Original Research Article

Screening of in vitro antibacterial activity of *Rumex vesicarius* leaves extracts against twelve pathogenic bacterial

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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 axonopodies, Siccibacter colletis, Edwardsielloa anguillarum, Aeromonas cavernicala, Enterobacter xiangfangenis* and *Vibro rotiferianus.* The plant extract showed highest 12mm zone of inhibition against *Staphylococcus constellatus* at the concentration of 20µg/disc and no zone of inhibition was found from *Aeromonas diversa*. In minimum inhibitory concentration (MIC) test, methanol extract of *Rumex vesicarious* in 200µg/ml concentration showed best result against *Vibrio rotiferianus*. It can be concluded that methanol extracts of *Rumex vesicarious* leaves may be used as natural antibacterial from treatment of some diseases, especially local skin diseases.

Keywords: *Rumex vesicarius* extract, Bacteria, MIC values

INTRODUCTION

Rumex vesicarius (L.) is an important medicinal plant (Alam, 2014) belonging to the family of Polygonaceae. The genus *Rumex* contains about 150 species extensively distributed among the World. The plant contains anthraquinones and flavonoids (Zhang *et al.*, 2012). The genus includes several eatable plant species that have medicinal importance of the treatment of some most risky diseases (Vermani and Sanjay, 2002). *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, calcules, heart troubles, pains, diseases of the spleen, hiccough, 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, triterepenoids and organic acids. This plant is a good source of minerals, viz; K, Na, Ca, Mg, Fe, Mn, Cu (Mostafa *et al*., 2011; Prasad and Ramakrishnan, 2012). 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 carotenodis, phenolic compounds and flavonoids will lead to the defense against non-communicable diseases in human beings; cancer, cardiovascular diseases and cataract (Rao, 2003; Matkowski, 2008). 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*.

Materials and Methods

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, Xanthomlnas axonopodies, Siccibacter colletis, Edwardsielloa anguillarum, Aeromonas cavernicala, Enterobacter xiangfangenis* and *Vibro rotiferianus*.

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 100gm 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.

Antibacterial activity of plan extracts

For doing the test, 250µl 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 20µg/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.

Determination of minimum inhibitory concentration

The rate of MIC was measured according to Owoseni and Ajayi (2010) 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.

RESULTS

Antimicrobial study

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

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

Name of Bacteria	Zone of inhibition (mm)	
Gram Negative	Plant extract	Kanamycin
Aeromonas diversa	5	7
Xanthomonas campestris	10	10
Xanthomonas axonopodies	8	10
Siccibacter colletis	5	10
Edwardsiella anguillarum	7	10
Aeromonas cavernicala	-	10
Enterobacter Xiangfangensis	6	10
Vibro notiferianus	7	11
Gram Positive		
Streptococcus constellatus	12	12
Steaphylococcus gallinarum	5	18
Steaphylococcus sciuri	10	18
Strephylococcus iniae	5	10

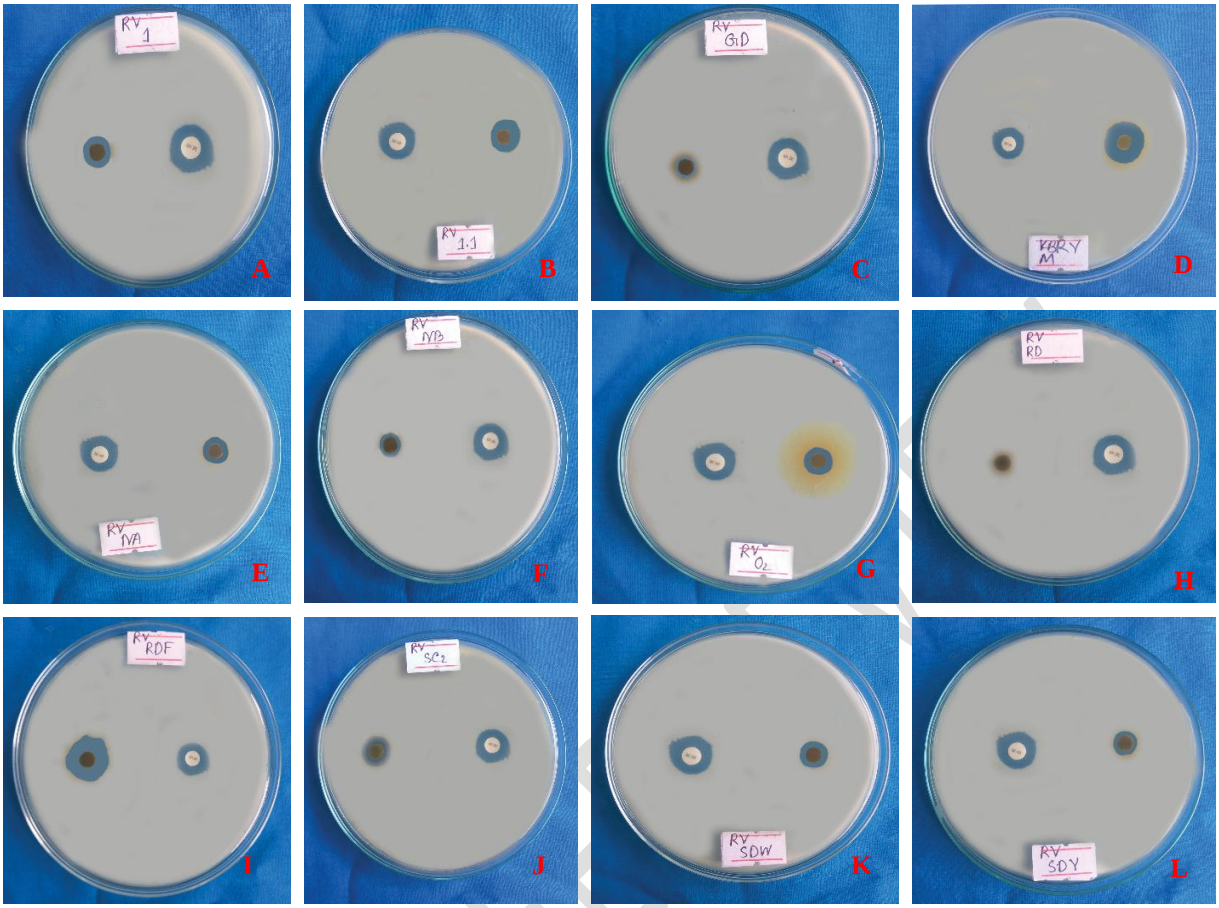

Figure 1: Antibacterial activities of *Rumex vesicarius* leaves extracts against the tested pathogenic bacterial. A. *Streptococcus constellatus*, B. *Staphylococcus gallinarum*, C. *Staphylococcus sciuri* D. *Streptococcus iniae* **E.** *Aeromonas diversa*, F. *Xanthomonas campestris*, G. *Xanthomlnas axonopodies*, H. *Siccibacter colletis*, I. *Edwardsielloa anguillarum*, J. *Aeromonas cavernicala*, K. *Enterobacter xiangfangenis* and L. *Vibro rotiferianus*

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 to30 µg/ml against the tested organisms. Detail results are presented in table 2.

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

Name of Bacteria	Methanol extract (µg/ml)	Kanamycin (µg/ml)	Negative control
Aeromonas diversa	150	20	-
Xanthomonas campestris	150	20	-
Xanthomonas axonopodies	200	20	-
Siccibacter colletis	200	30	-
Edwardsiella anguillarum	200	30	-
Aeromonas cavernicala	130	10	-
Enterobacter xiangfangensis	120	10	-
Vibrio rotiferianus	100	10	-
Streptococcus constellatus	200	30	-
Steaphylococcus gallinarum	160	30	-
Steaphylococcus sciuri	160	30	-
Steaphylococcus iniae	200	30	-

N.B: (-) = No inhibition

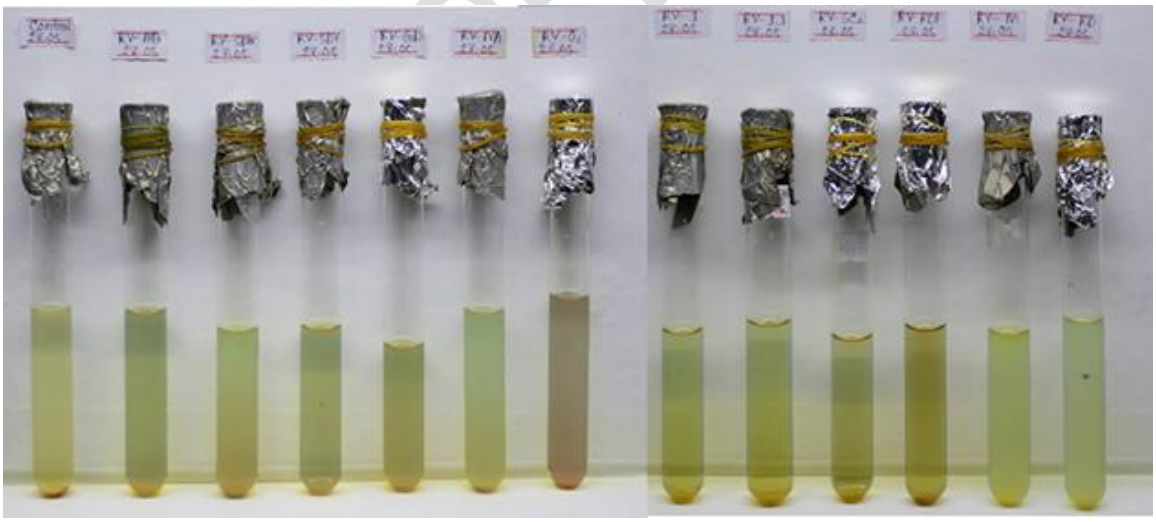

Figure 2: MIC values of *R. vesicarious* leave extract against some pathogenic bacteria

Discussion

Extracts from *R. vesicarious* 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 (Alam, 2014). 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 5mm 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 12mm diameter against *Streptococcus constellatus* and lowest inhibition zone was 5mm found of *Staphylococcus gallinarum* and *Staphylococcus iniae.* Our present findings support the previous investigation of Hasan and Sikdar (2016). 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 (Fatima *et al*., 2009). 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. vesicarious* leaves were effective against bacterial growth. Hussain *et al.,* (2010) 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*., (2009) 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* (Kongchum *et al*., 2016). Dhayanithi *et al.,* (2012) used the mangrove plant *Avicennia marina* (ethyl acetate: methanol 6:4) extract at the concentration of 125µg/ml to control *Vibrio alginolyticus*. Similarly, *Rumex vesicarious* methanol extract which was 200µg/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*., (2012) in *Abroma augusta* Lnn. Leaves extract. So, the previous results are similar to our present findings.

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. vesicarious* 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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Author Contributions

ASC, BS and MFH designed the experiments, developed the methodology and prepared the manuscript. ASC, AKD and MFH collected the data and carried out analysis. ASC, MAI, TN, SRS, BS and MFH assisted with manuscript preparation.

Conflict of interest

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

References

Alam, E. A. (2014). Volatile compounds of Egyptian fruits of *Rumex vesicarius* L. *International Journal of Pharmacy and Pharmaceutical Sciences*, *6*(12): 18-19.

Dhayanithi, N.B., Kumar, T.T.A., & Balasubramanian, T. (2012). In Vitro and Experimental Screening of Mangrove Herbal Extract against *Vibrio Alginolyticus* in Marine Ornamental Fish. *World Academy of Science Engineering and Technology*, *68*:1310-1314.

Fatima, N., Zia, M., Rehman, R., Rizvi, Z. F., Ahmad, S., Mirza, B., & Chaudhary, M. F. (2009). Biological activities of *Rumex dentatus* L: Evaluation of methanol and hexane extracts. *African Journal of Biotechnology*, *8*(24): 6945-6951.

Hasan M.F. and Sikdar B. (2016). Screening of antimicrobial, cytotoxic and pesticidal activities of *Coccinia grandis* (L.) Voigt. *Journal of Microbiology, Biotechnology and Food Sciences, 5*(6): 584-588.

Hussain, F., Ahmad, B., Hameed, I., Dastagir, G., Sanaullah, P., & Azam, S. (2010). Antibacterial, antifungal and insecticidal activities of some selected medicinal plants of polygonaceae. *African Journal of Biotechnology, 9*(31):5032-5036.

Kongchum, P., Chimtong, S., Chareansak, N., & Subprasert, P. (2016). Effect of Green Tea Extract on *Vibrio Parahaemolyticus* Inhibition in Pacific White Shrimp (*Litopenaeus vannamei)* Postlarvae. *Agriculture and Agricultural Science Procedia, 11*:117-124.

Matkowski, A. (2008). Plant in vitro culture for the production of antioxidants—a review. *Biotechnology advances*, *26*(6):548-560.

Mostafa, H. A. M., Elbakry, A. A., & Eman, A. A. (2011). Evaluation of antibacterial and antioxidant activities of different plant parts of *Rumex vesicarius* L. (Polygonaceae). *International Journal of Pharmaceuticals and Pharmacological Sci*ences *3*(2):109-18.

Owoseni, A. A., & Ajayi, A. (2010). Antimicrobial properties of ethanolic and aqueous extracts of *Cymbopogon citratus* on selected bacteria and fungi. *Journal of Medicine and Applied Biosciences, 2*:64-73.

Prasad, P. S. H., & Ramakrishnan, N. (2012). In vitro lipid peroxidation assay of *Rumex vesicarius* L. *International Journal of Pharmaceuticals and Pharmacological Sciences, 4*(Suppl 1):S368-S370.

Rao, B. N. (2003). Bioactive phytochemicals in Indian foods and their potential in health promotion and disease prevention. *Asia Pacific Journal of clinical nutrition*, *12*(1):9-22.

Saikot, F. K., Khan, A., Hasan, M.F. (2012). Antimicrobial and cytotoxic activities of *Abroma augusta* Lnn. leaves extract. Asian Pacific Journal of Tropical Biomedicine. *2012*: S1418-S1422.

Vermani, K. Sanjay, G. (2002). Herbal medicines for sexually transmitted diseases and AIDS. J *Ethnopharmacol*, *80*:49-66.

Zhang, H., Guo, Z., Wu, N., Xu, W., Han, L., Li, N., & Han, Y. (2012). Two novel naphthalene glucosides and an anthraquinone isolated from *Rumex dentatus* and their antiproliferation activities in four cell lines. *Molecules*, *17*(1):843-850.