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 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 study showed that the extract of methanol among twelve bacterial strains where highest zone of inhibition 12mm in *Staphylococcus constellatus* and no zone of inhibition in *Aeromonas diversa*. In MIC test methanol extract of *Rumex vesicarious* in 200µl/ml concentration showed better result against *Vibrio rotiferianus*. It can be conclude that methanol extracts of *Rumex vesicarious* 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 and Orhanp *et al*., 2009). *Rumex 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, 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, 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 and Matkowski, 2008). We aim in this study is investigate antibacterial screening against some pathogenic bacteria and determination of minimum inhibitory concentration of leaves extract of *Rumex vesicarius*.

Materials and Methods

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, Xanthomlnas axonopodies, Siccibacter colletis, Edwardsielloa anguillarum, Aeromonas cavernicala, Enterobacter xiangfangenis* and *Vibro rotiferianus* (Table1) and identified by using conventional biochemical tests.

Table 1: List of bacteria used in the present experiment

Gram Negative
Aeromonas diversa
Xanthomonas campestris
Xanthomonas axonopodies
Siccibacter colletis
Edwardsiella anguillarum
Aeromonas cavernicala
Enterobacter xiangfangensis
Vibro rotiferianus
Gram Positive
Streptococcus constellatus
Staphylococcus gallinarum
Staphylococcus sciuri
Streptococcus iniae

Plant samples

Fresh leaves of *Rumex vesicarius* 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.

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.

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.

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.

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

Gram Negative	**Zone of inhibition**	**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 *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 (µl/ml)**	**Negative control**
Gram Negative	200	-
Aeromonas diversa	200	-
Xanthomonas campestris	200	-
Xanthomonas axonopodies	200	-
Siccibacter colletis	200	-
Edwardsiella anguillarum	200	-
Aeromonas cavernicala	200	-
Enterobacter xiangfangensis	200	-
Vibrio rotiferianus	200	-
Gram Positive		
Streptococcus constellatus	200	-
Steaphylococcus gallinarum	200	-
Steaphylococcus sciuri	200	-
Steaphylococcus iniae	200	-

N.B: (-) = NO inhibition

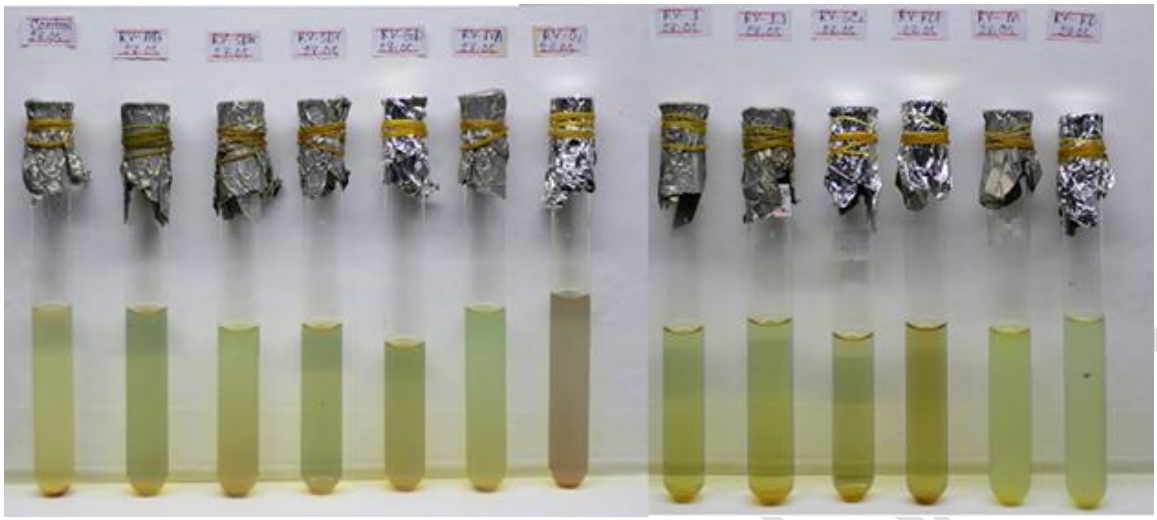

Figure 2: MIC values of *Rumex vesicarious* leaves extracts against some pathogenic bacteria

Discussion

Extracts of *Rumex vesicarious* 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 vesicarious* 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 vesicarious* 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 vesicarious* 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.

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