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Potential Assessment of *Rumex* spp. as a Source of Bioactive Compounds and Biological Activity

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Abstract: Secondary metabolites from the shoots and roots of three *Rumex* species collected from three different habitats were investigated (*Rumex dentatus* collected from cultivated land, *R. pictus* collected from the coastal desert and *R. vesicarius* collected from the inland desert) and tested for antioxidant activity as well as for anti-microbial activity against some human pathogenic bacteria. The present study indicated that the quantitative analysis of shoot and root extracts of three *Rumex* spp. were found to be rich in tannins and phenolics composition. The aerial parts of the three plants exhibited the highest significant values compared to the root parts. The MeOH extracts of *Rumex* species showed adequate antioxidant activity, wherein the IC_{50} values of the MeOH from the cultivated sample was 41.61 and 31.31 mg mL^{-1}, coastal samples were 34.99 and 23.99 mg mL^{-1}, while the sample of inland showed IC_{50} value of 41.59 and 31.67 mg mL^{-1}, for root and shoot, respectively. Furthermore, using a filter paper disc assay, the MeOH extracts of the three *Rumex* species showed a substantial anti-microbial inhibitory effect on the growth of 10 pathogenic bacteria. According to sensitivity, the tested organisms could be sequenced as following: *E. coli* < *K. pneumoniae* < *S. typhi* < *P. aeruginosa* for Gram-negative bacteria and *B. subtilis* < *S. pneumoniae* < *L. monocytoyenes* < *S. epidermis* < *S. aureus* < *B. cereus* for Gram-positive bacteria. In addition, the antibacterial performance of *R. dentatus* root and *R. vesicarius* shoot MeOH extract is 100% broad spectrum against Gram-negative bacteria. A shoot of *R. dentatus* and *R. pictus* MeOH extract against Gram-positive bacteria is 83.3% broad spectrum. A further study is recommended for more characterization of the major compounds and assesses their efficiency and biosafety.

Keywords: *Rumex* spp.; xerophytes; antibacterial; antioxidant; phytochemical.

1. Introduction

Medicinal plants are a major source of complex and highly structurally diverse chemical compounds (phytochemicals). In fact, plants represent an enormous reservoir of new, undiscovered and bioactive molecules, moreover, they represent an inexhaustible source of life-saving biological resources for the majority of the world's population [1,2]. Natural products sometimes have a pharmacological activity that can benefit treating diseases [3]. The demand for arable land, water, energy, and biological resources to provide a sufficient supply of food while protecting the quality of our environment is increasing as the world population continues to increase geometrically. As the world population expands, the food problem will become increasingly severe conceivably, with the number of malnourished reaching 3 billion [4].

Polygonaceae (knotweed family) is a family of flowering plants, most of the members are perennial herbaceous plants with swollen nodes, but trees, shrubs and vines are also present [5]. Meickle [6] reported that Polygonaceae comprises about 40 genera widely distributed, but chiefly in temperate regions of the northern hemisphere. Genus *Rumex* is considered one of the most important genera in this family, comprises about 150 species of annual, biennial and perennial herbs with a cosmopolitan distribution in temperate regions [7,8]. *Rumex* species has a long history of being used as a domestic herb. It's a gentle and safe laxative with a weaker action than rhubarb, so it's especially good for treating mild constipation. The plant has valuable cleansing properties and is useful for treating a wide range of skin problems. All parts of the plant can be used, though the root is most active medicinally [9,10].

Rumex dentatus L. (dentate dock) is an annual, glabrous herb producing a slender, erect stem up to 70 or 80 centimeters in maximum height. It is widely distributed in Europe, Asia and the Mediterranean region, including Egypt [8]. It occurs in waste places, shores, canal banks and cultivated fields. It has been used as a leafy vegetable in the Mediterranean diet [11,12]. *Rumex pictus* Forssk. (veined dock) is also an annual, glabrous herb, 10-30 cm, stems decumbent and richly branched at the base. It is widely distributed in Syria, Arabia and the Mediterranean coast of Egypt [8]. *Rumex vesicarius* L. (bladder dock) is an annual semi-succulent herb, pale green, 15-30 cm high. The plant is widely cultivated as a green leafy vegetable and medicinal properties in many parts of the world [8,13]. In Egypt, three plant species are growing wild and collected in spring and eaten fresh or cooked [14].

Rumex spp. is widely used as food, as a medicinal herb [15]. Previous chemical investigations have shown the presence of polyphenols, flavonoids, carotenoids, anthraquinones, tocopherols and ascorbic acid in different organs extract from *Rumex* spp. [16-19]. Little information has been reported on the chemical composition and biological activity of *R. pictus*. However, *Rumex* spp. are reported to have various biological activities, as antioxidant, antibacterial, hepatoprotective, antiemetic, cytotoxic and phytotoxic [20-23].

As a result, plant communities in arid regions have a long history of using sustainable renewable resources for uncultivated areas in order to increase food production, animal feed, and the manufacturing of local raw materials [24,25]. This study was carried out to (i) assess the in vitro anti-microbial and antioxidant activities of three wild-grown *Rumex* sp. (*Rumex dentatus, R. pictus* and *R. vesicarius*) collected from three heterogeneous habitats, (ii) qualitative and quantitative investigation of some chemical constituents of the three samples, and (iii) evaluate the allelopathic activity of the three species against the nuisance weed, Chenopodium murale.

2. Materials and Methods

2.1. Plant materials and extraction.

Composite samples of the three *Rumex* species during the flowering stage from three different habitats were collected in plastic bags; the first location was cultivated land habitat with clay soil near Mansoura City, Nile delta, Egypt (31°04′46.76″N 31°21′57.37″E), sandy habitat near Gamasa City was the second location, northern Mediterranean coast, Egypt (31°26′58.78″N 31°28′36.14″E).

While the third location was the inland wadi habitat with sandy and gravel soil north Eastern Desert (Wadi Araba), Egypt (28°59'57.75"N 32°6'57.95"E). The collected plants were identified according to Boulos [8] by Dr. Yasser A. El-Amier Lecture of Plant Ecology, Botany

Department, Mansoura University, Egypt. The plant materials were separated into root and shoot system, washed and dried at room temperature, reduced into small pieces before ground and packed in paper bags. The three samples of Rumex spp. were dried on air, then about 100 g powder of them were extracted in 70% hydro-methanol at room temperature (27°C ±2). The extraction was filtered, and evaporated under a vacuum at 55 °C, then dried to give dark gum.

2.2. Phytochemical analysis.

2.2.1. Qualitative phytochemical screening.

Rumex spp. (*Rumex dentatus, R. pictus*, and *R. vesicarius*) air-dried shoot and root powder were subjected to preliminary phytochemical screening for alkaloids, saponins, tannins, phenolics, anthraquinone, steroid, flavonoids, glucosides, coumarins, and terpenoids using the standard methods mentioned by Harborne [26], Trease and Evans [27] and Sofowora [28].

2.2.2. Quantitative estimation of some secondary compounds.

Total phenolics, tannins, alkaloids, flavonoids and saponins of *Rumex* spp. (*Rumex dentatus, R. pictus* and *R. vesicarius*) samples were quantitatively estimated spectrophotometrically [29-32].

2.3. Evaluation of antioxidant activity.

In terms of radical scavenging activity, the antioxidants activity of the samples was measured using stable radical DPPH [33]. A methanol solution of 1 mL of each Rumex sample was prepared, mixed well and incubated at room temperature (25°C) for 15 min in dark conditions at different concentrations (5, 10, 20, 30, 40 and 50 ppm), with the same volume of the methanol solution of 0.3 mM DPPH. As standard, catechol was used. Use of UV visible spectrophotometer to determine the absorption decrease of 517 nm (Spectronic 21 D model). The IC_{50} (the concentration of specimen required to reduce the absorbance of DPPH by 50%) was calculated graphically. The percentage inhibition of the DPPH radical was calculated as follows:

$$\%\ \text{Radical scavenging activity} = (1 - \frac{Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

2.4. Evaluation of antibacterial efficacy.

2.4.1. Bacterial strains.

From the Laboratory of Bacteriology, Department of Botany, Faculty of Science, Mansoura University, Egypt, five Gram-negative bacteria: *Pseudomonas aeruginosa* (ATCC9027), *Salmonella typhi* (ATCC25566), *Listeria monocytoyenes* (ATCC19116), *Escherichia coli* (ATCC10536), and *Klebsiella pneumoniae* (ATCC10031), and four Gram-positive bacteria; *Bacillus subtilis* (DMS1088), *Entrobacter cloeoae* (DMS30054), *Streptococcus epidermis* (EMCC1353t), and *Staphylococcus aureus* (ATCC6538) were taken.

2.4.2. Estimation of anti-microbial activity (Agar diffusion method)

The agar diffusion assay was applied according to the method described by Cappuccino and Sherman [34]. The sterilized Whatman no.1 filter paper discs (6 mm in diameter) were impregnated with 100 µl of the extract (100 µg/ml) and were then applied on the surface of agar plates freshly seeded with standard inoculate of young cultures. All Petri dishes were incubated at 37 °C for 24 hours. Experiments were done in triplicate; the diameter of the inhibition zone (mm) was estimated and compared with DMSO as control. Standard antibiotics of chloramphenicol, penicillin and clotrimazole were used.

3. Results and Discussion

3.1. Phytochemical constituents.

The data of preliminary qualitative phytochemical screening of three *Rumex* spp. revealed that different species of *Rumex* plant are a good source of bioactive compounds due to their content of various phytochemicals. The powder of *Rumex* spp. shoot and root parts were subjected to phytochemical screening and the results were recorded in Table 1. In this study, the use of methanolic solvent showed different responses for the presence of phytoconstituents and according to the intensity of color or precipitates produced, the terms of scores are using as -, +, ++, +++. The methanolic extract showed the presence of all the tested chemical constituents in the plant parts (shoot and root). However, steroids, glycosides and coumarins were absent in *R. vesicarius* root extract, anthraquinones and coumarins in the root extract of *Rumex dentatus* and *R. pictus* (Table 1). Plants are an abundant natural source of structurally diverse organic compounds that are not directly involved in normal growth, development, or reproduction but are thought to be required to adapt to their environment [35,36].

On the other hand, comparative study of wild *Rumex* spp. shoot and root extracts revealed that they were high in tannins and phenolics composition. However, the aerial parts of the three samples exhibited the highest significant values compared to root parts. The present results showed that the contents of total phenolics, tannins, total flavonoids, alkaloids and saponins were in the range of 16.71-4.15, 10.39-3.44, 6.67-2.25, 7.87-2.15 and 5.24-2.69 mg/g DW, respectively (Figure 1).

Table 1. Qualitative phytochemical analysis of extracts of *Rumex* spp.

Screening test	***Rumex* spp.**					
	R. dentatus		***R. pictus***		***R. vesicarius***	
	Shoot	**Root**	**Shoot**	**Root**	**Shoot**	**Root**
Alkaloids	++	+	+	+	+	+
Flavonoids	++	++	++	+	++	+
Phenols	+++	++	+++	++	+++	++
Saponins	+	+	+	+	+	+
Tannins	++	+	+	+	++	+
Steroids	+	+	+	+	+	-
Glycosides	++	+	+	+	+	-
Anthraquinones	+	-	+	-	+	++
Terpenes	+	+	+	+	+	+
Coumarins	+	-	+	-	+	-

Positive mark (+) indicates the presence of the phytochemical. Negative mark (−) indicates the absence of the phytochemical.

According to phytoconstituent data, the concentration of bioactive compounds in shoot and root of *Rumex* spp. followed the order phenolics > tannins > flavonoid > alkaloid >

saponins. Regarding the plant species, the concentrations of bioactive compounds were in *R. vesicarius* > *R. pictus* > *Rumex dentatus*. Moreover, different plant species had variable mineral concentrations. These results are in good accordance with previous studies, which showed that high phenolic content in xero-halophytes as they mainly protect plant cells from drought and salinity [37]. Our results were similar to those reported by Beddou *et al.* [38] on *R. pictus* and *R. vesicarius* and Shakir *et al.* [39] on *R. dentatus* and *R. hastatus*. Whereas Ammar *et al.* [40] reported that alkaloids, saponins and coumarins were all absent from *R. pictus* and *R. vesicarius*.

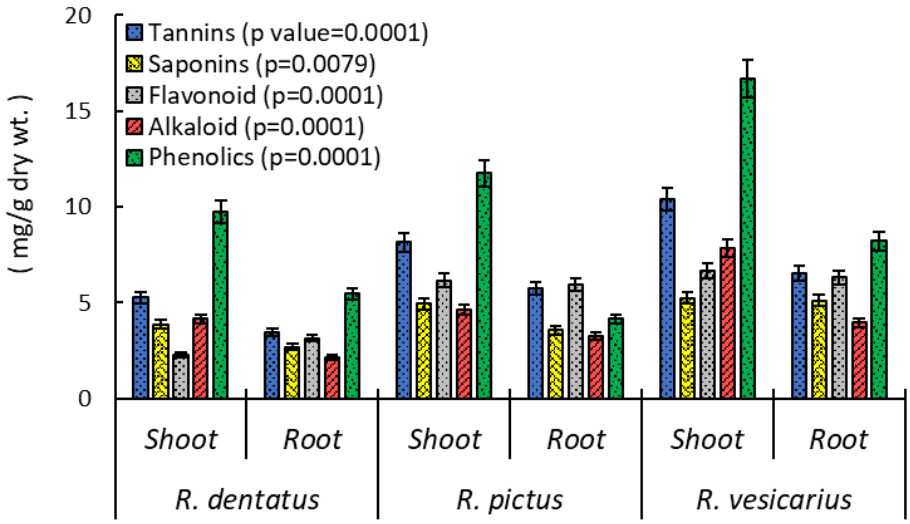

Figure 1. The concentration of the active constituents in mg/g dry weight for the *Rumex* spp.

3.2. Antioxidant activity of MeOH extract of Rumex species

The MeOH extract of three *Rumex* spp. showed a significant reduction in the DPPH color in a concentration-dependent manner with respect to control (Figure 2). At 50 mg mL^{-1}, the extracts of *Rumex dentatus*, *R. pictus* and *R. vesicarius* showed scavenging activities of 52.88%, 59.57% and 55.42%, for root and 62.78%, 72.96% and 64.66% for a shoot, respectively. However, the lowest concentration (5 mg/ml) shows the lowest antioxidant activity (Figure 2). The IC_{50} values of the MeOH from the cultivated sample were 41.61 and 31.31 mg mL^{-1} for root and shoot, coastal samples were 34.99 and 23.99 mg mL^{-1}. The IC_{50} values for the inland and coastal samples were 41.59 and 31.67 mg mL^{-1}, respectively. Based on the IC_{50} values, ascorbic acid (a normal antioxidant) showed a 4-fold increase in antioxidant activity compared to the MeOH extract of the root sample and a 3-fold increase in antioxidant activity compared to the shoot sample of *Rumex* spp. in three different habitats. The IC_{50} is inversely proportional to the antioxidant power, where the lower the IC_{50}, the higher the antioxidant activity (Figure 3).

The powerful antioxidant activity of *Rumex* MeOH extract can be attributed mainly due to secondary compounds [41]. The predominance of polyphenols may be the primary reason for the considerable observed antioxidant activity of *Rumex* spp. MeOH extract. Flavonoids, tannins and phenolic compounds have been found to be antioxidants in different plants, such as *Diplotaxis harra* [42], *Fagonia* species [43], *Rubus* fruits [44] and *Aizoon canariense* [45].

Moreover, the high content of phenolic compounds, flavonoids and tannins can be attributed to the antioxidant activity of the coastal and inland samples (Figure 1). Drought and salinity have been reported to be one of the key environmental factors, as both stresses can

lead to several morphological, physiological, biochemical and metabolic changes as a mechanism for acclimation to stressful conditions [46]. In general, this difference between activities of the three Rumex spp. could be confirmed with the environmental and habitat conditions [47,48].

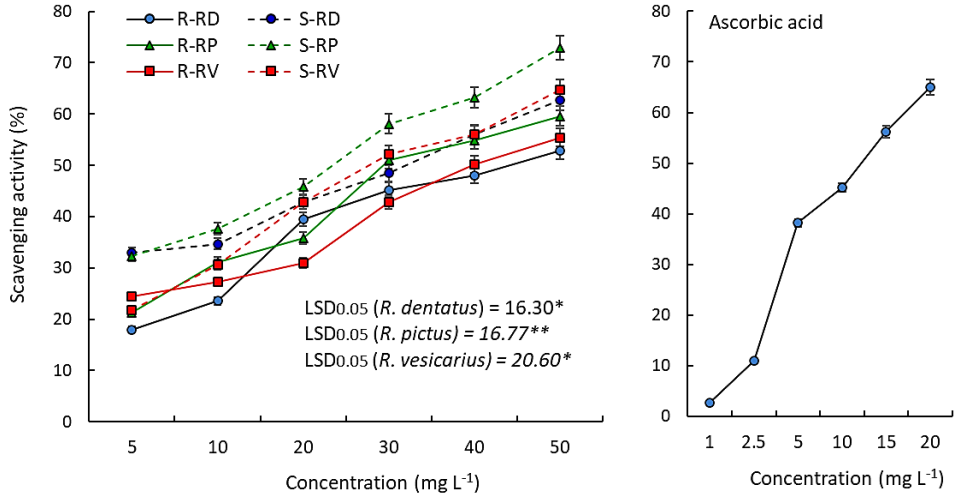

Figure 2. Scavenging activity percentage of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) by MeOH extract of *Rumex* species and ascorbic acid as standard. *values are means of triplicate ± standard error.

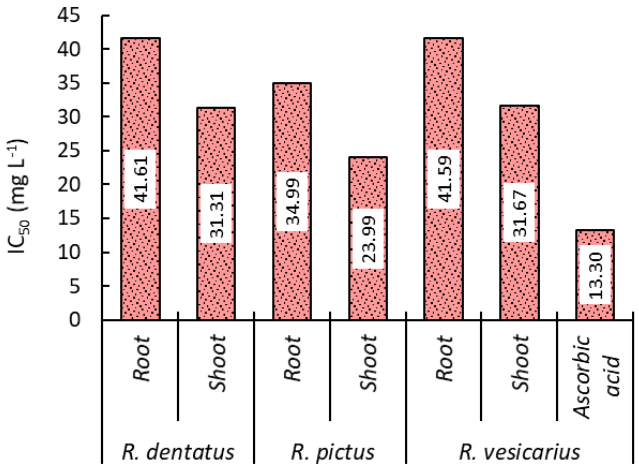

Figure 3. IC_{50} values of the different studied *Rumex* spp. and ascorbic acid (standard).

3.3. Antibacterial activity of MeOH extract of Rumex species.

In the agar disc diffusion assay, the antibacterial efficiency of *Rumex* species was assessed in vitro using ten different pathogenic strains. The root and shoot methanolic extract of the *Rumex* species had significant anti-microbial inhibitory activity on the growth of the Gram-positive and negative bacteria tested. Generally, Gram-negative bacteria were more sensitive to MeOH extract than Gram-positive bacteria, except *Bacillus subtilis* (Table 3). The *Escherichia coli* and *Bacillus subtilis* were the most affected bacterial strain, while *Pseudomonas aeruginosa* and *Bacillus cereus* were the most resistant to the MeOH of *Rumex* species (Tables 2 &3). According to their sensitivity, the organisms tested can be sequenced as follows: *E. coli* < K. pneumoniae < *S. typhi* < *P. aeruginosa* for Gram-negative bacteria and *B. subtilis* < *S. pneumoniae* < *L. monocytoyenes* < *S. epidermis* < *S. aureus* < *B. cereus* for Gram-positive bacteria. In addition, it was noted that the antibacterial performance of *R. dentatus* root and *R. vesicarius* shoot MeOH extract are 100% broad spectrum against Gram-negative bacteria. Whereas the shoot of *R. dentatus* and *R. pictus* MeOH extract against Gram-positive

bacteria is 83.3% broad spectrum each. Variant activities against the microorganisms test were showed by the antibiotics, ampicillin, cefotaxime and tetracycline. While *E. coli* was the most sensitive (30 mm) to cefotaxime, and *L. monocytoyenes* and *S. aureus* was the most susceptible (25 and 30 mm, respectively) to ampicillin (Tables 2 & 3).

From the obtained results, it could be concluded that the activity of the major compounds in the MeOH of *Rumex* species could be responsible for the observed potent anti-microbial activity, antioxidant activity, antitumor activity, laxative agents and anti-melanogenic activities [16,20,49-51]. Previous findings show that many wild plants against several bacterial and fungal strains were anti-microbial agents [52]. Alkaloids are known to have anti-microbial and anti-inflammatory effects [53,54]. David *et al*. [55] reported that flavonoids are beneficial for human health. They possess biological and pharmacological activities such as antioxidant and anti-microbial activity [53]. Laouini *et al*. [56] documented that polyphenols can display antibacterial effects against several pathogenic bacteria and antioxidant, antiallergic, anti-inflammatory, and anticancer agents.

Table 2. The antibacterial activities represented by the inhibition zone diameter (mm) of the extracted MeOH from *Rumex* spp. and standard antibiotics.

Plant MeOH extract (10 mg L^{-1})		**Gram-negative bacteria**			
Plant species	**Plant parts**	***Escherichia coli***	***Klebsiella pneumoniae***	***Pseudomonas aeruginosa***	***Salmonella typhi***
R. dentatus	Root	15	13	10	7
	Shoot	15	7	NA	NA
R. pictus	Root	10	12	NA	7
	Shoot	11	6	NA	12
R. vesicarius	Root	18	10	NA	8
	Shoot	11	6	9	12
Standard antibiotic (10 mg L^{-1})					
Ampicillin		20	5	NA	NA
Cefotaxime		30	20	10	10
Tetracycline		20	20	NA	10

Values are average (n = 3), NA: Not active.

Table 3. The anti-microbial activities represented by the inhibition zone diameter (mm) of the extracted MeOH from *Rumex* spp. and standard antibiotics.

Plant MeOH extract (10 mg L^{-1})		**Gram-positive bacteria**					
Plant species	**Plant parts**	***Bacillus cereus***	***Bacillus subtilis***	***Listeria monocytoyenes***	***Staphylococcus aureus***	***Staphylococcus epidermidis***	***Streptococcus pneumoniae***
R. dentatus	Root	9	12	6	NA	NA	15
	Shoot	NA	12	6	6	6	7.5
R. pictus	Root	NA	7	6	NA	NA	7
	Shoot	NA	29	8	10	6	8
R. vesicarius	Root	NA	11	6	NA	NA	6
	Shoot	NA	23	8	NA	6	6
Standard antibiotic (10 mg L^{-1})							
Ampicillin		5	20	25	30	10	10
Cefotaxime		10	5	20	22	20	11
Tetracycline		10	23	20	20	20	15

Values are average (n = 3), NA: Not active.

4. Conclusions

The phytochemical analysis of *Rumex* species growing naturally in three heterogeneous habitats in Egypt (cultivated land, coastal and inland desert) disclosed that MeOH extracts of these species are richer in bioactive compounds such as tannins and phenolics composition.

There was a comparable difference among *Rumex* species in a quantity of chemical composition. Whereas no significant variation was observed based on habitat, predominantly coastal and inland desert samples. So the variation is weakly correlated to habitat. Moreover, the MeOH extract inhibited a set of microbes and showed considerable antioxidant activity. Therefore, the three *Rumex* species are used in food preservation due to their antioxidant and anti-microbial activities. Further research is, however, recommended for characterizing authentic materials for the assessment of biosafety and efficiency as field scale of the major compounds.

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Conflicts of Interest

The authors declare no conflict of interest.

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