



Screening of *in vitro* Antibacterial Activity of *Rumex vesicarius* (L.) Leaves Extract against Twelve Pathogenic Bacterial

**Arnaba Saha Chaity¹, Md. Ashikul Islam¹, Tamanna Nasrin², Sathi Rani Sarker²,
Amit Kumar Dutta¹, Biswanath Sikdar¹ and Md. Faruk Hasan^{1*}**

¹*Department of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi-6205, Bangladesh.*

²*Department of Zoology, University of Rajshahi, Rajshahi-6205, Bangladesh.*

Authors' contributions

This work was carried out in collaboration among all authors. Authors ASC, BS and MFH designed the experiments, developed the methodology and prepared the manuscript. Authors ASC, AKD and MFH collected the data and carried out analysis. Authors ASC, MAI, TN, SRS, BS and MFH assisted with manuscript preparation. All authors read and approved the final manuscript.

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ABSTRACT

There is an alternative approaches from eradication of infections causes by pathogenic bacteria especially resistant bacteria. Methanol extract of *Rumex vesicarius* leaves were evaluated from *in vitro* antibacterial activity against twelve bacterial species were used which are four of them gram positive which are *Streptococcus constellatus*, *Staphylococcus gallinarum*, *Staphylococcus sciuri* and *Streptococcus iniae* and eight of them gram negative which are *Aeromonas diversa*, *Xanthomonas campestris*, *Xanthomonas axonopodias*, *Siccibacter colletis*, *Edwardsielloa anguillarum*, *Aeromonas cavernicala*, *Enterobacter xiangfangensis* and *Vibro rotiferianus*. The plant extract showed highest 12 mm zone of inhibition against *Staphylococcus constellatus* at the

*Corresponding author: E-mail: faruk_geb@yahoo.com;

concentration of 20 µg/disc and no zone of inhibition was found from *Aeromonas diversa*. In minimum inhibitory concentration (MIC) test, methanol extract of *Rumex vesicarius* in 200µg/ml concentration showed best result against *Vibrio rotiferianus*. It can be concluded that methanol extracts of *Rumex vesicarius* leaves may be used as natural antibacterial from treatment of some diseases, especially local skin diseases.

Keywords: *Rumex vesicarius* extract; bacteria; MIC values.

1. INTRODUCTION

Rumex vesicarius (L.) is an important medicinal plant [1] belonging to the family of Polygonaceae. The genus *Rumex* contains about 150 species extensively distributed among the World. The plant contains anthraquinones and flavonoids [2]. The genus includes several eatable plant species that have medicinal importance of the treatment of some most risky diseases [3]. *R. vesicarius* L. is a wild edible plant used as a sorrel and collected in spring time and consumed fresh, or cooked. The species used as an important medicinal value uses such as treatment of tumors, hepatic diseases, bad digestion, constipation, calculi, heart troubles, pains, diseases of the spleen, hiccup, flatulence, asthma, bronchitis, dyspepsia, piles, scabies, leucoderma, toothache and nausea. This plant is also used as antioxidant, cool, laxative, stomachic, tonic, analgesic, appetizer, diuretic, astringent, purgative, antispasmodic, aphrodisiac and antibacterial agents. The roasted seeds were taken for the cure of dysentery. Finally, the plant can be used also to reduce biliary disorders and control the levels of cholesterol. The importance of this medicinal plant is a reflection to its chemical composition since this plant contains many bioactive substances such as flavonoids (vitexin, isovitexin, orientin and isorientin), anthraquinones particularly of roots (emodin and chrysophanol), quinones, carotenoids, vitamins (especially vitamin C), proteins, lipids, carbohydrates, reducing sugars, phenols, tannins, saponins, triterpenoids and organic acids. This plant is a good source of minerals, viz; K, Na, Ca, Mg, Fe, Mn, Cu [4,5]. The earlier mentioned bioactive phytochemicals originate in *Rumex vesicarius* L. like, polyphenols, flavonoids, carotenoids, tocopherols and ascorbic acid that have a role as antioxidant and detoxifying agents. The intake of nutritional antioxidant phytochemicals like carotenoids, phenolic compounds and flavonoids will lead to the defense against non-communicable diseases in human beings; cancer, cardiovascular diseases and cataract [6,7]. We aim in this study to investigation, antibacterial screening for some

pathogenic bacteria and determination of minimum inhibitory concentration of leaves extract of *R. vesicarius* plants.

2. MATERIALS AND METHODS

2.1 Collection of Bacteria

Twelve pathogenic bacterial isolates were collected from Microbiology laboratory, Department of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi-6205, Bangladesh which was previously identified. Four of them gram positive which are *Streptococcus constellatus*, *Staphylococcus gallinarum*, *Staphylococcus sciuri* and *Streptococcus iniae* and eight of them gram negative which are *Aeromonas diversa*, *Xanthomonas campestris*, *Xanthomonas axonopodis*, *Siccibacter colletis*, *Edwardsiella anguillarum*, *Aeromonas cavernicola*, *Enterobacter xiangfangensis* and *Vibrio rotiferianus*.

2.2 Plant Samples

Fresh leave of *R. vesicarius* collected from the Rajshahi University Campus of Bangladesh. The surface of the leaves were sterilized with 70% alcohol, which rinsed of sterile distilled water. The leaves were dried for making powder form with the help of grinding machine. The 100 gm of plant leave powdered dried was extracted by 250 ml methanol using conical flask, for shaking and stirring for 14 days. To obtain the huge quantity of extracts the content was pressed of the marking cloth and the whole mixture was then filtered using Whitman filters paper after that the remaining filtrate were dehydrated *in vacuo* to afford a blackish mass. Then remaining output extracts and fraction was collected in vials and conserved in a refrigerator at 4°C carefully.

2.3 Antibacterial Activity of Plant Extracts

The antibacterial activities the plant extract was determined by moderate disc diffusion method [8]. screening For doing the test, 250 µl of fresh

broth culture containing isolated bacteria was pour sensibly on a nutrient agar plate and spread with a disinfected glass spreader. Discs were saturated on isolated organism cultured plates. The amount of 20 µg/disc of each plant extracts were taken with the help of micropipette and Kanamycin 5g/disc use as a control and incubate at 37°C for 14 hrs. Finally diameters of zone of inhibition of plant extracts were measured using by mm scale.

2.4 Determination of Minimum Inhibitory Concentration

The rate of MIC was measured according to Owoseni and Ajayi [9], in which different concentrations of *R. vesicarius* species methanol extract (serial dilutions of the extracts was prepare (25, 50 and 100%). The tubes were then incubated for 48 h at 37°C.

3. RESULTS

3.1 Antimicrobial Study

The study showed that the extract of methanol at a concentration of 20 µg/disc has zone of inhibition produced in case of 12 bacterial strains. The highest zone of inhibition was found to be 12 mm against *Staphylococcus constellatus* at the concentration of 20 µg/disc while no zone of inhibition was found in *Aeromonas diversa* at the same concentration. The control standard Kanamycin (5 µg/disc) showed zone of inhibition of 7-18 mm against the tested bacteria. The results of antimicrobial activities are presented in Table 1.

3.2 MIC Value Determination

In MIC test, twelve bacteria were used against *R. vesicarius* leaves extract. The MIC values were 100, 120, 130, 140, 150, 160 and 200 µg/ml respectively, against the tested gram positive and gram negative bacteria. Negative controls exhibited no inhibition against all the organisms. The standard antibiotic kanamycin had MIC value varying 10 to 30 µg/ml against the tested organisms. Detail results are presented in Table 2.

4. DISCUSSION

Extracts from *R. vesicarius* leave demonstrated significant inhibitory effect against all the bacteria except *Aeromonas cavernicala*. Ether extract of

R. vesicarius is consider as a very rich source of many valuable volatile compounds [1]. The highest inhibition zone of gram negative bacteria was 10 mm diameters found against *Xanthomonas campestris* and it indicate that it is weak bacteria. Similarly the lowest inhibition zone of gram negative bacteria was 5 mm found of *Aeromonas diversa* and *Siccibacter colletis* which indicates those are strong bacteria. The plant extract showed no inhibition zone of *Aeromonas cavernicala*. At the same time the highest inhibition zone of gram positive bacteria was 12 mm diameter against *Streptococcus constellatus* and lowest inhibition zone was 5 mm found of *Staphylococcus gallinarum* and *Staphylococcus iniae*. Our present findings support the previous investigation of Hasan and Sikdar [10]. The maximum zone of inhibition (12.1 mm) was observed against *Bordetella* by methanol extract of *R. dentatus* followed by activity against *Salmonella* and *Bacillus* (zone of inhibition 11 mm and 11.1 mm respectively) by the same extract [11]. The probable for developing antimicrobial from plants appears satisfying as it will lead to the development of a phytomedicine to act against microbes. Plant-based antimicrobials have huge therapeutic potential as they play vital role with fewer side effects that are often associated with synthetic antimicrobials. This study showed that extracts of *R. vesicarius* leaves were effective against bacterial growth. Hussain et al., [12] informed that methanol extracts of different species of *Rumex* genus for example *R. persicaria*, *R. hastatus* and *R. dentatus* have antibacterial activities but their inhibitory effects varied of gram negative and gram positive bacteria. However, these extracts did not shows detectable antibacterial activity *Aeromonas cavernicala*. Fatima et al., [11] also reported the antibacterial potential for gram positive and gram negative bacteria for methanol extracts of roots and leaves of *R. dentatus* plants. Green tea aqueous extract 10% (v/v) was used to control the *Vibrio parahaemolyticus* [13]. Dhayanithi et al., [14] used the mangrove plant *Avicennia marina* (ethyl acetate: methanol 6:4) extract at the concentration of 125 µg/ml to control *Vibrio alginolyticus*. Similarly, *Rumex vesicarius* methanol extract which was 200 µg/ml concentration used to control the pathogenic bacteria but gives the best result in *Vibrio rotiferianus*. The similar results was also reported by Saikot et al., [15] in *Abroma augusta* Leaves extract. So, the previous results are similar to our present findings.

Table 1. Antibacterial activity of *R. vesicarius* leaves extracts against the tested bacteria

Name of bacteria	Zone of inhibition (mm)	
	Plant extract	Kanamycin
Gram negative		
<i>Aeromonas diversa</i>	5	7
<i>Xanthomonas campestris</i>	10	10
<i>Xanthomonas axonopodias</i>	8	10
<i>Siccibacter colletis</i>	5	10
<i>Edwardsiella anguillarum</i>	7	10
<i>Aeromonas cavernicala</i>	-	10
<i>Enterobacter Xiangfangensis</i>	6	10
<i>Vibro notiferianus</i>	7	11
Gram positive		
<i>Streptococcus constellatus</i>	12	12
<i>Staphylococcus gallinarum</i>	5	18
<i>Staphylococcus sciuri</i>	10	18
<i>Staphylococcus iniae</i>	5	10

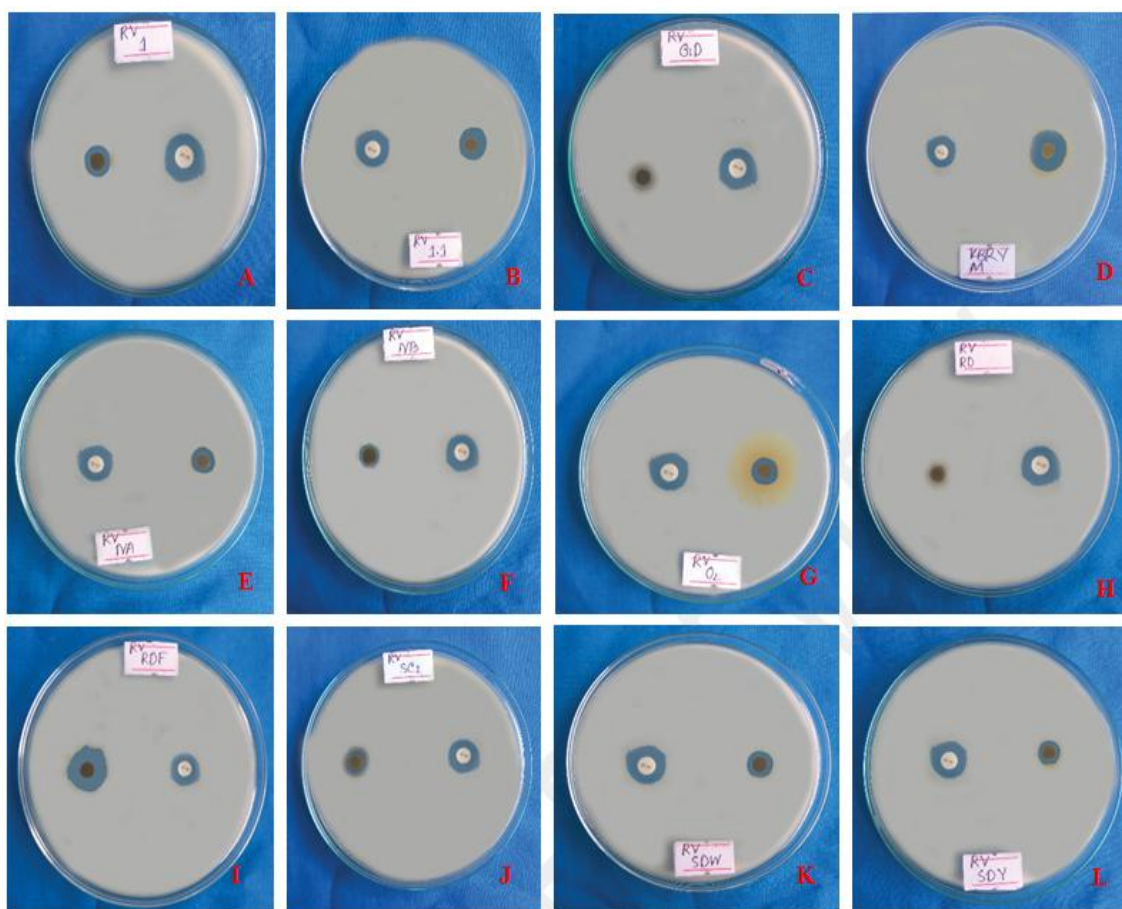


Fig. 1. Antibacterial activities of *Rumex vesicarius* leaves extracts against the tested pathogenic bacterial. A. *Streptococcus constellatus*, B. *Staphylococcus gallinarum*, C. *Staphylococcus sciuri* D. *Streptococcus iniae* E. *Aeromonas diversa*, F. *Xanthomonas campestris*, G. *Xanthomonas axonopodias*, H. *Siccibacter colletis*, I. *Edwardsiella anguillarum*, J. *Aeromonas cavernicala*, K. *Enterobacter xiangfangensis* and L. *Vibrio rotiferianus*

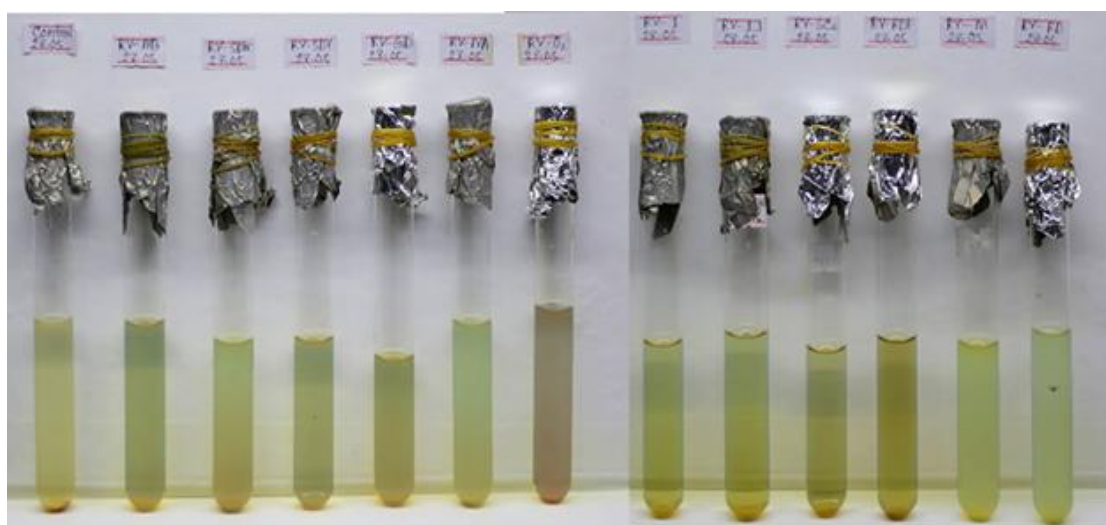


Fig. 2. MIC values of *R. vesicarius* leaf extract against some pathogenic bacteria

Table 2. MIC values of *R. vesicarius* leaf extract against the tested bacteria

Name of bacteria	Methanol extract ($\mu\text{g/ml}$)	Kanamycin ($\mu\text{g/ml}$)	Negative control
<i>Aeromonas diversa</i>	150	20	-
<i>Xanthomonas campestris</i>	150	20	-
<i>Xanthomonas axonopodias</i>	200	20	-
<i>Siccibacter colletis</i>	200	30	-
<i>Edwardsiella anguillarum</i>	200	30	-
<i>Aeromonas cavernicala</i>	130	10	-
<i>Enterobacter xiangfangensis</i>	120	10	-
<i>Vibrio rotiferianus</i>	100	10	-
<i>Streptococcus constellatus</i>	200	30	-
<i>Steaphylococcus gallinarum</i>	160	30	-
<i>Steaphylococcus sciuri</i>	160	30	-
<i>Steaphylococcus iniae</i>	200	30	-

N.B: (-) = No inhibition

5. CONCLUSION

These results suggested that there are bioactive compound present in *Rumex* genus. These compounds probably contain high biological activity. Several previous experiments on different parts of plant of different species of *Rumex* confirm that, *Rumex* genus was strong antibacterial agents. Results of this study revealed that the methanol extract from leaves of *R. vesicarius* was exhibiting antibacterial activity, which might be helpful in inhibiting the resistant bacterial infections and can be used in alternative agent of medicine. However, further studies are required to find the mechanism of extract of antibacterial efficacy and to analyze the active compounds responsible from this biological activity.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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