Studii şi Cercetări Ştiinţifice 2017, **18** (4), pp. 367 – 376
Chimie şi Inginerie Chimică, Biotehnologii, Industrie Alimentară

Scientific Study & Research
Chemistry & Chemical Engineering, Biotechnology, Food Industry *ISSN 1582-540X*

ORIGINAL RESEARCH PAPER

PHYTOCHEMICAL SCREENING, *IN VITRO* ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY OF *RUMEX VESICARIUS* L. EXTRACT

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Received: June, 18, 2017
Accepted: November, 16, 2017

Abstract: *Rumex vesicarius* L. (Polygonaceae), an edible plant has many important medicinal uses in the southeast of Algeria. The medicinal importance of this plant is a reflection of its chemical composition since the plant contains many bioactive substances. This research investigated the polyphenols profiling (total phenolic content, flavonoids, and condensed tannins), antioxidant and antibacterial properties of ethanolic extracts of seed, flower, leaf and stem from *Rumex Vesicarius* L. The total phenolic, flavonoid, condensed tannins contents, and antioxidant activity of all the parts extract was quantified. All extracts showed the presence of phenolic compounds and exhibited different levels of free radical scavenging activity against ABTS and superoxide radicals. In addition, for antibacterial activity, the following bacteria were tested: *Salmonella typhimurium* (ATCC 13311), *Escherichia coli* (ATCC 35210), *Bacillus subtilis* (ATCC 10907), *Staphylococcus epidermidis* (ATCC 12228), *Staphylococcus aureus* (ATCC 29213) and *Pseudomonas aeruginosa* (ATCC 27853). Results showed that all extracts possessed concentration-dependent antioxidant activity. The study concludes that *Rumex vesicarius* L possess diverse therapeutic potentials that might be used as natural antioxidant and antibacterial.

Keywords: *ABTS, antibacterial activity, metal chelating scavenging, phenolic content, Rumex vesicarius* L*, superoxide radical*

INTRODUCTION

The increasing demand for herbal medicines encourages collectors and traders to decimate natural populations of important medicinal plants [1]. Have been an integral part of pharmacotherapy throughout the history of mankind, and they have served as an invaluable source for the discovery of bioactive molecules [2].

Rumex vesicarius L. of the family Polygonaceae, are the most abundant of the nine known *Rumex* species [3], is an annual, glabrous herb, 15 - 30 cm height, branched, leaves elliptic, ovate (or) oblong with monoecious flowers. The plant is widely cultivated as a green leafy vegetable in many parts. It also is 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 [4]. It is used in the treatment of liver diseases, cancer, cardiovascular diseases and cataract [5, 6], digestive problems, toothache, nausea, pain, anti-inflammatory, antitumor as well as antischistosomal, and antimicrobial activities [7, 8]. It was also found to have an aphrodisiac effect [9, 10]. Previous chemical investigations have shown the presence of polyphenols, flavonoids, carotenoids, tocopherols and ascorbic acid in different organs extract from *Rumex vesicarius* L. Seham et al [11] indicate that the *Rumex vesicarius* L content the flavones and flavonols while catechins (flavanols) and flavanone the *n*-butanol extract.

Polyphenols play diverse roles such as functioning as antioxidant, antimicrobial, anti-allergic, anti-inflammatory and anticancer agents [12]. These compounds are the major low molecular weight bioactive components usually found in mushroom species, responsible for their antioxidant properties [13]. Phenolic compounds, tannins, anthocyanin, and flavonoids can play the role in free radical scavenging inhibition through different mechanisms. Previous research has reported that polyphenols and flavonoids inhibit the inflammation process by the production of pro-inflammatory molecules [14]. Bioactivities and bioavailability of these compounds may be affected by reaction between polyphenols and food matrix components [15]. In addition, the polyphenols play a role as antibacterial. This antimicrobial activity of the might be helpful in eliminating many pathogenic microorganisms that have acquired the ability to survive existing antibiotics at clinically relevant concentrations [16].

Reactive oxygen species (hydroxyl, superoxide, peroxyl, alkoxyl, hydroperoxyl, nitrogen dioxide, nitric oxide and lipid peroxyl; and non-radicals like hydrogen peroxide (H_2O_2), hypochlorous acid, ozone, singlet oxygen, peroxynitrite, nitrous acid, lipid peroxide, dinitrogen trioxide) are partially reduced or excited forms of atmospheric oxygen [17]. They function in cells as signaling molecules but are also thought of as the unavoidable toxic byproducts of aerobic metabolism. Reactivity of free radicals is generally stronger than non-radical species though radicals are less stable and disrupt the biological function of biomolecules. Reactive oxygen species include radicals like superoxide and ferrous ion destroy a lot of biomolecules such as protein lipids cause mutation in a living cell which cause diseases [18]. The main targets of ROS during oxidative stress are thought to be DNA, RNA, proteins, and lipids. Different ROS have different degrees of reactivity toward these cellular components, and the availability of free iron in the form of Fe^{2+} is considered paramount for ROS toxicity due to the role of iron in the Fenton reaction that drives the formation of hydroxyl radicals [16].

This paper reports the evaluation of phenolic content, flavanoids, the antioxidant and antibacterial capacity of ethanolic extract of *Rumex vesicarius* L (stem, flower, and

seed) applying different analytical methodologies. We report our findings and relate them to the phytochemical studies of the plant as well as its medicinal uses.

MATERIALS AND METHODS

Plant material and extraction

The different parts (stem, flower, and seed) of *Rumex vesicarius* L were collected from southeast of Algeria (33° 07" 00" N 7° 11' 00" E), state of El Oued on Mars 2016. The plant material was washed, reduced into small pieces before being ground and powdered into particles of small size. Then the powder was put in a hot air oven at 60 °C until complete drying (Heraeus Series 600, Thermo Fisher USA). Depending on the physical characteristics of the samples, the time ranged from 18 to 30 h. 80 g of plant material was extracted with 600 mL of ethanol for 5 h in Soxhlet. The extracts were filtered and evaporated under vacuum at 55 °C before being dried and lyophilized for 10 h. The raw extract was stored at -4 °C.

Chemicals

All chemicals were purchased from Biochem and Sigma Aldrich. The antibacterial activity was screened against four bacteria obtained from Pasteur Institut from Algiers Algeria.

Determination of total phenolic content

The total phenolic contents of stem flower and seed were determined by the Folin-Ciocalteu method [19]. Briefly, 0.25 mL of each extract and standard (gallic acid) of were mixed with 1.25 mL of 1 N Folin-Ciocalteu reagent. After 5 min, 1 mL of sodium carbonate aqueous solution (7.5 %, w/v) was added to the mixture and completed the reaction for 120 min at room temperature. The absorbance was read at 765 nm using a UV-visible spectrophotometer (Shimadzu UV-1800, Japan). The results were expressed in equivalent milligrams of gallic acid per gram of dry weight of plant extract (mg GAE·g^{-1} DW). The experiment was conducted in triplicate and the results were expressed as means ± SD (standard deviation).

Total flavonoids content

According to the colorimetric method assay we quantification the flavonoids content of different extracts [20]. 4 mL of distilled water was added to 1 mL of each extract. Then, 5 % of sodium nitrite solution (0.3 mL) was added followed by 10 % aluminum chloride solution (0.3 mL). After, the mixture was incubated at room temperature for 5 min, and then 2 mL of 1 M NaOH was added. Immediately, the volume of reaction mixture was made to 10 ml with distilled water. The absorbance of the pink color developed was determined at 510 nm by UV-Visible spectrophotometer (Shimadzu UV-1800, Japan). A calibration curve was prepared with catechin and the results were expressed as mg catechin equivalents per gram of dry weight (mg CE·g^{-1} DW).

Determination of condensed tannins content

The spectrophotometric method is used for determination of condensed tannins content [21]. 0.5 mL of different extracts or standard (catechin) were added to the mixture of 3 mL of 4 % vanillin - methanol (4 %, v/v), 1.5 mL of hydrochloric acid. The resulting mixture was allowed to stand for 15 min at room temperature. The absorbance was measured at 500 nm using spectrophotometer (Shimadzu UV-1800, Japan). Total condensed tannins content was calculated as mg catechin equivalent per gram of dry weight (mg CTE·g^{-1} DW).

ABTS radical scavenging assay

The antioxidant activity of different extracts stems, flowers and seeds from *Rumex vesicarius* L were evaluated by ABTS scavenging assay radical [22]. ABTS reagent was prepared by 10 mL (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 of extracts or standard were added to 1.588 µL diluted ABTS solution to react in the dark at room temperature for 10 min. The absorbance is a reader at 732 nm. The percentage inhibition of ABTS radical as calculated following the Equation 1:

$$\text{ABTSradical scavenging activity} = [(Abs_{control}\text{-}Abs_{sample})/ Abs_{control}] \times 100 \quad (1)$$

where:
$Abs_{control}$ is the absorbance of ABTS radical + ethanol
Abs_{sample} is the absorbance of ABTS radical + ethanol extract or standard

Scavenging activity of superoxide radicals

The inhibition of NBT (Nitroblue tetrazolium chloride) reduction by photochemical generated O_2^- used for evaluated the scavenging activity of superoxide radicals [23]. To reaction mixture constituted of 2 µM of riboflavin, 6 µM EDTA, 50 µM NBT and 3 µg of sodium cyanide in 67 mM phosphate buffer (*p*H = 7.8) in a final volume of 3 mL. Firstly the absorbance was measured at 530 nm, the tubes were illuminated uniformly with the incandescent lamp at 530 nm. The sample extract was added to the reaction mixture, in which O_2^- radicals are scavenged. Quercetin used as a positive control and the percentage of scavenging inhibition was calculated as % (Equation 1).

$$\% \text{ inhibition} = [(A_{control} - A_{sample})/A_{control}] \times 100 \quad (2)$$

where:
$A_{control}$: Absorbance of control
A_{sample} : Absorbance of extract

Antibacterial activity

Antibacterial activity of stem, flower, leaf, and seed of *Rumex vesicarius* L was evaluated. The following bacteria were tested: *Salmonella typhimurium* (ATCC 13311), *Escherichia coli* (ATCC 35210), *Bacillus subtilis* (ATCC 10907), *Staphylococcus epidermidis* (ATCC 12228), *Staphylococcus aureus* (ATCC 29213) and *Pseudomonas aeruginosa* (ATCC 27853). All strains were obtained from the laboratory of pathology,

hospital central of El Oued (Algeria). The sensitivity of different bacterial strains to various extracts was measured in terms of zone of inhibition using agar-diffusion assay. The bacterial suspension was adjusted with sterile saline to a concentration of approximately $1.0x10^7$ cells·mL^{-1}. The plates containing Mueller-Hinton agar is spread with 0.2 mL of the inoculums. Wells (6 mm diameter) were cut out from agar plates using a sterilized stainless steel borer [24]. In this method, extracts were dissolved in a small quantity of ethanol and were then prepared to the solutions at concentrations of 500 mg·mL^{-1} with sterile water firstly. These solutions were diluted in sterile water to form different concentrations of samples (50 and 100 mg·mL^{-1}) for stem, flower and seed ethanolic extracts. Finally, each well was filled with 100 µL of the solution and the plates inoculated with different bacteria were incubated at 37 °C for 24 h and diameter of the resultant zone of inhibition was measured.

Statistical Analysis

Experimental values are given as means ± standard deviation (SD) of three replicates for antioxidant activity and antibacterial activity. Statistical significance was determined by one-way variance analysis (ANOVA). Statistical calculations were carried out by OriginPro Version 9.1 software (OriginLab Corporation). Values of $p < 0.05$ were regarded as significant.

RESULTS AND DISCUSSION

Total phenolic content, flavonoids and condensed tannins

Extraction is an important step for obtaining extracts with acceptable phytochemical concentration and strong antioxidant activity [25]. The extracts of stem, flower, leaf, and seed of *Rumex vesicarius* L were found to be rich in phenolics composition. The total phenolic content is given in Table 1. Seed extract (SE) was founded to have the highest value 72.15 ± 1.75 mg GE·g^{-1} DW, following by the flower extract (FE) 63.55 ± 1.25 mg GE·g^{-1} DW, leaf extract (LE) 56.55 ± 1.15 mg GE·g^{-1} DW and the lowest value in stem extract (STE) 48.85 ± 1.18 mg GE·g^{-1} DW. Similar results were observed in the quantification of total flavonoids, the content of total flavonoids was also found to vary significantly ($p < 0.05$) and content ranged from 9.43 ± 0.2 mg CE·g^{-1} DW to 16.88 ± 0.5 mg CE·g^{-1} DW. The Total flavonoids in increasing order were: SE > FE > LE > STE. The condensed tannins content of different parts extract have been reported as mg catechin equivalents CE·g^{-1} of the dry weight of (+)-catechin equivalents per gram of dried extract, respectively (Table 1). We showed that SE has the greatest and STE has the lowest amount of condensed tannins content. Moreover, FE and LE have higher amounts of condensed tannins than STE.

Table 1. *Total phenolic content, flavonoid and condensed tannins of SE, FL, LE, and STE extract from Rumex vesicarius* L

Phytochemical	Extract parts			
	SE	FL	LE	STE
Total phenolic content [mg GAE·g^{-1} DW]	72.15±1.75	63.55±1.25	56.55±1.15	48.85±1.18
Total flavonoid [mg CE·g^{-1} DW]	44.74±1.21	41.58±1.12	33.87±1.11	30.64±1.07
Condensed tannins [mg CE·g^{-1} DW]	35.25±0.95	32.82±1.05	27.95±0.91	24.17±0.85

ABTS radical scavenging assay

Previous works have reported that ABTS assays are valid methods to determine the antioxidant properties of food and beverages [26]. Figure 1 and Table 2 showed the dose-response curves of ABTS scavenging activities of seed, flower, leaf and leaf extracts and reference antioxidant (BHT) on ABTS radicals. As shown in Figure 1, the ABTS radical scavenging activity of all extracts started with low values 12.05 ± 0.58 %, 9.84 ± 0.46 %, 8.04 ± 0.53 % and 27.09 ± 1.15 % respectively at a concentration of extract 25 µg·mL^{-1}. After a rapid growth, reached to stabilization (82.18 ± 1.7) %, (62.14 ± 1.38) %, (56.88 ± 1.13) %, 54.74 ± 1.14 and 96.7 ± 2.43 % respectively at a concentration of extract 600 µg·mL^{-1},. The abilities of scavenging ABTS radicals were in descending order: BHT > SE > FE > LE > STE.

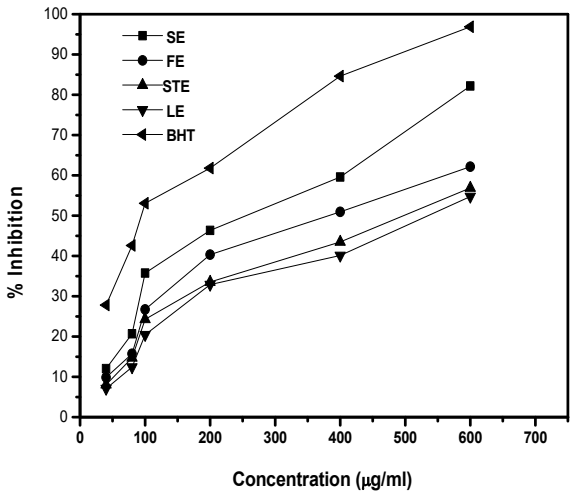

Figure 1. *ABTS radical scavenging activity of SE, FE, LE, and STE of Rumex vesicarius* L *and BHT*

Scavenging activity of superoxide radicals

The antioxidant activity against superoxide radical results for the different extracts are presented in Figure 2 and Table 2 and was compared with the standard (quercetin).

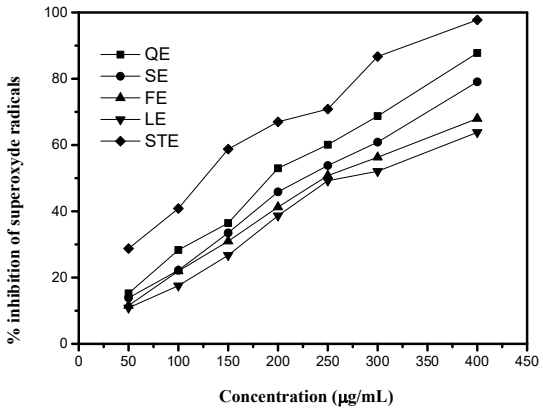

Figure 2. *Superoxide radical scavenging activity of SE, FE, LE, and STE of Rumex vesicarius* L *and Quercetin*

The results for Figure 2 are expressed in terms of IC_{50}. The SE show the best results in antioxidant potential with values of (190.59 ± 3.57, higher than FE IC_{50} = 224.71 ± 5.57 µg·mL^{-1}, followed by the LE IC_{50} = 238.09 ± 4.18 µg·mL^{-1}. The STE shows a moderate antioxidant potential IC_{50} = 265.45 ± 4.76 µg·mL^{-1}, the quercetin show an antioxidant activity higher than different extracts IC_{50} = 125.71 ± 3.57 µg·mL^{-1}.

Table 2*. Scavenging activity of superoxide radicals and ABTS of SE, FE, LE, and STE of Rumex vesicarius* L. *and standards (quercetin) expressed as % inhibition IC_{50} values (µg·mL^{-1})*

Extracts and standards	**superoxide radical IC_{50} = [µg·mL^{-1}]**	**ABTS radical IC_{50} = [µg·mL^{-1}]**
SE	255.13±7.29	260.81±4.75
FE	383.71±9.75	389.36±5.41
LE	501.68±10.50	509.52±6.82
STE	539.27±10.18	541.25±6.37
Quercetin	94.88±2.25	-
BHT	-	92.12±2.25

Data are expressed as means ± standard deviation of triplicate samples. Values with a different row are significantly ($P < 0.05$)

Antibacterial activity

The results of the antibacterial effect of ethanolic extracts of different organs of *Rumex vesicarius* L. in terms of zone of inhibition (mm) were presented in Table 3. The extracts of stem, flower, leaf and seed of *Rumex vesicarius* L. showed a moderate antibacterial activity against all the six bacterial strains tested against *Escherichia coli* (ATCC 35210), *Bacillus subtilis* (ATCC 10907), *Staphylococcus epidermidis* (ATCC 12228), *Staphylococcus aureus* (ATCC 29213) and *Pseudomonas aeruginosa* (ATCC 27853). The strongest antimicrobial activity of ethanolic seed, flower, leaf and stem extracts of *Rumex vesicarius* L was against *Escherichia coli* (ATCC 35210) and *Pseudomonas aeruginosa* (ATCC 27853), while the antimicrobial activity against *Bacillus subtilis* (ATCC 10907), *Salmonella typhimurium* (ATCC 13311) and

Staphylococcus aureus (ATCC 29213) was moderate. For the extracts, the general order of their antibacterial activity would be SE> FE > LE> STE. The previous study obtained similar results for the antibacterial activity of *Rumex vesicarius* L extract and reported that this plant exhibit excellent antimicrobial activity against several bacteria [27, 28]. Polyphenols comprise a wide variety of molecules with polyphenol structure, potentially useful structures for the development of new chemotherapeutic agents [29]. In recent year, several studies reported the antimicrobial and resistance modifying potentials of these compounds [30]. Phenolic compounds have demonstrated permeative action by destabilizing the lipopolysaccharide membrane Nevertheless, as occurs with antioxidant activity, it is difficult to attribute the antibacterial activity of a complex mixture of bioactive compounds, as with the *Rumex vesicarius* L extracts, to a single or particular constituent, especially taking into account the great variability in the composition of bioactive compounds [31].

Table 3. *Antimicrobial activity of SE, FL, LE, and STE extract from Rumex vesicarius L tested at 50 and 150 mg·mL^{-1} expressed as a zone of inhibition (mm)*

Bacteria	Diameter of zone of inhibition [mm]							
	SE		FE		LE			STE
	50 mg·mL^{-1}	100 mg·mL^{-1}	50 mg·mL^{-1}	100 mg·mL^{-1}	50 mg·mL^{-1}	100 mg·mL^{-1}	50 mg·mL^{-1}	100 mg·mL^{-1}
Salmonella typhimurium (ATCC 13311)	10±0.3	13±0.5	9±0.4	12±0.5	9±0.4	11±0.4	10±0.4	10±0.4
Bacillus subtilis (ATCC 10907)	9±0.2	11±0.5	8±0.4	10±0.5	8 ±0.4	10±0.5	10±0.5	9±0.5
Escherichia coli (ATCC 35210)	12±0.4	15±0.6	11±0.5	14±0.6	10±0.5	13±0.5	11±0.4	13±0.4
Staphylococcus epidermidis (ATCC 12228)	11±0.4	14±0.5	10±0.4	13±0.3	10±0.3	12±0.4	10±0.5	11±0.4
Staphylococcus aureus (ATCC 29213)	10±0.3	13±0.4	9±0.3	12±0.5	9±0.5	10±0.5	9±0.5	10±0.5
Pseudomonas aeruginosa (ATCC 27853)	12±0.5	14±0.5	11±0.4	13±0.6	11±0.5	11±0.5	10±0.4	12±0.4

Data are presented as the mean ± standard deviation of three determinations

CONCLUSION

In the present research paper, a thorough examination of a phenolic profile in vitro study on the antioxidant and antibacterial potential of seed, flower, leaf, and seed ethanolic extract obtained by Soxhlet technique of *Rumex vesicarius* L was undertaken for the first time. This study demonstrates a higher total phenolic content, flavonoids, and condensed tannins content and could be regarded as a possible source of these natural products. The free radical scavenging activity of different extracts was studied by their ability to bleach the stable ABTS and O_2^- free radicals. In addition, the ABTS and O_2^- radical scavenging abilities of the extracts were significantly less than those of standards. Moreover, the antimicrobial activity of seed extract was stronger than that of

the other three tested extract (flower, stem and leaf) against *Escherichia coli* (ATCC 35210), *Bacillus subtilis* (ATCC 10907), *Staphylococcus epidermidis* (ATCC 12228), *Staphylococcus aureus* (ATCC 29213) and *Pseudomonas aeruginosa* (ATCC 27853). Results obtained in this study on phenolic composition, antioxidant and antimicrobial activity of a different ethanolic extract of *Rumex vesicarius* L involucral bracts support the traditional medicinal use of this plant and provide grounds for further investigation its use as a functional food. In the future, the extract with different parts will be separated and purified, and their structure-bioactivity relationship will be studied further.

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