

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

Abstract

There is an alternative approaches for eradication of infections causes by pathogenic bacteria especially resistant bacteria. Methanol extracts of *Rumex vesicarius* leaves were evaluated for in vitro antibacterial activity against twelve bacterial species were used in which four of them are Gram positive (*Streptococcus constellatus*, *Staphylococcus gallinarum*, *Staphylococcus sciuri* and *Streptococcus iniae*) and the remaining eight are Gram negative (*Aeromonas diversa*, *Xanthomonas campestris*, *Xanthomonas axonopodias*, *Siccibacter colletis*, *Edwardsielloa anguillarum*, *Aeromonas cavernicala*, *Enterobacter xiangfangensis* and *Vibrio rotiferianus*). The study showed that the extract of methanol among twelve bacterial strains where highest zone of inhibition 12 mm in *Staphylococcus constellatus* and no zone of inhibition in *Aeromonas diversa*. In MIC test methanol extract of *Rumex vesicarius* in 200µl/ml concentration showed better result against *Vibrio rotiferianus*. It can be concluded that methanol extracts of *Rumex vesicarius* leaves may be used as natural antibacterial for treatment of some diseases, especially local skin diseases.

INTRODUCTION

The genus *Rumex*, (family: Polygonaceae) contains about 150 species extensively distributed around the World. The main chemical elements of *Rumex* are anthraquinones and flavonoids (Zhang *et al.*, 2012). The genus includes several eatable plant species that have medicinal importance for the treatment of some most risky diseases (Vermani and Sanjay, 2002; or and ? Orhanp *et al.*, 2009). *Rumex vesicarius* L. is a wild edible plant used as a sorrel, it is usually 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, cooling, 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 in 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

Comment [e1]: Please provide the summary of extraction method

Comment [e2]: Please rephrase your results and provide the adequate information
Please always separate the unit from the number

Comment [e3]: Please rephrase

Comment [e4]: How many extracts did you use?

Comment [e5]: In general, rephrase your abstract

Comment [e6]: Please check the text referencing format

Comment [e7]: Check and rephrase

(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 carotenoids, phenolic compounds and flavonoids will lead to the defense against non-communicable diseases in human beings; cancer, cardiovascular diseases and cataract (Rao, 2003; or and ? and Matkowski, 2008). Hence, this study was designed to ~~We aim in this study is investigate antibacterial screening against~~ activities of *Rumex vesicarius* against some pathogenic bacteria and determine the minimum inhibitory concentration of leaves extract of *Rumex vesicarius*.

Comment [e8]: You can abbreviate after the first full name as *R. vesicarius*

Materials and Methods

Comment [e9]: Or rephrase

Microbial isolates

Different twelve pathogenic microbial isolates 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 cavernicala*, *Enterobacter xiangfangensis* and *Vibro rotiferianus* (Table1) and identified by using conventional biochemical tests.

Comment [e10]: Please rephrase and how did you get this isolates?

Table 1: List of bacteria used in the present experiment

Gram Negative
<i>Aeromonas diversa</i>
<i>Xanthomonas campestris</i>
<i>Xanthomonas axonopodis</i>
<i>Siccibacter colletis</i>
<i>Edwardsiella anguillarum</i>
<i>Aeromonas cavernicala</i>
<i>Enterobacter xiangfangensis</i>
<i>Vibro rotiferianus</i>
Gram Positive
<i>Streptococcus constellatus</i>
<i>Staphylococcus gallinarum</i>
<i>Staphylococcus sciuri</i>
<i>Streptococcus iniae</i>

Plant samples

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

Comment [e11]: Why ?

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. 20µl/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 formed due to using plant extracts and then measured by mm scale.

Comment [e12]: Please specify your method of extraction and rephrase the English please

Determination of minimum inhibitory concentration

The rate of MIC was measured according to Owoseni and Ajayi (2010) in which different concentrations of *Rumex vesicarius* species methanolic extract (serial dilutions of the extracts was prepare (25, 50 and 100%). Then the tubes were then incubated for 48 h at 37°C.

Comment [e13]: Please check the correct way of presenting antibacterial activity methodology.

RESULTS

Antimicrobial study

The study showed that the extract of methanol at a concentration of 5g/disc has zone of inhibition produced in case of 12 bacterial strains where highest zone of inhibition 12mm in *Steaphylococcus constellatus* and no zone of inhibition in *Aeromonas diversa* standard Kanamycin (5µg/disc) showed zone of inhibition of 7-18 mm.

Comment [e14]: You have been using methol extract why changing to methanolic extract here? Please you need to check your methodology

Table 2: Antibacterial activity of *Rumex vesicarius* leaves extracts against the tested pathogenic bacterial

Gram Negative	Zone of inhibition	Kanamycin
<i>Aeromonas diversa</i>	5	7
<i>Xanthomonas campestris</i>	10	10
<i>Xanthomonas axonopodies</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

UNDER PEER REVIEW

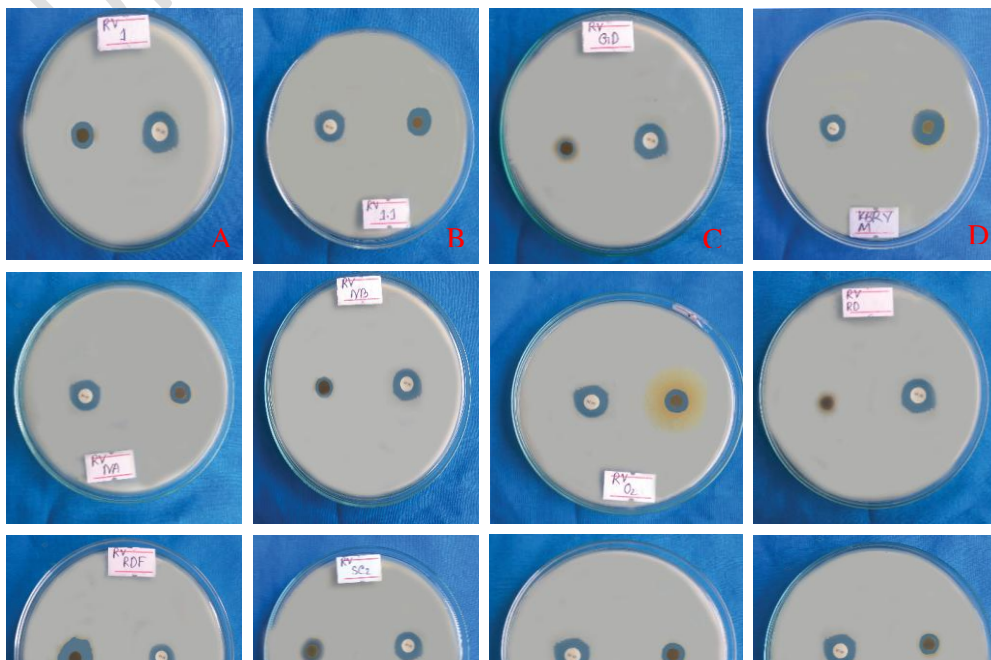


Figure 1: Antibacterial activities of *Rumex vesicarius* leaves extracts against the tested pathogenic bacterial. A. *Streptococcus constellatus*, B. *Staphylococcus gallinarum*, C. *Staphylococcus sciuri* D. *Streptococcus iniae* E. *Aeromonas diversa*, F. *Xanthomonas campestris*, G. *Xanthomonas axonopodius*,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

MIC value determination

In MIC test twelve bacteria were used against *Rumex vesicarius* leaves extract. The plant extract showed best minimum inhibition against the bacteria *Vibrio rotiferianus*.

Table 3: MIC values of *Rumex vesicarius* leaves extracts against the tested pathogenic bacterial

Micro-organism Name	MIC (μ l/ml)	Negative control
Gram Negative	200	-
<i>Aeromonas diversa</i>	200	-
<i>Xanthomonas campestris</i>	200	-
<i>Xanthomonas axonopodius</i>	200	-
<i>Siccibacter colletis</i>	200	-
<i>Edwardsiella anguillarum</i>	200	-
<i>Aeromonas cavernicala</i>	200	-
<i>Enterobacter xiangfangensis</i>	200	-
<i>Vibrio rotiferianus</i>	200	-
Gram Positive		

<i>Streptococcus constellatus</i>	200	-
<i>Steaphylococcus gallinarum</i>	200	-
<i>Steaphylococcus sciuri</i>	200	-
<i>Steaphylococcus iniae</i>	200	-

PEER REVIEW

N.B: (-) = NO inhibition

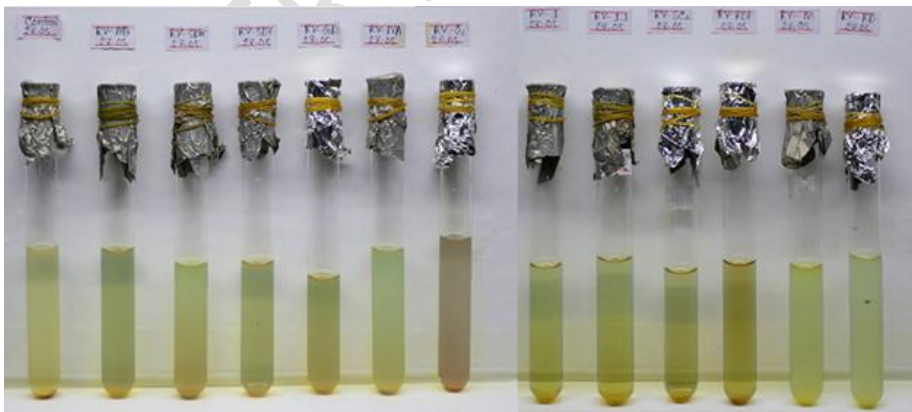


Figure 2: MIC values of *Rumex vesicarios* leaves extracts against some pathogenic bacteria

Discussion

Extracts of *Rumex vesicarius* leaves demonstrated significant inhibitory effect against all the bacteria except *Aeromonas cavernicala*. The highest inhibition zone of gram negative bacteria was 10 mm diameter 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 against *Aeromonas diversa* and *Siccibacter colletis* which indicate those are strong bacteria. But the extract show no inhibition zone against *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 against *Staphylococcus gallinarum* and *Staphylococcus iniae*. Our present result also support the previous investigation. The maximum zone of inhibition (12.1 mm) was observed against *Bordetella* by methanol extract of *Rumex 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 *Rumex vesicarius* leaves were effective against bacterial growth. Ahmad *et al.* informed that methanol extracts of different species of *Rumex* genus for example *R. persicaria*, *R. hastatus* and *R. dentatus* had antibacterial activities but their inhibitory effects varied against Gram negative and Gram positive bacteria (Hussain *et al.*, 2010). However, these extracts did not show detectible antibacterial activity *Aeromonas cavernicala* which is Gram negative bacteria. Fatima *et al.* also explained that methanol extracts of roots and leaves of *Rumex dentatus* had antibacterial potential against Gram positive and Gram negative bacteria (Fatima *et al.* , 2009). Green tea aqueous extract 10% (v/v) was used to control the *Vibrio parahaemolyticus* (Kongchum *et al.*, 2016). *Avicennia marina* ethyl acetate: methanol (6:4) extract 125µg/ml concentration was used to control *Vibrio alginolyticus*. Similarly, *Rumex vesicarius* methanol extract which was 200µl/ml concentration used to control the pathogenic bacteria but gives the best result in *Vibrio rotiferianus*.so the previous result more or less similar to my result.

Conclusion

These results suggested that there are bioactive compounds 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 were strong antibacterial agents. Results of this study revealed that the methanol extract from leaves of *Rumex vesicarius* were exhibit 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 for this biological activity.

Comment [e15]: Your methodology has question mark likewise your discussion

References

- 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).
- Hussain, F., Ahmad, B., Hameed, I., Dastagir, G., Sanullah, 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). *Int. J. Pharm. Pharm. Sci*, 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. *J. Med. Appl. Biosci*, 2, 64-73.
- Prasad, P. S. H., & Ramakrishnan, N. (2012). In vitro lipid peroxidation assay of *Rumex vesicarius* L. *Int J Pharm Pharm Sci*, 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).
- 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

Comment [e16]: Check your referencing format please
Some you abbreviate your Journal name, some without abbreviation and some are italicized why someone are not.
Some have page number and some without number of pages.