotene molecules and causing decrease in absorbance at 470 nm. The pres-

ence of different antioxidants can hinder the extent of  $\beta$ -Carotene blanching by neutralization of the linoleate-free and other free radicals formed in the system<sup>[35]</sup>. The results of inhibition activity (50 %) of extracts and the positive control (BHT and BHA) were showed in Table 1, the high value obtained in SE ( $IC_{50} = 133.93 \pm 5.23 \mu\text{g/mL}$ ), FE ( $IC_{50} = 188.32 \pm 7.03 \mu\text{g/mL}$ ), STE and the lowest inhibition found in LE ( $IC_{50} = 194.12 \pm 8.55 \mu\text{g/mL}$ ). The interaction of a potential antioxidant with  $\beta$ -Carotene depends on organ extracts. The results indicated and supported that the presence of phenolic content with high concentration in the extracts of can moderately prevent the degradation of  $\beta$ -Carotene caused by radical reactions. Thus, consumption of such underutilization of the antioxidant can protect the oxidation and degradation of cellular macromolecules due to free-radical attacks.

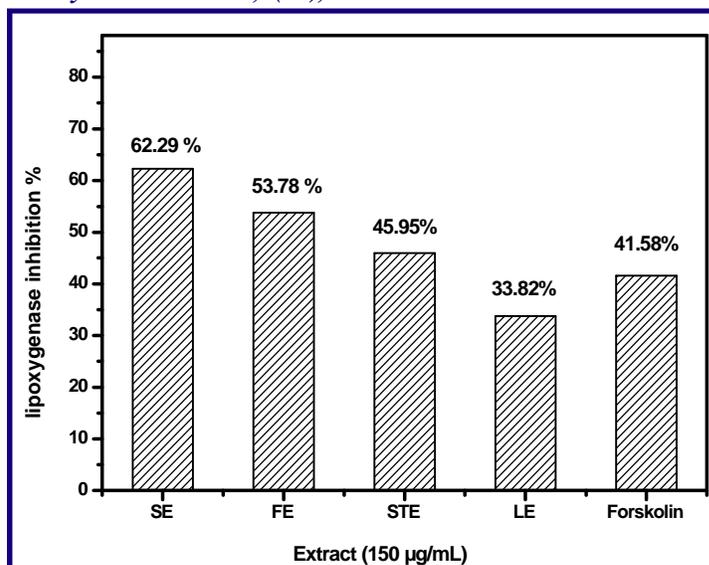
**Table 1:  $\beta$ -Carotene bleaching activities of SE, FE, STE and LE of *Rumex vesicarius* L and standards (BHT). Antioxidant activity was expressed as % inhibition  $IC_{50}$  values ( $\mu\text{g/ml}$ )<sup>a</sup>**

Samples Conc. ( $\mu\text{g/ml}$ )	Reaction time (min)		Antioxidant activity $IC_{50} =$ ( $\mu\text{g/ml}$ )	
	30	120		
SE	50	16.56 $\pm$ 0.50	9.35 $\pm$ 0.11	226.34 $\pm$ 7.14
	100	34.25 $\pm$ 1.27	24.35 $\pm$ 1.43	
	300	75.32 $\pm$ 3.68	64.97 $\pm$ 2.43	
FE	50	12.37 $\pm$ 0.32	07.40 $\pm$ 0.29	262.72 $\pm$ 9.03
	100	23.55 $\pm$ 2.01	17.60 $\pm$ 1.02	
	300	68.21 $\pm$ 2.12	57.41 $\pm$ 1.85	
STE	50	14.21 $\pm$ 0.02	05.92 $\pm$ 0.06	279.52 $\pm$ 8.15
	100	21.61 $\pm$ 0.21	15.31 $\pm$ 0.73	
	300	62.23 $\pm$ 2.05	54.09 $\pm$ 1.05	
LE	50	12.45 $\pm$ 0.31	04.14 $\pm$ 0.15	295.74 $\pm$ 8.41
	100	18.65 $\pm$ 1.06	13.86 $\pm$ 0.42	
	300	58.12 $\pm$ 1.45	51.34 $\pm$ 1.65	
BHT	40	53.45 $\pm$ 1.31	32.67 $\pm$ 1.32	85.84 $\pm$ 1.82
	100	73.55 $\pm$ 1.22	56.23 $\pm$ 1.54	
	200	89.21 $\pm$ 1.74	79.35 $\pm$ 1.97	

<sup>a</sup>Data are expressed as means  $\pm$  standard deviation of triplicate samples. Values with different row are significantly ( $P < 0.05$ ).

### Anti-inflammatory Activity

The 15-lipoxygenase inhibiting activity was measured using the 96-well microplate- based ferric oxidation of xylenol orange(FOX) assay. In a preliminary screening to select samples for dose-response study ,extracts and the reference compounds (Forskolin) were tested at a single concentration of 150  $\mu\text{g/mL}$ . The results presented in Figure 5 show that all the extracts investigated had a high level of 15-lipoxygenase inhibitory effect. For the concentration at 150  $\mu\text{g/mL}$ , the percentage of inhibition of SE, FE, ETE, LE and Forskolin 62.45 $\pm$ 1.53%, 53.78 $\pm$ 1.42%, 45.95 $\pm$ 1.23%, 33.82 $\pm$ 1.07% and 41.58 $\pm$ 1.38% respectively.



**Figure 5: Percentage inhibition of LOX of the SE, FE, STE, LE of *Rumex vesicarius* L and Forskolin**

### Cholinesterase inhibitory activity

AChE inhibitors are well utilized for the management of mild to moderate Alzheimer’s disease, and there are several researchers focused on the search of new AChE inhibitors from the herbal resources. Table 2 shows the acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities of the extracts, compared with those of galantamine used as a standard. The results of AChE and BChE inhibitory activities of SE, FE, STE and LE were expressed as percentage of inhibition and galanthamine equivalents (mg GALE/ mL). Against AChE enzyme, all extracts were found to be active. The most SE active SE demonstrating a  $IC_{50} = 0.524 \pm 0.005 \text{ mg/mL}$ , followed by FE  $IC_{50} = 0.672 \pm 0.007 \text{ mg/mL}$ , STE  $IC_{50} = 0.789 \pm 0.008 \text{ mg/mL}$  and LE  $IC_{50} = 0.855 \pm 0.008 \text{ mg/mL}$ . Against BChE enzyme, all extracts displayed activity inhibition on BChE. the most active extract was found to be SE  $IC_{50} = 0.084 \pm 0.00 \text{ mg/mL}$ , as well. FE demonstrated the best activity  $IC_{50} = 0.112 \pm 0.00 \text{ mg/mL}$ , followed by the STE  $IC_{50} = 0.141 \pm 0.00 \text{ mg/mL}$  and FE  $IC_{50} = 0.214 \pm 0.004 \text{ mg/mL}$ .

**Table 2: Acetylcholinesterase and butyrylcholinesterase inhibitory activities of SE, FE, STE and LE of *Rumex vesicarius* L.**

Sample	AChE assay $IC_{50}$ (mg/mL) <sup>a</sup>	BChE assay $IC_{50}$ (mg/mL) <sup>a</sup>
SE	0.524 $\pm$ 0.005	0.084 $\pm$ 0.00
FE	0.672 $\pm$ 0.007	0.112 $\pm$ 0.00
STE	0.789 $\pm$ 0.008	0.141 $\pm$ 0.00
LE	0.855 $\pm$ 0.008	0.214 $\pm$ 0.004
Galantamine	0.006 $\pm$ 0.00	0.056 $\pm$ 0.00

<sup>a</sup>Values expressed are means  $\pm$  SD

### DISCUSSION

*Rumex vesicarius* L is a good source of bioactive compounds due to its content of various phytochemicals, however most of the literature

report activities from this medicinal plant<sup>[27]</sup>. For this reason our study was focused on original of *Rumex vesicarius* L organs: leaf, flower, stem and seed. Furthermore, to the best of our knowledge leaf, flower, stem and seed extracts have been never investigated for and antioxidant, anti-inflammatory and anticholinesterase activities. In all samples examined, higher levels of total phenolic content and flavonoids were obtained in increasing order was: SE > FE > STE > LS. Thus, compared to total phenolic compounds, the contribution of flavonoids in antioxidant activity seems to be below and organ dependent<sup>[28,29]</sup>. Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups<sup>[30]</sup>. The phenolic compounds may contribute directly to antioxidative action<sup>[31]</sup>. The protective effects of phenolic compounds and flavonoids are directly related to their ability to scavenge free radicals. It is known that polyphenolic compounds have inhibitory effects on inflammatory, Cholinesterase, mutagenesis and carcinogenesis<sup>[32]</sup>. Phenolic compounds from plants are known to be good natural antioxidants. However, the activity of synthetic antioxidants was often observed to be higher than that of natural antioxidants<sup>[33]</sup>. 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 into account the various mechanisms of antioxidant action<sup>[34]</sup>. In this study, the antioxidant activities of the seed, flower, stem and leaf from *Rumex vesicarius* L evaluated by using in vitro antioxidant models, FRAP assay, ABTS radical and  $\beta$ -Carotene bleaching test. The antioxidant activity of the SE, FE, STE and LE of *Rumex vesicarius* L could be attributed to their chemical composition and the polarities of these chemicals. It is extremely important to point out that the most antioxidant activities from plant sources are correlated with phenolic compounds, correlation between antioxidant activity and polyphenolic contents were consistent with previous studies<sup>[35]</sup>. These compounds are known to act as antioxidants not only because of their ability to donate hydrogen or electrons but also because they are stable radical intermediates. Our findings showed the richness of *Rumex vesicarius* L on phenolic content. Lipoxygenase process is an important event for many diseases like asthma, atherosclerosis, cancer, tumor, angiogenesis, peptic ulcer, neuropathic and inflammatory pain. Inhibition of lipoxygenase activity results in down regulation of the pro-inflammatory activity of leukocytes and platelets, which may cause a diminished or delayed outcome of the inflammatory reaction. Lipoxygenase is a family of non heme containing dioxygenase group of enzymes that are widely distributed in plants and animals, these enzymes have key role in the biosynthesis of a variety of bioregulatory compounds such as hydroxyeicosatetraenoic acid, leukotrienes, lipoxins, hepxoylines<sup>[36]</sup>. Therefore, lipoxygenases are potential targets for the acute and chronic inflammatory disease and autoimmune disorders<sup>[37]</sup>. Leukotrienes are the downstream products of arachidonic acid that exert pivotal biological functions as well as pathogenic effects in a wide range of inflammatory processes. From a mechanistic point of view, seed, flower, stem and leaf extracts elicited

marked inhibitory activity in soybean lipoxygenase assay. Recently, several compounds that have been isolated from medicinal herbs showed strong anti-inflammatory activities, this activity could be, to some extent, a consequence of a certain amount of phenolic compounds found in the extract. Namely, phenolics, due to their renowned free radical scavenging capacity, could be involved in transforming arachidonic acid into pro-inflammatory agents, since these reactions are based on free radical mechanisms<sup>[38]</sup>.

On the other hand, many investigations have shown that different phenolic flavonoid molecules modulate the activity of and lipoxygenase<sup>[1]</sup>, the inhibition of these enzymes by flavonoids may be one of the most important mechanisms of their anti-inflammatory activity. The literature also supports that isoflavone glycosides show significant inhibition of chemical modulators formed during inflammatory response<sup>[39]</sup>.

Against AChE and BchE enzyme, seed, flower, stem and leaf extracts of *Rumex vesicarius* L were found to be active. Medicinal plants have been previously reported as having acetylcholinesterase inhibitor ability and a number of references reported the main components as phenolics<sup>[40]</sup>. However, the AChE and BchE inhibitory activities of *Rumex vesicarius* L we studied have never been reported before. Recently, the inhibition of this enzyme was targeted as a new approach to intercede in the progression of Alzheimer's disease.

## CONCLUSION

In the present study, thorough examination of phenolic content, flavonoids and in vitro studies on antioxidant, anti-inflammatory and anticholinesterase activity of seed, flower, stem and leaf extract of *Rumex vesicarius* L were undertaken for the first time. The four organs showed notable phenolic content and could be regarded as a possible source of these natural products. Furthermore, in vitro assays showed moderate antioxidant activity, sufficient ability to inhibit the 15-LOX enzymes and high inhibition of AchE and BchE enzyme. The results presented here strongly support traditional use of *Rumex vesicarius* L as a medicinal plant. Indeed, there is a current need for availability of new plant derived bioactive molecules, thus these plant extracts may be a great natural source for active components agents in some applications including food and medicinal. The identification and quantification of the detected main phenolic compounds and its relationship to the total antioxidant of the extracts should constitute the further work in our laboratory with this plant.

## Abbreviation

ABTS: 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt  
DPPH: 1,1-Diphenyl-2-picryl-hydrazyl  
FRAP: The ferric reducing antioxidant power  
AChE: acetylcholinesterase  
BChE: butyrylcholinesterase

SE: Seed extract

FE: Flower extract

STE: Stem extract

LE: Leaf extract

15-Lipoxygenase: 15-LOX

FOX: ferrous oxidation xylenol orange

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**Source of support: Nil, Conflict of interest: None Declared**