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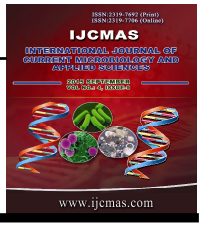

Original Research Article

In vitro screening of Phytochemical, Antibacterial and Antioxidant activities of *Rumex vesicarius* L.

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Keywords

Rumex vesicarius L., phyto-chemicals, antibacterial, antioxidant, DPPH

A B S T R A C T

The present investigation was deliberated to evaluate the phytochemical, antibacterial and antioxidant activities of ethnobotanical herb *Rumex vesicarius* L. The preliminary qualitative phytochemical screening of *Rumex vesicarius* L. revealed that the presence of bioactive components alkaloids, anthraquinones, carbohydrates, flavonoids, phenols, proteins, saponins, tannins and triterpenoids. The ethyl acetate extract of *Rumex vesicarius* L. exhibited significant antibacterial activity. The results of *in vitro* antioxidant activity of *R. vesicarius* L. revealed that the maximum percentage of DPPH inhibition was exhibited by ethyl acetate extract followed by distilled water extract and the lowest inhibition percentage was recorded by n-hexane extract at 1000µg concentration. The present investigation also supports the folkloric usage of the *Rumex vesicarius* L., which possesses several known and unknown bioactive compounds with huge biological activity.

Introduction

From the time immemorial plants have been used by human societies for medicinal and food purposes. Today also human food is plant based; nearly 12 crops, vegetables and greens contribute 85-90% of world's caloric intake (Maikhuri *et al.*, 2008). Green Leafy Vegetables (GLV) is the store house of vitamins like beta carotene, ascorbic acid, folic acid and riboflavin as well as minerals such as iron, calcium and phosphorus. They also contain an immense variety of bioactive-nonnutritive health promoting factors as antioxidants, phytochemicals, essential fatty acids and dietary fibre (FAO, 1988).

Rumex vesicarius L. (Polygonaceae) is a wild edible green eaten fresh (or) cooked and used in daily diet and commonly known in Arabic as "Humaidah of hummayd" and in English as "Bladder dock". It is distributed in many parts of Saudi Arabia and desert and semi-desert areas of North Africa, Asia and Australia. In Saudi Arabia, *R. vesicarius* L. is widely used as food, as a medicinal herb and as an antidote to scorpion stings (Al-Yahya *et al*., 1990). *R. vesicarius* L. is considered as a dietary complementary plant because this plant is a rich source of β-carotenes. The plant also contains carotenoids and vitamins

(particularly vitamin C), proteins, lipids and organic acids. The plant is also a good source of minerals such as K, Na, Ca, Mg, Fe, Mn and Cu (Al-Rumaih, 2002).

According to ethno medicinal uses, *Rumex vesicarius* L. used to treat tumors, hepatic diseases, bad digestion, constipation, calculus, heart troubles, pains and acts as aphrodisiac agent, the plant is also used as good cooling, laxative, analgesic, appetizer, diuretic, astringent, purgative antispasmodic and antibacterial agents (Nadkarni, 1954; Mossa *et al.*, 1987). It is also used to reduce biliary disorders and controls cholesterol levels (Mostafa *et al.*, 2011). Consequently, the present investigation was deliberated to evaluate the phytochemical, antibacterial and antioxidant activities of *Rumex vesicarius* L.

Materials and methods

Collection of plant

The fresh and healthy whole plants of *Rumex vesicarius* L. were collected from Al - Zulfi, Riyadh Province, Kingdom of Saudi Arabia (Figure 1).

Figure.1 Study plant: *Rumex vesicarius* L. (Green Leafy Vegetable – an edible plant)

Scientific classification

Class	:	Dicotyledons
Order	:	Polygonales
Family	:	Polygonaceae
Genus	:	*Rumex*
Species	:	*vesicarius* L.

Identification of plant

The collected plant were carefully examined and identified with the help of regional floras (Miller and Cope, 1996; Collenette, 1999; Mossa *et al*., 2000; Chaudhary, 2001) and further authenticated by expert taxonomist.

Preparation of plant extracts

The plants were cleaned, dried under shade, ground to a coarse powder and stored in an airtight container at 25 °C for further investigation. Plant extracts such as distilled water, ethyl acetate and n –hexane extracts of *Rumex vesicarius* L. (concentration 500 µg/ml) were prepared.

Qualitative phytochemical screening

Qualitative phytochemical analyses of plant extracts were done by following the method described by Harborne (1973) and Trease and Evans, (1983).

Evaluation of antibacterial efficacy

The antibacterial efficacy of distilled water, ethyl acetate and n –hexane extracts of *R. vesicarius* L. was evaluated by well agar method (Perez *et al*., 1990). The important human pathogens such as *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi* and *Staphylococcus aureus* were obtained from culture collections in Department of Medical Laboratories, College of Science Al-Zulfi, Majmaah University, Kingdom of Saudi Arabia.

The young bacterial inoculums was prepared and used during the research period. The fresh bacterial culture was spread on the nutrient agar medium (Himedia) by spread plate technique. One well of 5mm size made into the agar plates with the help of sterile

cork borer, the wells were loaded with 200µl (concentration 500 µg/ml) of distilled water, ethyl acetate and n- hexane extracts of *R. vesicarius* L. All the plates were incubated at 37ºC for 24 hours. After incubation, the plates were observed for formation of clear inhibition zone around the well indicated the presence of antibacterial activity. The zone of inhibition was calculated by measuring the diameters of the inhibition zone around the well. The standard antibiotics such as chloramphenicol, kanamycin, and tetracycline were used as positive control and the solvents such as distilled water, ethyl acetate and n – hexane were used as negative control. All the experiments were performed in triplicate sets.

Evaluation of antioxidant activity

The scavenging of 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) free radicals was used for measuring the antioxidant activity of the extracts, according to the method of Koleva *et al*. (2002). Different concentrations of solvent extracts and quercetin standard namely 125, 250, 500 and 1000 µg/ml were prepared in respective corresponding solvent. DPPH (150 µm in ethanol) was used as free radical, about 10 µl of each concentration of test sample solvent extract were mixed with 190 µl of DPPH in clean and labelled test tubes separately. Same procedure was followed with standard quercetin, and the standard test tubes set up were maintained separately. All the experiments were performed in triplicate sets.

After vortexing, the tubes with mixture were incubated at 37^0C in dark for 30 minutes. The decrease in absorbance of the test mixture (due to quenching of DPPH free radicals) was measured at 517nm using UV-Visible spectrophotometer, and the inhibition percentage was calculated. The scavenging activity of the extract against the stable DPPH was calculated by using the following formula:

$$\% \text{ of DPPH Inhibition} = \frac{\text{A control} - \text{A test}}{\text{A control}} \times 100$$

Where A control = Absorbance of control reaction set up.

(Containing all reagents except the test extract)

A Test = Absorbance of sample extracts set up.

Results and discussion

Medicinal plants are considered to be a chemical factory as it contains multitude of chemical compounds like alkaloids, carbohydrates, flavonoids, lipids, phenols, proteins, glycosides, saponins and oils (Hill, 1952).

The preliminary qualitative phytochemical screening of *Rumex vesicarius* L. revealed that the presence of bioactive components alkaloids, anthraquinones, carbohydrates, flavonoids, phenols, proteins, saponins, tannins and triterpenoids. However quinones were absent in tested three extracts. Flavonoids, phenols, proteins and tannins were detected in all the tested three extracts (Table 1).

These results were in agreement with previous findings of Alfawaz (2006), Panduraju *et al.* (2009), Amira *et al.* (2011), Hariprasad and Ramakrishnan **(**2011) and Husain Khan *et al*. (2014), as were found that the presence of alkaloids, carbohydrates, anthraquinones, protein, cardiac glycosides, saponins, glycosides, flavonoids, tannins, steroids, ascorbic acid, tocopherols, cysteine, glutamic acid, proline, terpenoids, phenylalanine and histidine in various extracts of *R. vesicarius* L.

The identified bioactive phytochemicals such as phenols and flavonoids are considered to be antimicrobial, antioxidant and anticancer agents (Rao, 2003; Alberto *et al.*, 2006; Matkowski, 2008; Stevic *et al*., 2010; Imran *et al.*, 2011; Husain Khan *et al*., 2014).

Table.1 Qualitative phytochemical analysis of extracts of *Rumex vesicarius* L**.**

S. No.	Phytocompounds tested	Distilled water extract	Ethyl acetate extract	n-Hexane extract
1	Alkaloids	-	+	-
2	Anthraquinones	-	+	-
3	Carbohydrates	+	+	-
4	Flavonoids	+	+	+
5	Phenols	+	+	+
6	Proteins	+	+	+
7	Quinones	-	-	-
8	Saponins	+	-	-
9	Tannins	+	+	+
10	Triterpenoids	+	+	-

(+) Presence, (-) absence

During the last decade, infectious diseases have played a significant role in the death of millions around the world. Screening of plants for antimicrobial agents has gained much importance because lately WHO is keenly interested in the development and utilization of medicinal plant resources in the traditional system of medicine. There has been a rising interest in the research for natural products from plants for the discovery of new antimicrobial and antioxidant agents.

Table 2 and Plate 1 illustrated the results of antibacterial activity of *Rumex vesicarius* L. extracts and the ethyl acetate extract had maximum antibacterial activity with zone of inhibition range 12.1 to 19.5 mm. The growth of *Staphylococcus aureus* was strongly inhibited by ethyl acetate extract.

The distilled water extract showed moderate antibacterial activity against tested pathogens. On the other hand n- hexane extract did not show any activity except *Klebsiella pneumoniae*. Table 3 and Plate 2 demonstrated the results of antibiotic sensitivity test on bacterial pathogens. The inhibition activity of the plant extracts were compared with standard antibiotics. Our results are agreement with the findings of Panduraju *et al.* (2009) who found that aqueous, methanol and petroleum ether extracts of *R. vesicarius* L. leaves had variable degree of inhibition against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*., Previously, Elegami *et al.* (2001) found that chloroform extract of *R. vesicarius* L. showed significant activity against *Bacillus subtilis* even though no activity against *Escherichia coli* and *Staphylococcus aureus*. Likewise Mostafa *et al.* (2011) also reported roots and flower extracts of *R. vesicarius* L. has considerable antibacterial activity.

Table.2 Antibacterial efficacy of *Rumex vesicarius* L. by well agar method

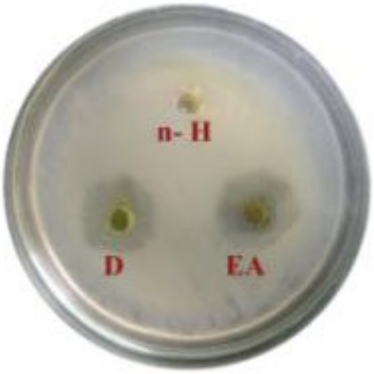
S. No.	Name of the bacterial pathogens	Zone of inhibition (Diameter in mm)		
		Distilled water extract	Ethyl acetate extract	n-Hexane extract
1	*Escherichia coli*	10.9 ± 0.6	12.1±1.1	-
2	*Klebsiella pneumoniae*	11.2 ± 1.0	16.1±0.5	7.5±0.7
3	*Salmonella typhi*	11.5 ± 0.4	16.2±0.3	-
4	*Staphylococcus aureus*	12.1 ± 0.4	19.5±0.8	-

Values are expressed as Mean ± Standard Deviation (n=3) - - no activity

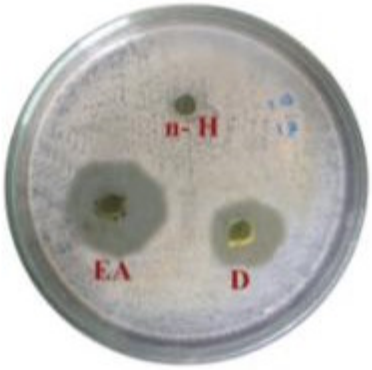
Plate.1 Antibacterial efficacy of *Rumex vesicarius* L. by well agar method

Escherichia coli

Klebsiella pneumoniae



Salmonella typhi



Staphylococcus aureus

D- Distilled water extract, EA - Ethyl acetate extract, n-H- n-Hexane extract

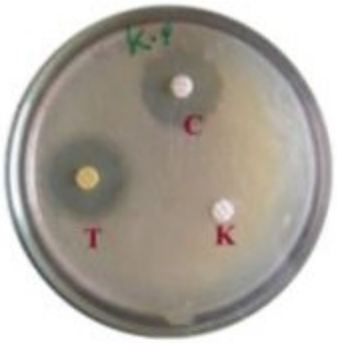
Table.3 Antibiotic sensitivity test on tested bacterial pathogens

S. No.	Name of the bacterial pathogens	Zone of inhibition (Diameter in mm)		
		Chloramphenicol	Kanamycin	Tetracycline
1	*Escherichia coli*	14.5 ± 0.7	17.3 ± 1.2	15.1 ± 0.8
2	*Klebsiella pneumoniae*	17.9 ± 0.9	-	18.5 ± 0.4
3	*Salmonella typhi*	-	-	-
4	*Staphylococcus aureus*	-	15.2 ± 1.3	10.1 ± 0.5

Values are expressed as Mean ± Standard Deviation (n=3)
- - no activity

Plate.2 Antibiotic sensitivity test on tested bacterial pathogens

Escherichia coli



Klebsiella pneumoniae

Salmonella typhi

Staphylococcus aureus

C - Chloramphenicol, K - Kanamycin, T – Tetracycline

Oxidative damages caused by free radicals are responsible for causing a large number of diseases including ulcerative colitis (Ramakrishna *et al*., 1997), cataracts and chronic inflammatory diseases (Halliwell and Gutteridge, 1999), Alzheimer's disease (Smith *et al*., 2000), Parkinson's disease (Bolton *et al*., 2000), alcohol induced liver disease (Arteel, 2003), atherosclerosis (Upston *et al*., 2003), cancer (Kinnula and Crapo, 2004), mild cognitive impairment (Guidi *et al*., 2006), cardiovascular disease

(Singh and Jialal, 2006), aging (Hyun *et al*., 2006) and neural disorders (Sas *et al*., 2007). The most commonly used synthetic antioxidants presently used are butylated hydoxy anisole (BHA), butylated hydoxy toluene (BHT), Propyl gallate (PG) and butylate dhydro quinone. However, these synthetic antioxidants have side effects such as liver damage and carcinogenesis. Many plants have been identified as having potential antioxidant activities and it is of interest to investigate the antioxidant properties of herbal infusions especially those traditionally used in folk medicine.

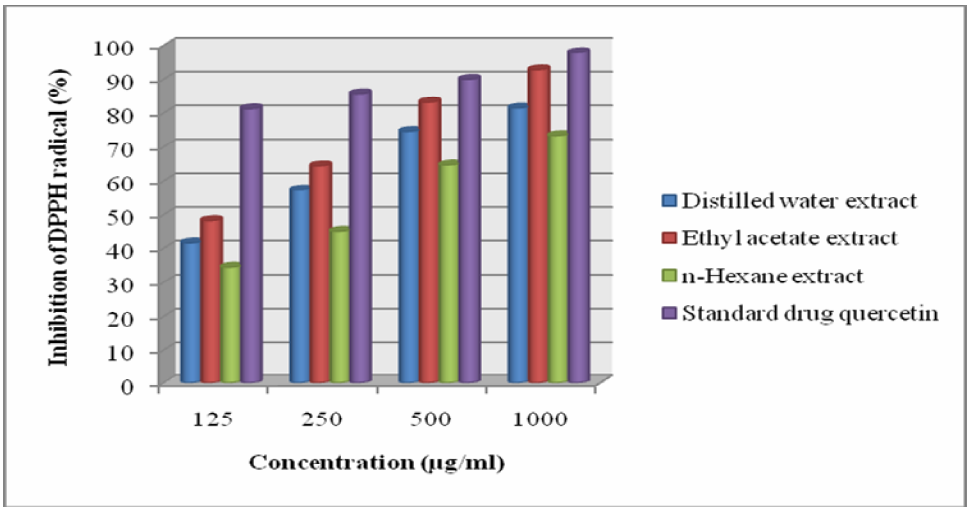
The results of *in vitro* antioxidant activity of *R. vesicarius* L. was studied by DPPH method were presented in Table 4 and Figure 2. The maximum percentage of DPPH inhibition was exhibited by ethyl acetate extract (92.81 ± 0.95%) followed by distilled water extract inhibiting 81.47 ± 0.80 % and the lowest inhibition percentage was recorded by n-hexane extract (73.40 ± 0.58 %) at 1000µg concentration. All the doses possessed significant antioxidant activity, the activity was dose dependent. The inhibition was compared with standard antioxidant quercetin. The present antioxidant activity results were in harmony with the findings of El-Bakry *et al*. (2012) and Husain Khan *et al*. (2014) who established that *R. vesicarius* possess significant antioxidant activity.

Table.4 Antioxidant activity of *Rumex vesicarius* L. by DPPH method

S. No.	Concentration (µg/ml)	Inhibition of DPPH radical (%)			
		Distilled water extract	n-Hexane extract	Ethyl acetate extract	Standard drug quercetin
1.	125	41.57 ± 0.40	34.53 ± 0.15	48.29 ± 0.63	81.26 ± 1.74
2.	250	57.31 ± 0.60	45.17 ± 1.43	64.43 ± 1.35	85.75 ± 0.55
3.	500	74.64 ± 0.99	64.70 ± 1.30	83.21 ± 1.50	89.98 ± 0.71
4.	1000	81.47 ± 0.80	73.40 ± 0.58	92.81 ± 0.95	97.90 ± 1.43

Values are expressed as Mean ± SEM (n=3)

Figure.2 Antioxidant activity of *Rumex vesicarius* L. by DPPH method



The medicinal importance of this plant is a reflection to its chemical composition since this plant contains many bioactive substances (Hariprasad and Ramakrishnan**,** 2011; Husain Khan *et al*., 2014; Ankita *et al*., 2015). The present investigation also complements the ethnobotanical usage of the *Rumex vesicarius* L., which possesses several known and unknown bioactive compounds with biological activity.

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