International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 3, Issue 2, 2011

Research Article

EVALUATION OF ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF DIFFERENT PLANT PARTS OF *RUMEX VESICARIUS L.* (POLYGONACEAE)

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Received: 05 Dec 2010, Revised and Accepted: 08 Jan 2011

ABSTRACT

The main aim of this research work is to evaluate antibacterial and antioxidant activities of different plant parts of *Rumex vesicarius L.*. Different extracts of different organs of *Rumex vesicarius L.* (Leaves, Stems, Roots, Flowers, Whole plant parts and Fruits), were screened for their antibacterial activity against six human pathogenic bacterial isolates by disk diffusion assay. The pattern of inhibition, activity index and proportion index showed highly significant variations according to variations of solvents used for extraction; plant parts used and tested bacterial isolates. Ether extract of roots was found to be the most effective against *Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus* and *Streptococcus pyogenes* (inhibition zones=26.500, 22.000, 41.5000 and 21.500 mm respectively), methanol extract of roots was found to be the most effective against *Streptococcus pneumoniae* (inhibition zone =18.000 mm) and ethanol extract of flowers was found to be the most effective one against *Escherichia coli* (inhibition zone =15.875 mm). Antioxidant activity was determined spectrophotometrically using total antioxidant activity and DPPH scavenging activity methods. Stems were found to have the highest total antioxidant capacity (17.458 GA equivalents "ppm"). Regarding DPPH scavenging activity, fruits were found to be the most effective plant parts (IC_{50} =0.731 mg/ml). Preliminary phytochemical screening on crude extracts and chemical examination of successive extractives solvents of different plant parts showed variations in the presence and amount of active ingredients under investigation within different extracts of different plant parts. Total phenolics were estimated, fruits extract was found to be the richest one in this regard (15.633 mg GAE_s/g F.W.). Total anthraquinones were estimated, the highest amount of anthraquinones was found to be in roots extract (352.941 µg/g F.W.). Total flavonoids were estimated, fruits extract was found to be the highest containing one in this regard (25.995 µg/g F.W.). HPLC analysis of flavonoids was carried out using Quercetin as standard, whole plant parts extract was found to contain the highest amount of Quercetin (82.452 µg/g D.W.). HPLC analysis of anthraquinones was carried out using Emodin as standard, leaves extract was found to contain the highest amount of Emodin (16.937 µg/g D.W.).

Keywords: *Rumex vesicarius* L., Antibacterial activity, Antioxidant activity, Phenolics, Anthraquinones, Flavonoids.

INTRODUCTION

Rumex vesicarius L. is a wild edible plant used as a sorrel and collected in spring time and eaten fresh [1], or cooked [2]. *Rumex vesicarius* L. has many important medicinal uses such as 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. The plant also used as cooling, laxative, stomachic, tonic, analgesic, appetizer, diuretic, astringent, purgative, antispasmodic and antibacterial agents. The roasted seeds were eaten for cure of dysentery. Finally, the plant can be used also to reduce biliary disorders and control cholesterol levels [1-6].

The medicinal importance of this plant is a reflection to its chemical composition since the plant contains many bioactive substances such as flavonoids (vitexin, isovitexin, orientin and isorientin). The plant also rich in anthraquinones particularly in roots (emodin and chrysophanol). The plant also contains carotenoids, vitamins (especially vitamin C), proteins, lipids and organic acids. This plant is a good source of minerals "K, Na, Ca, Mg, Fe, Mn, Cu" [7-10].

The previously mentioned bioactive phytochemicals (such as polyphenols, flavonoids, carotenoids, tocopherols and ascorbic acid) have a role as antioxidant and detoxifying agents. The intake of dietary antioxidant phytochemicals like carotenodis, phenolic compounds and flavonoids will lead to the protection against non-communicable diseases in human beings "cancer, cardiovascular diseases and cataract" [11-12].

Plants containing flavonoids and anthraquinones (such as quercetin and emodin) are good antibacterial agents against many human pathogenic bacteria such as *Escherichia coli, Streptococcus sp, Staplylococcus aureus* and *Pseudomonas aeruginosa* [13-16]. The previously mentioned bacterial strains are causative agents of many dangerous diseases such as vomiting, diarrhrea, urinary infections, gastroenteritis "*Escherichia coli*", ears and eye diseases may also be caused by bacteria "*Pseudomonas*", infections around nose and spreading over the face, piles, carbuncles may be also caused by bacteria "*Streptococci*", bacteria also considered to be the major cause of impetigo "*Staphylococcus*", urinary tract infections may be also caused by bacteria "*Klebsiella*" [17-18].

From the previously mentioned, there were a need to evaluate antibacterial and antioxidant activities of such an edible plant rich in bioactive substances specially flavonoids and anthraquinones.

Therefore this study was aimed to evaluate antibacterial and antioxidant activities of different plant parts of *Rumex vesicarius L.* and to investigate these plant parts chemically to determine bioactive agents that may be responsible for these biological activities (antibacterial and antioxidant activities).

MATERIALS AND METHODS

Plant materials

Rumex vesicarius L. samples were collected during 2009 and 2010 at the flowering stage (April) and ripening fruiting stage (August) from 60 km away from Ain Sokhna, Quatamia- Ain Sokhna desert road, Egypt. Plant specimens were botanically identified and authenticated by comparing with herbarium specimens, and the identified plant specimen was available in the plant herbarium of Botany and Microbiology Department, Faculty of science, Helwan University, Helwan, Egypt (Number : 1057). All experimental studies on the plant were carried out in Prof. Dr. / Hisham Afifi Lab., Plant Physiology Unit, Botany Department and Central Services Lab., National Research Centre, Giza, Egypt.

Tested microorganisms

Antibacterial activity of different extracts of different plant parts of *Rumex vesicarius* L. was investigated against six human pathogenic bacterial isolates, obtained from Clinical Pathology Department, Faculty of Medicine (Kasr El- Eini) Cairo University, Egypt. These included three gram-negative bacteria including *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Klebsiella pneumoniae* (ATCC 700603), three gram-positive bacteria including *Streptococcus pneumoniae* (ATCC 49619),

Staphylococcus aureus (ATCC 25923) and *Streptococcus pyogenes* (ATCC 19615). The purity and viability of cultures were checked by culturing on nutrient agar slants, incubated at 37ºC for 24 hours. Cultures were subcultured regularly (every week) and stored at 4ºC [18-19].

Inoculum preparation

A loopful of isolated colonies was inoculated into 4 ml peptone water and incubated at 37ºC for 4 hours. The turbidity of actively growing bacterial suspension was adjusted to match the turbidity standard of 0.5 MC farland units prepared by mixing 0.5 ml of 1.75% (w/v) barium chloride dehydrate with 99.5 ml 1% (v/v) sulphuric acid. This turbidity was equivalent to approximately 1-2X10^8 colony-forming units per milliliter (cfu/ml), the suspension was then used for further testing [19].

Antibacterial bioassay

The antibacterial bioassay was carried out following Disc Diffusion Method according to [19]. The concentration of each extract per disc equals 50 mg/disc in case of different plant parts and positive controls (synthetic drugs; Cefotaxime, Cephradine and "Amoxycilin, Flucloxacilin"), while in case of Quercetin and Emodin (natural compounds used as positive controls) different lesser concentrations were used (12.5, 50, 100 µg/disc). Negative controls were petroleum ether, ether, chloroform, methanol, ethanol, water and empty discs. The diameter of inhibition zone (measured in mm) is indicated by clear area in the Petri dish which was devoid of bacterial cells growth was measured. Each Petri dish contains four centered disks, r value of each disk=5 mm, one layer, Whattman number 1 filter paper.

Determination of activity and proportion indexes

Calculations were carried out following the methods of [20-21].

Antioxidant bioassay

Total antioxidant activity was performed using phosphomolybdenum reagent solution method of [22] and adopted by [23]. The antioxidant capacity was expressed as Gallic Acid Equivalent (GAE) by using the standard Gallic acid graph.

DPPH (1.1 diphenyl-2 picryl hydrazyl) scavenging activity was carried out by using the method of [24].

Preliminary phytochemical screening and Chemical examination of successive extractives_solvents (according to increasing in polarity gradients):

Flavonodis; Anthraquinones; Tannins; Alkaloids; Saponins; Carbohydrates and/or glycosides; Irodoids; Coumarins; Chlorides and Sulphates; Sterols and/or Triterpenes; Cardiac glycosides and sublimable substances were investigated for their presence/amount within different plant parts [25-37].

Assay for total phenolics

Total phenolics were estimated following the method of [24] involving Folin–Ciocalteu reagent and Gallic acid as standard. 1 ml of each extract of different plant parts contains 66.7 mg F.W.. Concentrations of phenolic compounds were calculated according to the following equation that was obtained form the standard Gallic acid graph.

Absorbance = 0.0167 Gallic acid (ug) + 0.017 (R^2: 0.99)

Assay for total flavonoids

Total flavonoids were determined using the method of [24]. 1ml of each extract of different plant parts contains 66.7 mg F.W.. Concentration of flavonoid contents were calculated according to the following equation that was obtained from the standard Quercetin graph:

Absorbance = 0.0228 Quercetin (ug) – 0.0045 (R^2: 0.9979)

Assay for total anthraquinones

Total anthraquinones were estimated using the method of (38). 1 ml of each extract of different plant parts contains 66.7 mg F.W.), using Emodin as standard.

HPLC analysis of flavonoids

Flavonodis were extracted according to [39], using HPLC-grade chemicals. HPLC analysis was carried out following the method of [40], using Quercetin (Sigma) as standard, with some modifications to fit conditions of Central Services Lab, National Research Centre, Egypt, as following: Filtration through membrane filter (0.4µm); The filtrate was subjected to separation by HPLC instruments under the following conditions (conditions in case of standard were the same with that used for all samples): Mobile phase, acetonitrile(86%): methanol (100%), 75:25 (v/v); Buffer 14% (Pot. Dihydrogen Phosphate: Phosphoric acid , 2:1 v/v); Flow rate: 1 ml/minute; Agilent 1100 series (Waldborn, Germany); Quaternary pump (G1311A); Degasser (G1322A): Thermostated Autosampler (G1329A); Variable wave lengths detector (G1314A); Column Zorabax 3005 B C_{18} column"25X4.6 mm, 5µ"(Agilent Technologies, USA). Concentration of Quercetin in each sample = 6 mg/ml, while concentration of different plant parts in each sample = 50 mg/ml. Injection volume 20 µL. Wave length was adjusted at 370 nm for separation of different compounds.

HPLC analysis of anthraquinones

Anthraquinones were extracted according to [41], using HPLC-grade chemicals for extraction. HPLC analysis was carried out following [42], using Emodin (Aldrich) as standard, with some modifications to fit conditions of Central Services Lab, National Research Centre, Egypt, as mentioned before with little changes; Concentration of Emodin in each sample = 0.1 mg/ml, while concentration of different plant parts in each sample = 100 mg/ml. Wave length was adjusted at 440 nm, using fluorescence detector for separation of different compounds.

Statistical analysis

Statistical analysis of all results was done using Fisher analysis of variance methodology. A least significant difference test was applied at 5% and 1% probability level to determine differences among treatment means [43]. The MSTAT computerized package program was subjected to the regular statistical analysis of variance [44], using two designs -1- Anova-1 completely randomized design (CRD) -2- Factorial implemented in completely randomized design. Each reading = mean of three replicates ± SD.

RESULTS AND DISCUSSION

Antibacterial activity studies of successive extractives solvents (petroleum ether, ether, chloroform, methanol and ethanol) of different plant parts of *Rumex vesicarius* L. (Tables: 1-6 and Figures 1-3) revealed that, there were highly significant variations (at 5% and 1% levels) within antibacterial activities of different extracts of different plant parts.

It was found that, ether extract of roots was the most effective against *Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus* and *Streptococcus pyogenes* (inhibition zones = 26.500, 22.000, 41.500 and 21.500 mm, activity indexes = 1.981, 0.636, 1.456 and 0.688 respectively); While methanol extract of roots was found to be the most effective one against *Streptococcus pneumoniae* (inhibition zone= 18.000 mm, activity index = 0.328) and ethanol extract of flowers was found to be the most effective one against *Escherichia coli* (inhibition zone = 15.875 mm, activity index= 0.508).

The proportion index of antibacterial activity of successive extractives solvents of different plant parts of *Rumex vesicarius* L. on pathogenic bacterial isolates under investigation reached its highest value (1) using - a– Ether extracts of leaves, stems and whole plant parts - b- ethanol extracts of leaves and fruits -c- Ethanol extracts of roots and fruits.

The positive controls in these experiments were Quercetin and Emodin (natural products) and it was found that, Quercetin is a potent antibacterial agent, while Emodin has lesser effect at the used concentrations. In addition to three synthetic drugs, Cefotaxime was the most effective one, followed by Amoxycilin, Flucloxacilin, while Cephradine was the least effective one.

These results of antibacterial activity studies were parallel to findings of [45], who found that, aqueous, methanol and petroleum ether extracts of *Rumex vesicarius* L. leaves have variable effects against both gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), while (3) found that, chloroformic extract of *Rumex vesicarius* L. (whole plant parts) has its effect against *Bacillus subtilis,* but it has no effect against *Escherichia coli* and *Staphylococcus aureus*, while in our results, the chloroformic extract of whole plant parts has affected them, this may be related to variation on the concentration used (100 mg/ml "0.1 ml/cup" against 50 mg/disc in our studies), or may be due to other factors such as, locality of the plant (Sudan against Egypt in our studies).

Several previous experiments on different plant parts of different species of *Rumex* confirm that, they were potent antibacterial agents against both gram-positive and gram-negative bacteria [46-49].

Table 1: Antibacterial activity of petroleum ether extract of different plant parts of *Rumex vesicarius* L. on pathogenic bacterial isolates under investigation.

Pathogenic bacterial isolates	Plant parts					
	Whole plant parts	Leaves	Stems	Flowers	Roots	Fruits
1	0.000	0.000	10.625± 0.543	0.000	0.000	0.000
2	2.125± 0.543	8.500± 0.543	0.000	0.000	0.000	0.000
3	4.875± 0.543	5.750± 0.543	0.000	3.625± 0.543	0.000	0.000
4	2.625± 0.543	0.000	0.000	0.750± 0.543	3.500± 0.543	6.000±0.543
5	0.000	4.875± 0.543	0.000	0.000	4.000± 0.543	4.500±0.543
6	0.000	3.000± 0.543	0.000	3.500± 0.543	4.500± 0.543	0.000
L.S.D. (0.05)a,b,ab respectively	0.362, 0.362, 0.887					
L.S.D. (0.01)a,b,ab respectively	0.482, 0.482, 1.180					

L.S.D.(0.05,0.01) a,b,ab respectively= Least Significant Differences at 0.05 and 0.01 levels, a- plant parts, b- types of bacteria, ab – interaction between the two factors. Each reading "inhibition zone in mm" = mean of triplicates ± SD.

1-Escherichia coli (ATCC 25922) *2- Pseudomonas aeruginosa* (ATCC 27853) *3- Klebsiella pneumoniae* (ATCC 700603) *4- Streptococcus pneumoniae* (ATCC 49619) *5- Staphylococcus aureus* (ATCC 25923) 6- *Streptococcus pyogenes* (ATCC 19615).

Table 2: Antibacterial activity of ether extract of different plant parts of *Rumex vesicarius* L. on pathogenic bacterial isolates under investigation.

Pathogenic bacterial isolates	Plant parts					
	Whole plant parts	Leaves	Stems	Flowers	Roots	Fruits
1	7.375± 0.415	4.750± 0.415	6.750± 0.415	0.000	0.000	0.000
2	9.500± 0.415	6.375± 0.415	6.875± 0.415	0.000	**26.500± 0.415**	0.000
3	8.750± 0.415	7.000± 0.415	7.250± 0.415	0.000	**22.000± 0.415**	0.000
4	6.250± 0.415	4.375± 0.415	6.125± 0.415	16.125± 0.415	14.500± 0.415	0.000
5	11.000± 0.415	9.250± 0.415	10.250± 0.415	15.375± 0.415	**41.500± 0.415**	0.000
6	8.000± 0.415	5.250± 0.415	6.250± 0.415	0.000	**21.500± 0.415**	0.000
L.S.D.(0.05) a,b,ab respectively	0.276, 0.276, 0.677					
L.S.D. (0.01) a,b,ab respectively	0.370, 0.370, 0.901					

Table 3: Antibacterial activity of chloroform extract of different plant parts of *Rumex vesicarius* L. on pathogenic bacterial isolates under investigation.

Pathogenic bacterial isolates	Plant parts					
	Whole plant parts	Leaves	Stems	Flowers	Roots	Fruits
1	3.250± 0.346	4.625± 0.346	0.000	0.000	0.000	0.000
2	0.000	0.000	0.000	0.000	0.000	0.000
3	0.000	0.000	0.000	0.000	0.750± 0.346	0.000
4	1.250± 0.346	0.000	6.000± 0.346	6.000± 0.346	6.500± 0.346	0.000
5	0.875± 0.346	0.000	3.875± 0.346	5.250± 0.346	1.500± 0.346	0.000
6	5.750± 0.346	1.500± 0.346	6.000± 0.346	5.375± 0.346	5.750± 0.346	0.000
L.S.D.(0.05) a,b,ab respectively	0.231, 0.231, 0.566					
L.S.D. (0.01) a,b,ab respectively	0.307, 0.307, 0.752					

Table 4: Antibacterial activity of methanol extract of different plant parts of *Rumex vesicarius* L. on pathogenic bacterial isolates under investigation

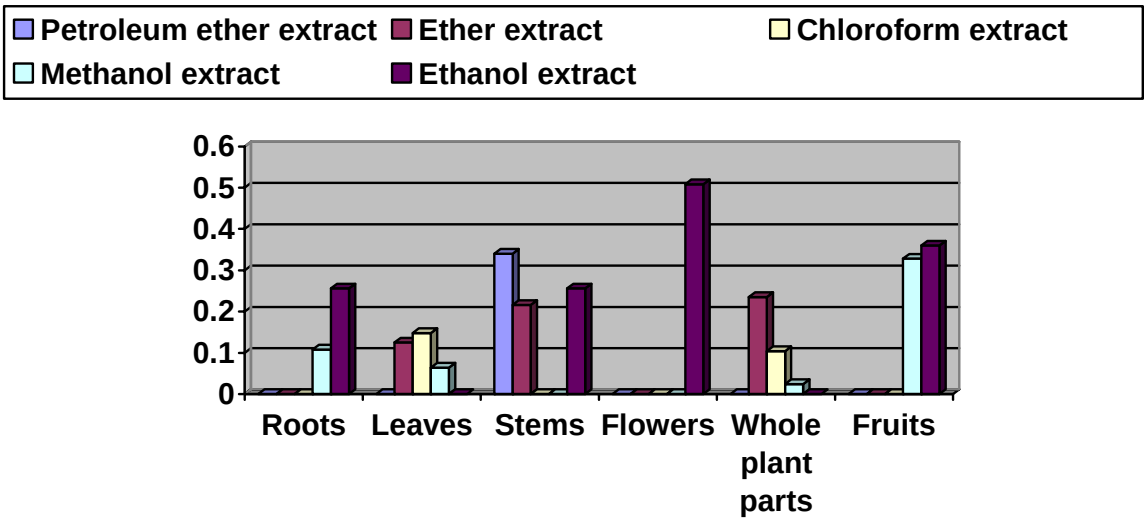
Pathogenic bacterial isolates	Plant parts					
	Whole plant	Leaves	Stems	Flowers	Roots	Fruits
1	0.750± 0.404	2.000±0.404	0.000	0.000	3.375±0.404	10.250±0.404
2	0.000	6.750±0.404	4.625±0.404	0.000	0.000	9.625±0.404
3	8.125±0.404	7.250±0.404	7.125±0.404	3.750±0.404	6.500±0.404	9.375±0.404
4	7.000±0.404	5.625±0.404	5.000±0.404	8.250±0.404	**18.000±0.404**	10.000±0.404
5	9.625±0.404	11.750±0.404	8.625±0.404	5.000±0.404	7.000±0.404	10.000±0.404
6	10.875±0.404	6.875±0.404	7.625±0.404	12.125±0.404	14.500±0.404	18.125±0.404
L.S.D.(0.05)a,b,ab respectively	0.269, 0.269, 0.659					
L.S.D. (0.01) a,b,ab respectively	0.358, 0.358, 0.877					

Table 5: Antibacterial activity of ethanol extract of different plant parts of *Rumex vesicarius* L. on pathogenic bacterial isolates under investigation

Pathogenic bacterial isolates	Plant parts					
	Whole plant	Leaves	Stems	Flowers	Roots	Fruits
1	0.000	0.000	8.000±0.281	**15.875±0.281**	8.000±0.281	11.250±0.281
2	0.000	0.000	10.375±0.281	11.375±0.281	8.000±0.281	9.500±0.281
3	4.500±0.281	0.000	16.250±0.281	11.125±0.281	9.000±0.281	11.250±0.281
4	6.000±0.281	8.125±0.281	8.375±0.281	13.125±0.281	10.250±0.281	12.625±0.281
5	5.625±0.281	8.000±0.281	9.500±0.281	15.875±0.281	9.250±0.281	7.000±0.281
6	6.000±0.281	8.000±0.281	0.000	0.000	4.375±0.281	9.750±0.281
L.S.D.(0.05) a,b,ab respectively	0.187, 0.187, 0.459					
L.S.D.(0.01) a,b,ab respectively	0.249, 0.249, 0.610					

Table 6: Antibacterial activity of different antibacterial agents (Positive controls) on pathogenic bacterial isolates under investigation.

Bacteria	Cephradine (50 mg/disc)	Amoxycillin, Flucloxacillin (50 mg/disc)	Cephotaxime (50 mg/disc)	Quercetin			Emodin		
				100 µg/disc	50 µg/disc	12.5 µg/disc	100 µg/disc	50 µg/disc	12.5 µg/disc
1	0.000	0.000	**31.250 ±0.539**	11.500 ±0.539	8.250 ±0.539	6.375 ±0.539	0.000	0.000	0.000
2	0.000	0.000	**13.250 ±0.539**	0.000	0.000	0.000	0.000	13.375 ±0.539	0.000
3	0.000	11.375 ±0.539	**34.625 ±0.539**	1.125 ±0.539	5.250 ±0.539	0.000	0.000	0.000	0.000
4	8.250 ±0.539	**54.875 ±0.539**	28.250 ±0.539	8.875 ±0.539	5.750 ±0.539	0.000	0.000	0.000	0.000
5	11.250 ±0.539	11.125 ±0.539	**28.500 ±0.539**	8.500 ±0.539	21.000 ±0.539	14.000 ±0.539	0.000	0.000	2.250 ±0.539
6	0.000	**31.250 ±0.539**	6.000 ±0.539	0.000	25.625 ±0.539	14.625 ±0.539	0.000	0.000	0.000
L.S.D.(0.05) a,b,ab respectively	0.356, 0.291, 0.872								
L.S.D. (0.01) a,b,ab respectively	0.471, 0.384, 1.153								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Chloroform extract
Methanol extract
Ethanol extract
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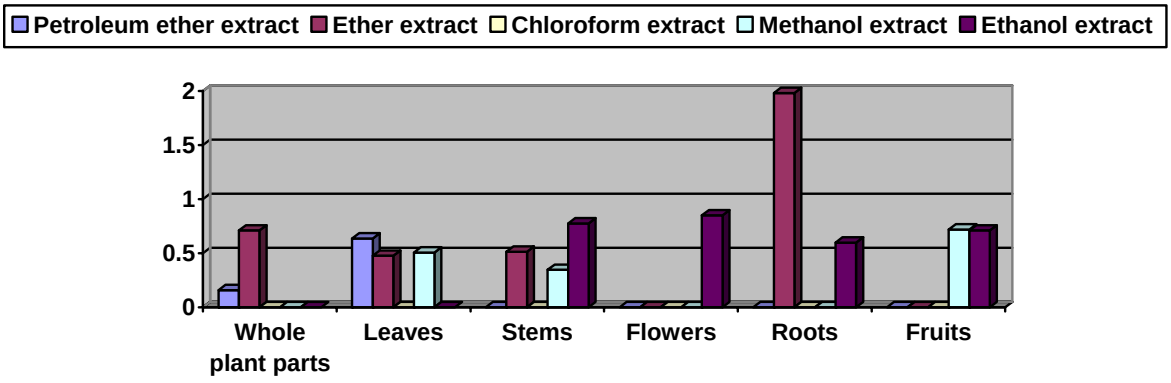
0.3
0.2
0.1
0
Roots
Leaves
Stems
Flowers
Whole plant parts
Fruits

(a)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Ether extract
Chloroform extract
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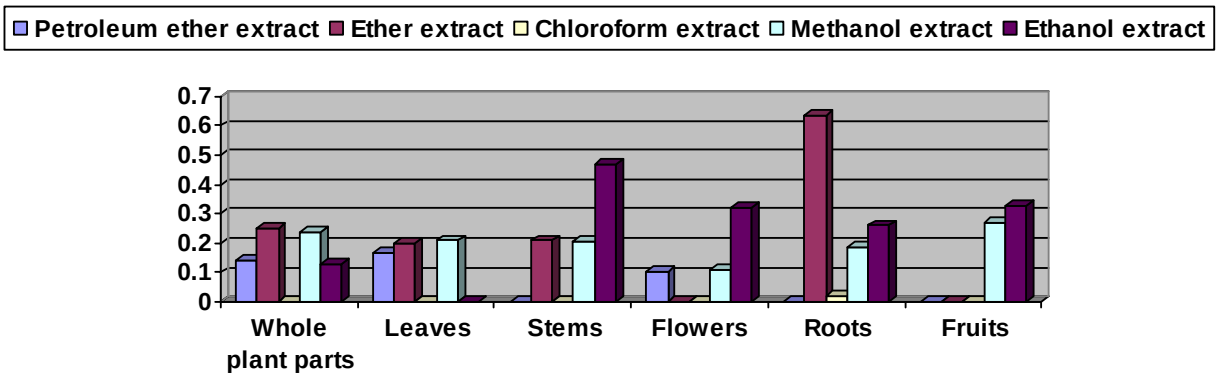
Leaves
Stems
Flowers
Roots
Fruits

(b)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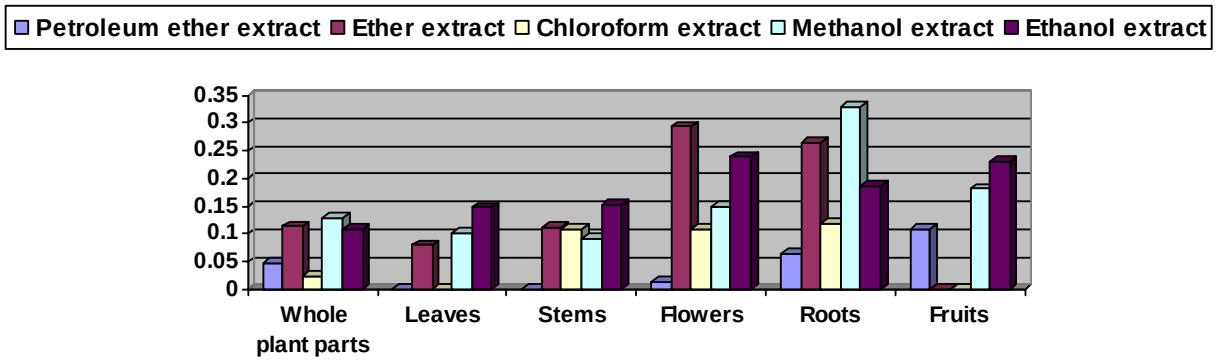
Whole plant parts
Leaves
Stems
Flowers
Roots
Fruits

(c)



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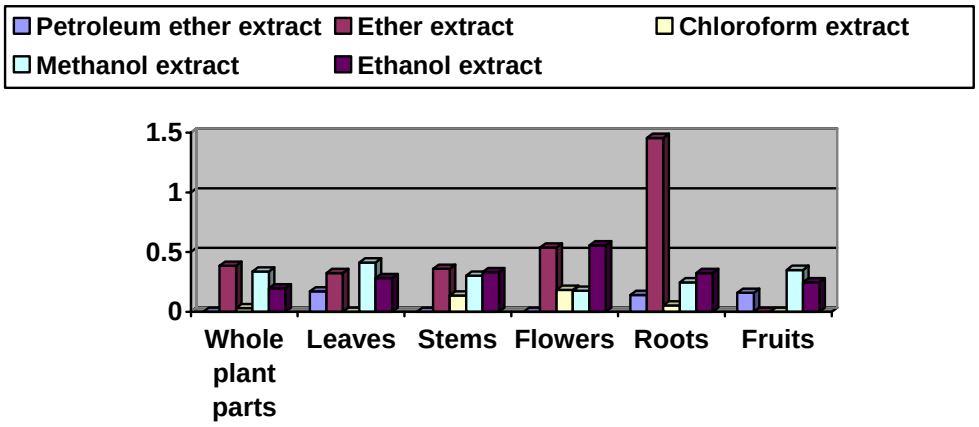

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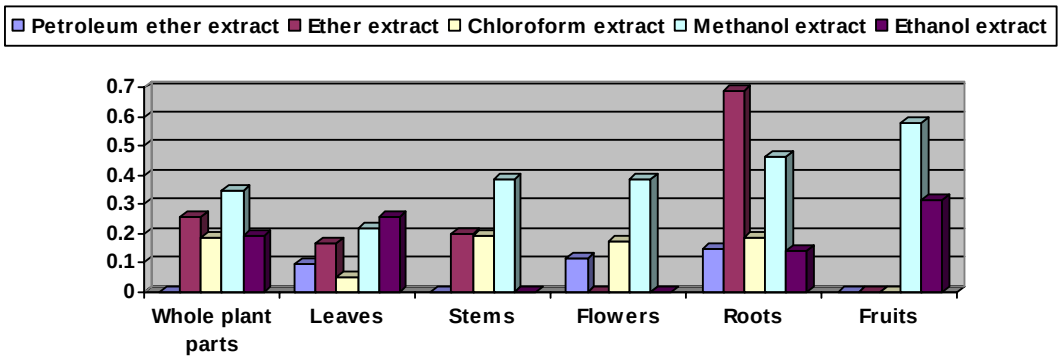

(f)

Fig. 1: Antibacterial activity index of successive extractives solvents of different plant parts of *Rumex vesicarius* L. on different bacterial isolates.

(a) *Escherichia coli* (ATCC 25922) (b) *Pseudomonas aeruginosa* (ATCC 27853) (c) *Klebsiella pneumoniae* (ATCC 700603). (d) *Streptococcus pneumoniae* (ATCC 49619) (e) *Staphylococcus aureus* (ATCC 25923) (f) *Streptococcus pyogenes* (ATCC 19615).

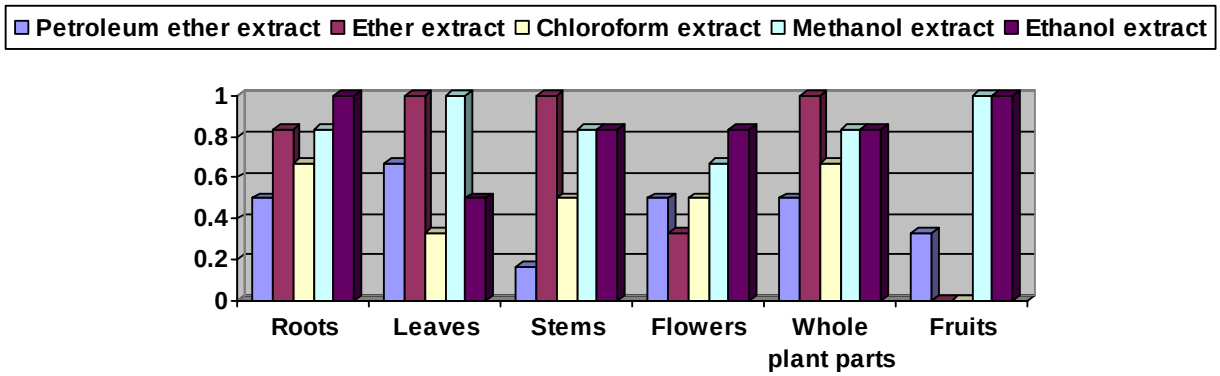

Fig. 2: Proportion index of antibacterial activity of successive extractives solvents of different plant parts of *Rumex vesicarius* L. on pathogenic bacterial isolates under investigation.

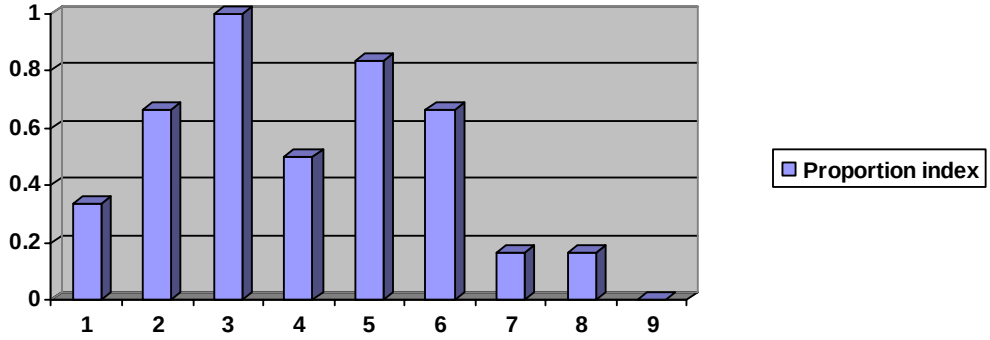

Fig. 3: Proportion index of antibacterial activity of different antibacterial agents " Positive controls" 1, 2, 3 = Cephradine, (Amoxycillin, Flucloxacillin) and Cephotaxime " 50 mg/disc " respectively, 4-6 = Quercetin at concentrations of 12.5, 50, 100 µg/disc respectively and 7-9 Emodin at concentrations of 12.5, 50, 100 µg/disc respectively.

Regarding antioxidant activity studies (Table:7), there were highly significant variations (at 5% and 1% levels) within different plant parts using total antioxidant activity method, while using DPPH scavenging activity method there were significant variations (at 5% level only) within different plant parts.

Total antioxidant activity (Table:7) was estimated, it was found that, stems extract was found to have the highest amount of antioxidants (17.458 GAEs "ppm"), followed by fruits extract (15.890 GAE_s "ppm"), while roots extract was found to have the least amount (12.363 GAEs "ppm").

DPPH scavenging activity of different plant parts (Table:7) revealed that, the least IC_{50} "the highest the effectiveness" was obtained using fruits (IC_{50} = 0.731 mg/ml), followed by leaves (IC_{50}= 1.120 mg/ml), while flowers were the least effective plant part used (IC_{50}= 1.593 mg/ml). The positive controls in these experiments were Quercetin and Emodin, it was found that Quercetin is a potent antioxidant agent (IC_{50} = 0.801 mg/ml), while Emodin has no effect at the used concentrations.

These results of antioxidant activity of different plant parts of *Rumex vesicarius* L. were in coincidence with other results on closely related species of *Rumex,* since the previous studies found that, different extracts of roots of *R. japonicus* and *R. patientia*, leaves of *R. Pulcher* and *R. acetosella* and whole plant parts of *R. dentatus*, were considered to be antioxidant agents, the chemical compositions of some of these extracts were studied, with special reference to studies of flavonoids and anthraquinones in case of roots of *R. patientia*, since there were a similarity between their results and the obtained results in this study [50-54].

Concerning preliminary phytochemical screening results, it was found that, all plant parts were rich in flavonoids, anthraquinones, alkaloids, tannins, sterols and / or triterpenodis, carbohydrates and/or glycosides, chlorides and sulphates and sublimable substances. There were variations in the presence and/or amount of these active ingredients within different plant parts.

The obtained results of chemical examinations of successive extractives solvents study (Table:8) showed a positive relationship between chemical compositions of successive extractives solvents and the obtained results of antibacterial activity studies. It was found that, ether extract of roots was rich in flavonoids and ethanolic extract of roots was rich in flavonoids and anthraquinones, these substances were known as antibacterial agents (such as Quercetin and Emodin), this may be the reason for the highly antibacterial activities obtained using these extracts. These results were in harmony with results of [11, 12, 14-16], since they found that, flavonoids and polyphenols were good antioxidant and antibacterial agents, since *Rumex vesicarius* L. is rich in polyphenols, so it can be found that, there were a positive relationship between chemical composition and the obtained biological activity results of this plant, so the presence or absence of flavonoids may be a determining factor of this biological activity.

Table 7: Antioxidant activity of different plant parts of *Rumex vesicarius* L. (Total antioxidant activity and DPPH scavenging activity methods).

Plant parts	Total antioxidant activity (Gallic acid equivalent "ppm")	DPPH scavenging activity (IC_{50})
Leaves	14. 553±0.630	1.120±0.260
Stems	**17.458±0.630**	1.198±0.260
Roots	**12.363±0.630**	1.375±0.260
Flowers	13.184±0.630	**1.593±0.260**
Whole plant parts	15.633±0.630	1.175±0.260
Fruits	15.890±0.630	**0.731**±0.260
		Quercetin (positive control) = 0.801±0.260
L.S.D.(0.05)	**0.461**	**0.163**
L.S.D.(0.01)	**0.646**	**0.226**

Table 8: Chemical examination of successive extractives solvents of different plant parts of *Rumex vesicarius* L.

Extracts of different plant parts	Sterols and / or Triterpenes	Tannins	Flavonoids	Alkaloids	Carbohydrates and /or Glycosides	ıntharaquinones	Saponins
Petroleum ether extr							
Roots	++	-	-	-	+	-	-
Stems	++	-	-	-	++	-	-
Flowers	++	-	-		+	-	-
				-			
Leaves	++	-	-	-	++	-	-
Whole plant parts	++	-	-	-	++	-	-
Fruits	++	-	-	-	++	-	-
Ether extract:							
Roots	-	-	++	-	+	-	-
Stems	-	-	++	-	++	-	-
Flowers	-	-	++	-	+	-	-
Leaves	-	-	++	-	++	-	-
Whole plant parts	-	-	++	-	++	-	-
Fruits	-	-	+	-	++	-	-
Chloroform extract:							
Roots	-	++	++	-	+	-	-
Stems	-	++	++	-	++	-	-
Flowers	-	++	++	-	+	-	-
Leaves	-	++	++	-	++	-	-
Whole plant parts	-	++	++	-	++	-	-
Fruits	-	+	+	-	++	-	-
Methanol extract							
Roots	-	-	++	++	+	++	-
Stems	-	-	++	++	++	-	-
Flowers	-	-	-	++	+	-	-
Leaves	-	++	++	++	++	-	-
Whole plant parts	-	++	++	++	++	-	-
Fruits	-	++	+	++	+	+	-
Ethanol extract							
Roots	-	-	++	++	+	++	-
Stems	-	-	++	++	++	+	-
Flowers	-	-	++	++	+	++	-
Leaves	-	-	++	++	++	+	-
Whole plant parts	-	-	++	++	++	++	-
Fruits	-	+	++	++	+	++	-

Regarding chemical compositions of different plant parts study "total phenolic contents, total flavonoid contents, Total anthraquinones, HPLC analysis of flavonoids and HPLC analysis of anthraquinones" (Table:9), there were highly significant variations (at 5% and 1% levels) in chemical compositions within different plant parts.

Concerning total phenolic contents studies (Table: 9), it was found that, all plant parts were rich in phenolics particularly fruits (15.633 mg GAE_s/g F.W.). These results also confirm the positive relationship between polyphenolics and antioxidant activity of *Rumex vesicarius* L. (16).

Total flavonoid contents (Table:9) reached its highest value in case of fruits extract (25.995 µg/g F.W.).

Total anthraquinones studies (Table:9) revealed that, all plant parts were rich in anthraquinones and roots extract was the highest containing plant parts in this regard (352.941 µg/g F.W.).

The obtained results of these studies on *Rumex vesicarius L.* were parallel to (1,7) they found that, *R. vesicarius L.* contains high amount of flavonoids glycosides (vitexin, isovitexin, orientin and isoorientin) and anthraquinones (emodin and chrysophanol). They also found that, roots were rich in anthraquinones, this confirm our results regarding total anthrquinones studies and antibacterial activity studies, since roots were also the most effective plant parts regarding antibacterial activity.

(8,9) found that, *Rumex vesicarius L.* is a good source of minerals, proteins, lipids, vitamins and organic acids, so this plant is a wild edible plant with high nutritional value, the obtained results; to great extents; complementary to the previously mentioned results of others, since the plant is not only an edible plant with highly nutritional values, but also a good antibacterial and antioxidant source rich in phenolics, flavonoids and anthraquinones.

HPLC analysis of flavonoids (Table:9)"using Quercetin as standard" revealed that, there were variations within different plant parts, whole plant parts extract was the highest containing one in this regard (82.452 µg/g D.W.), followed by fruits extract (48.264 µg/g D.W.), while roots extract was the least containing one (4.662 µg/g D.W.).

HPLC analysis of anthraquinones (Table:9) using Emodin as standard" revealed that, leaves extract was found to contain the highest amount of Emodin (16.937 µg/g D.W.), followed by fruits extract (12.128 µg/g D.W.), while whole plant parts extract was found to contain the least amount (8.094 µg/g D.W.). Meanwhile, other remaining plant parts were devoid of this compound.

It can be concluded from results of the present study that, different plant parts of *R. vesicarius* L. have variable antibacterial and antioxidant activities. These variations were closely related to variations in chemical compositions within different plant parts.

In the next studies, we will try to use cells and tissues cultures of *R. vesicarius* L. for the *in vitro* production of some bioactive substances that may be responsible for these biological activities (antibacterial and antioxidant activities) and we will try to increase their amounts compared to the plant.

Table 9: Total phenolics (mg GAE_s/g F.W.), total flavonoids (µg/g F.W.), total anthraquinones (µg/g F.W.), HPLC analysis of flavonoids (using Quercetin as standard "µg/g D.W.") and HPLC analysis of anthraquinones (using Emodin as standard "µg/g D.W.") of different plant parts of *Rumex vesicarius* L.

Plant parts	Total phenolics	Total flavonoids	Total anthraquinones	Quercetin	Emodin
Leaves	6.667±0.320	11.924±1.850	61.765±6.500	7.489±1.000	**16.937±0.100**
Stems	**5.102±0.320**	1.932±1.850	**36. 275±6.500**	12.625±1.000	0.000
Roots	14.360±0.320	**1.668±1.850**	**352. 941±6.500**	**4.662±1.000**	0.000
Flowers	14.261±0.320	14.753±1.850	184. 216±6.500	30.783±1.000	0.000
Whole plant parts	10.417±0.320	11.223±1.850	87. 255±6.500	**82.452±1.000**	**8.094±0.100**
Fruits	**15.633±0.320**	**25.995±1.850**	245.098±6.500	48.264±1.000	12.128±0.100
L.S.D (0.05)	**0.235**	**1.340**	**4.722**	**0.727**	**0.076**
L.S.D. (0.01)	**0.330**	**1.879**	**6.621**	**1.019**	**0.107**

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Abbreviations:

GAE : Gallic Acid Equivalent.
DPPH : 1.1-Diphenyl-2- picryl hydrazyl.
HPLC: High Performance Liquid Chromatography.
IC_{50}: Concentration that gives 50% inhibition.
ppm, µg/g, mg/g and mm: part per million, microgram/ gram, milligram/gram and millimeter respectively.
D.W. : Dry Weight.
F.W. : Fresh Weight.