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Antioxidant, Antimicrobial and Antidiarrhoeal Activity of Methanolic Extract of *Rumex maritimus* L. (Polygonaceae)

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ABSTRACT

The aim of this current study was to evaluate phytochemistry along with pharmacological activities of the whole plant of *Rumex maritimus* L. (Family: Polygonaceae). Phytochemical analysis of the extract of *R. maritimus* indicates the presence of Reducing Sugar, Glycoside, Gum, Tannin and Alkaloid type compounds and the pharmacological attention of these compounds prompted us to check *R. maritimus* for possible anti-oxidant, antimicrobial and antidiarrheal activities in a dose dependent manner. The dried plants were subjected to successive extraction with methanol and the extract was used to investigate the activities. The extract of plants produced good diarrheal inhibition in castor oil induced diarrhea in mice (250mg/kg and 500mg/kg body weight) which was comparable to the standard drug Loperamide at the dose of 3 mg/kg of body weight. The alcoholic extract was showed anti-bacterial activity against the tested microorganisms (both gram positive as well as negative bacteria). This study reveals the potent anti-oxidant activity that is (IC_{50}~80 µg/ml) to that of standard drug ascorbic acid (IC_{50} about ~7 µg/ml) in-vitro when tested in 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) scavenging method. The obtained results provide a support for the use of this plant in traditional medicine and its further investigation.

INTRODUCTION

Bioactive Guided Approach- Once the plant medication was provided to the ancient people in the crude form often exhibited many unwanted effects due to the presence of some toxic components beyond the active constituent. Wide-ranging phytochemical evaluation and isolation of active component(s) in the pure form thus become necessary to avoid untoward effects and to ensure safe use of herbal medicines. With technological advancement, phytochemical studies of medicinal plants got a rapid pace and the presence of many chemical compounds comes in light. These plant-derived compounds often played an important role in directing laboratory synthesis of many new classes of drug molecules. Sometimes the plant components became the starting material in the synthetic process of industrial production of many drug molecules like the application of diosgenin isolated from Mexican Yam for laboratory synthesis of oral contraceptive progesterone reduced the cost of progesterone from a value of $80 per gram to $1.75 per gram (Goldstein *et al*., 1974). *R. maritimus* is generally described as a biennial herb & is native to the United States and its most active growth period in the spring and summer. At maturity, the typical it will reach up to two feet high & a maximum height at 20 years. It can be propagated by seed, sprigs. The whole plant contains various chemicals which have particular medicinal importance. The plant contains- high level of oxalic acids, glycosides, tannins, gums, alkaloids etc. Plant is used as aphrodisiac, astringent, carminative, treat bloater & applied externally to burns; have purgative, refrigerant and antipruritic properties. The seed contains about 5% tannin. Plants contain high levels of oxalic acid, which gives many members of this genus, this aroma acid in lemon. The leaves should not be consumed in large quantities, since oxalic acid can block other nutrients in food, especially calcium, so it can be used for mineral deficiencies. The fruit contain rumarin, rutin and hyperin. Seeds contain 5.1% tannin. Roots also contain chrysophanic acid, saccharose and tannin.

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MATERIALS AND METHODS

Plant collection and Identification

For this present investigation the plant *Rumex maritimus* L. was collected from road side area of Nirala residential area Khulna City, Khulna, Bangladesh in February 2010 and was identified by Bangladesh National Herbarium, Mirpur, Dhaka. (Accession number-29865) and a voucher specimen was deposited in Khulna University laboratory.

Drying and grinding

Unexpected materials were separated from the collected medicinal plants and these were dried by shade drying for seventy days to ensure the active constituents free from decomposition as well as to avoid any photochemical degradation. The whole plants were ground into a coarse powder with the help of an appropriate grinder. In an airtight container, grinded powder was stored and kept in a cool, dark and dry place for analysis until commence.

Cold extraction (Methanol extraction) & Evaporation

The plants were extracted by cold extraction method. 100 gm grinded powder was soaked in 500 ml of methanol in a glass container for eight days accompanying regular shaking and stirring. Plant debris was separated from the extract by filtration by a piece of clean, white cotton material & it was done two times.

The filtrate (methanol extract) obtained was taken into rotary evaporator to evaporate methanol.

Then this filtrate was taken into beaker, the opening of beaker was wrapped by a sheet of aluminum foil to which perforation was done for evaporation of the rest of the methanol & was kept in dry & cool place for several days & at last evaporation was done under table fan until dried. It rendered concentrate of deep purple type and it was designated as crude extract of methanol.

Phytochemical Tests

Testing of different chemical groups present in extract, represent the preliminary photochemical studies- The chemical group tests, which are performed according to Evans (1989). In each test 5 % (w/v) solution of extract in methanol was taken unless otherwise mentioned in individual test.

Test for antimicrobial activity

To inhibit the development of microorganisms, some researchers use the diameter of zone of inhibition and/or the minimum weight of extract. Though, a great amount of factors like the extraction methods, inoculum volume, and culture medium composition (Bauer *et al*., 1966) pH and incubation temperature can stimulate outcomes. Among the above revealed techniques the disc diffusion (Bauer *et al.*, 1966) is a widely accepted in vitro investigation for preliminary screening of test agents which may possess antimicrobial activity. On the other hand, no difference between bacteriostatic and bactericidal activity can be demonstrated by this method (Roland, 1982).

The total experiments were carried out three times and the mean of the reading were recorded. Microorganisms used for the activity test both gram positive and gram-negative bacterial strains were taken. The bacterial strains used for the investigation are listed in Table 1. These organisms were collected from the Microbiology Laboratory of Pharmacy Discipline, Khulna University, Bangladesh.

Table 1: List of gram positive and Gram negative strains.

Gram negative	Gram positive
1. *Shigella dysenteriae*	1. *StaphyIococcuss saprophyticus*
2. *Salmonella typhi*	2. *Streptococcus pyogenes*
3. *Pseudomonas spp.*	3. *Streptococcus agalactiae*
4. *Enterococcus coli*	

Methodology

Preparation of media- Nutrient agar media was prepared by adding water to a dehydrated product that contains all the ingredients. Practically all media are available commercially in powdered form (Pelczar, 1986).

Anti Oxidant test

For assessing the DPPH radical scavenging activity, the modified method described by Gupta *et al.,* (2007) was used.

Stock solution (5mg/ml) of *R. maritimus* were prepared in and serial dilutions were carried out to obtain concentrations of 1, 5, 10, 25, 50,100, 200, 400 µg/mL. In this assay, an equal amount sample solution was added to an equal amount of 0.1mM methanolic DPPH solution, to occur. After about half an hour incubation period, the absorbance was took against a blank at 517 nm with a double beam Analykjena UV/visible spectrophotometer (Specord 205, Germany). Substances which perform this reaction can be considered as antioxidants and accordingly radical scavengers (Dehpour, 2009). The radical scavenging activity was expressed as the inhibition percentage (I%) and calculated as per the equation:

$$I\ (\%) = \{(A_{blank} - A_{sample})/A_{blank}\} \times 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound. IC_{so} value is the concentration of sample required to scavenge 50% DPPH free radical and was calculated from the plot of inhibition (%) against extract concentration. All the tests were done in triplicate and average of the absorptions was noted. Ascorbic acid was used as positive control standard.

Ascorbic acid was used at standard compound to study the DPPH radical scavenging activity test. Ascorbic acid or Vitamin C is the most powerful anti-oxidant. Ascorbic acid was found active during this study while tested for DPPH radical scavenging. The percent hang-up of DPPH radical by the extracts was compared to a synthetic antioxidant i.e. Butylated Hydroxyanisole (BHA). All the fractions showed less radical scavenging activity that was compared with BHA. The activity of the extract which showed the highest DPPH radical scavenging activity (ethyl acetate) was about half of BHA at 100 ppm

concentration. At about 200 ppm, the ethyl acetate fraction shows nearly 93% DPPH scavenging activity (data not given). Even though, when compared to BHA, the concentration required for an equivalent activity was higher for this fraction, there was wide spread settlement that BHA and butylated hydroxytoluene (BHT) needed to be exchanged with natural antioxidants considering their potential health hazards and harmfulness (Kahl and Kappus, 1993).

Anti-diarrheal Activity Screening

In pathological term, diarrhoea occurs due to passage of excess of water in faeces. In the ileum and colon active Na^+K^+ ATPase mediated salt absorption occurs, water follows isoosmotically. In addition glucose facilitated Na^+ absorption take place in the ileum. This mechanism remains intact even in severe diarrhoeas. Diarrhoea associated with carcinoma (secreting 5-HT) and medullary carcinoma of thyroid (secreting calcitonon). It is mediated by cAMP. Excess of bile acids also causes diarrhea by activating adenylcyclase (Tripathi, 2001).

Table 2: Statistic analysis of effect of *R. maritimus* on castor oil (0.5 ml each mouse at 6 hr.) induced diarrhoea in mice.

Group	Treatment	Mean of feaces	Standard Deviation (SD)	Standard Error (SE)	t-test (P-Value)
I (control)	Distilled Water	9.8	1.32	0.66	---
II (Positive control)	Loperamide 3mg/kg	4	0.632	0.316	7.92 (P<0.001)
III (Test Group-I)	*R. maritimus* (250mg/kg)	6.6	1.74	0.87	2.93
IV (Test Group-II)	*R. maritimus* (500mg/kg)	4.8	1.72	0.86	4.61

Diarrhoea was defined by the presence of stool or any fluid material that stained the absorbent paper placed beneath the cage. Time taken before the first defecation is the 'Latent period'. The total count stool and latent period of test group are compared with positive control group. Anti-diarrhoeal agent increase latent period and decrease total number of stool. The effect of *R. maritimus* on castor oil (0.5 ml each mouse at 6 hr.) induced diarrhoea in mice in relative to standard, when we observed the t-value (P-value) along with Standard Deviation (SD) with Standard Error (SE). In table 5, shows the statistic analysis of effect of *R. maritimus* on castor oil (0.5 ml each mouse at 6 hr.) induced diarrhoea in mice.

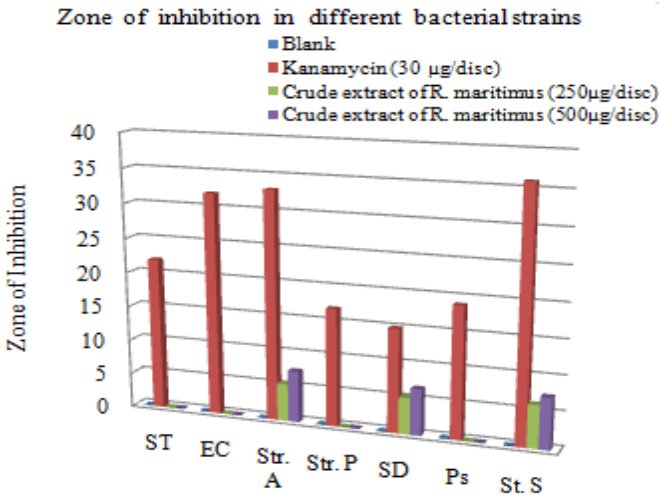
RESULTS AND DISCUSSION

For Phytochemical analysis

The crude extract was tested for the presence of different chemical groups and the following groups were identified- glycosides, tannins, gums, alkaloids, high level of reducing sugar etc.

For Anti-microbial activity

The crude extract of *R. maritimus* was tested for anti-microbial activity against a number (07) of both gram positive and gram-negative bacteria. A standard antibiotic disc of Kanamycin was used for comparison purpose. The crude extract of 500µg/disc showed anti-microbial activity against *Streptococcus agatectiae*, *Shigella dysentrae* and *Staphylococcus saprophyticus* (Table 1). Anti-microbial activity observed not only for gram positive but gram negative bacteria also. Other family member's of Polygonaceae, like *R. dentatus*, *R. nepalensis*, *R. alveollatus*, *R. obtusifolius* have their anti-bacterial activity, to judge these evidence we use whole plant of *R. maritimes* and finally got anti-microbial action. It is a preliminary investigation and further study should be done for more scientific evidence.



[*Staphylococcus saprophyticus*- St. S, *Pseudomonas spp.*- Ps, *Shigella dysentrae*- SD, *Streptococcus pyogens*- Str. P, *Streptococcus agatectiae*- Str. A, *Enterococcus coli*- EC, *Salmonella typhe*- ST.]

Fig. 1: Zone of inhibition in different bacterial strains caused by crude extract of *R. maritimus* and Kanamycin.

For Antioxidant activity

The methanolic extract of *R. maritimus* showed comparable antioxidant activity (IC_{50}~80 µg/ml) against DPPH assay (M. S. Blois, 1958) to that of standard drug ascorbic acid (IC_{50}~7 µg/ml). In figure 2, DPPH radical scavenging test of crude extract of *R. maritimus* and Figure 3, DPPH radical scavenging test of Ascorbic acid (Standard). The free radicals are created in aerobic cells due to ingestion of oxygen in cell progress (Rice-Evans, 2004, Morton, 1992).

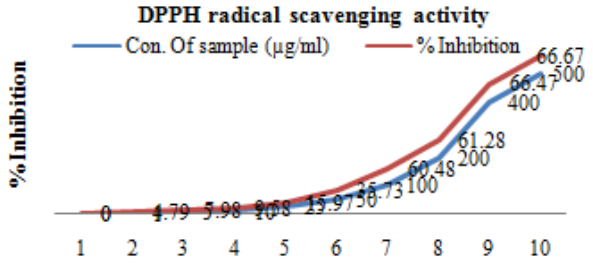


Fig. 2: DPPH radical scavenging test of crude extract of *R. maritimus*

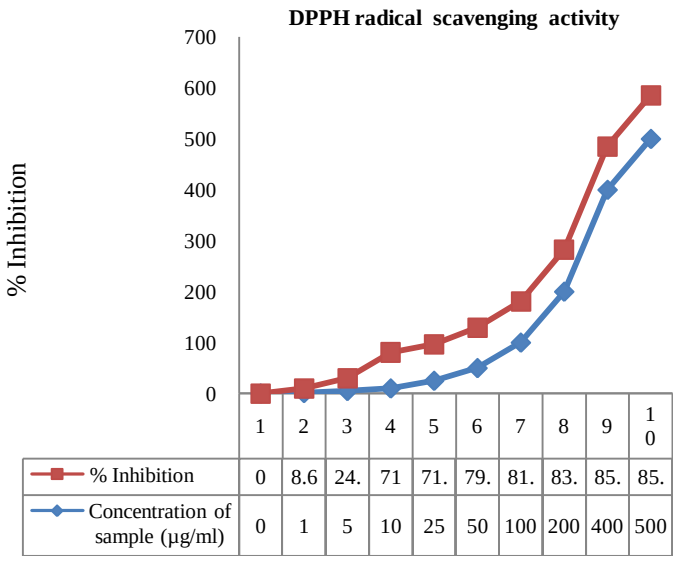


Fig. 3: DPPH radical scavenging test of Ascorbic acid (Standard).

Free radicals cause reduction in membrane fluidity, loss of enzyme receptor action and impairment to membrane protein prominent for death (F. Franca, 1996). These free radicals are involved in various sicknesses like ageing, cancer, CVS diseases, diabetes, rheumatoid arthritis, epilepsy & ruin of essential fatty acids. Antioxidant helps in treatment of above disorders (Jain and Lata, 1996). In this study, percentage inhibition of free radical scavenging activity was increased with the increase concentrations of both crude extract and ascorbic acid. It showed maximum inhibition 85.71% at 500µg/ml and 50% inhibition at concentration near than 7µg/ml (figure 4).

From the above test and analytical method, it can be concluded that, the crude extract of *R. maritimus* has anti-oxidant activity. The free radical scavenging property may be one of the mechanism by which the plants are effective in traditional medicine. Further study is necessary to identify the constituents which are responsible for this property as well as studies with other models, such as lipid per-oxidation and in-vitro assays are essential to characterize them as biological anti-oxidants.

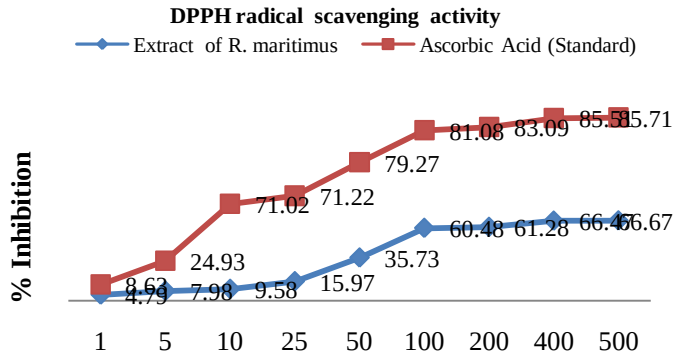


Fig. 4: Percentage inhibition of crude extract of *R. maritimus* and with ascorbic acid (Standard).

For Antidiarrhoeal activity

In the castor oil-induced diarrheal mice, the methanolic extract of *R. maritimus* at the doses of 250 mg/kg and gave large number of stools of stools along with less delay in first defecation i.e. shorter latent period comparing with standard drug, Loperamide. In figure 5 shows, the latent period of *R. maritimus* extract on castor oil induce diarrhoea in mice & in figure 6 shows: the effect of *R. maritimus* extract on the basis of mean stool count on castor oil induce diarrhea in mice.

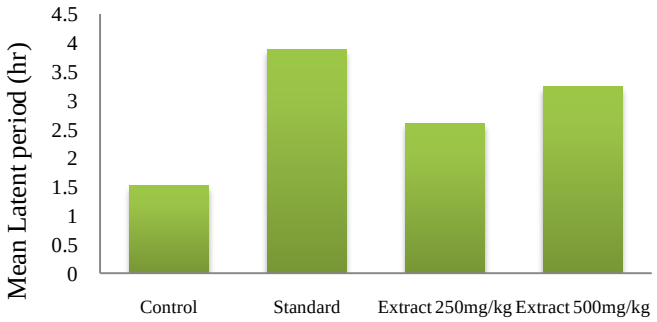


Fig. 5: The latent period of *R. maritimus* extract on castor oil induce diarrhoea in mice.

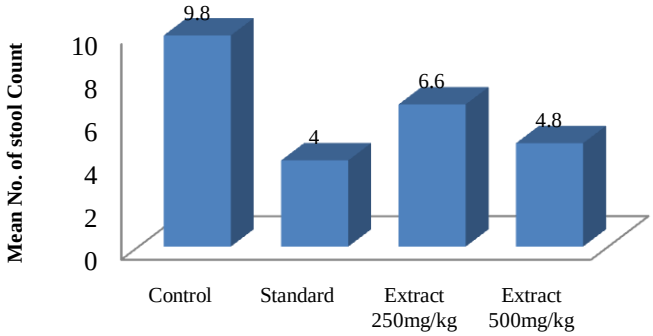


Fig. 6: The effect of *R. maritimus* extract on the basis of mean stool count on castor oil induce diarrhea in mice.

The ricinoleic acid creates irritating and inflammatory actions on the intestine which leads to the discharge of prostaglandins (Yoshio *et al.*, 1999) and exciting peristaltic (decreasing Na+ and K+ absorption) activity and diarrhoea (Zavala *et al.*, 1998). Loperamide is an opiate/alkaloid analogue (Tripathi, 2008) which inhibits prostaglandin synthesis and interruption diarrhoea induced with castor oil (Sunil *et al.*, 2001). The presence of alkaloids of *R. maritimus* may have same pathway as Loperamide does. Furthermore, antidysenteric and antidiarrhoeal belongings of medicinal plants were found due to alkaloids as well as tannins, saponins, flavonoids, sterols and/or triterpenes and reducing sugars (Havagiray *et al.*,. 2004). These ingredients are also present in *R. maritimus* responsible for antidiarrhoeal activity. So it can be declared that it has a little antidiarrhoeal activity though the activity increased in a dose dependent manner.

CONCLUSION

The whole plant crude extract was tested for their activity against a number of both gram positive and gram negative bacteria and found moderate antimicrobial activity which compared to that of a standard antibiotic kanamycin (30µg/disc). The methanolic extract of *R. maritimus* was investigated (250µg/disk & 500µg/disk) which showed such antimicrobial activity against from both gram positive & gram negative bacteria. The crude extract of *R. maritimus* has moderate anti-oxidant activity which can be used effectively in medicine.

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REFERENCES

A. A. Dehpour, M. A. Ebrahimzadeh, S. F. Nabavi and S. M. Nabavi. Antioxidant Activity of Methanol Extract of Ferula assafoetida and Its Essential Oil Composition. Grasas Aceites, 2009; 60(4), 405-412.

Bauer AW, Kirbey WMM, Sherries JC, Truck M. Antibiotic susceptibility testing by standardized single disc method. Am J Clin Pathol, 1996; 45:493-496.

C. A. Rice-Evans. Flavonoids and Isoflavones: Absorption, Metabolism and Bioactivity. Free Radical Biology and Medicine, 2004; 36(7): 827-82

Evans WC. 1989. Trease and Evan's Pharmacognosy, l3th ed. United Kingdom: University Press, Cambridge, 546.

F. Franca, E. L. Lago and P. D. Marsden. Plants Used in the Treatment of Leishmanial Ulcers Due to Leishmania (Viannia) braziliensis in an Endemic Area of Bahia, Brazil, Revista-Sociedade Brasileira de Medicinal Tropical, 1996; 29(3): 229-232.

Ghani A. 1998. Medicinal Plants of Bangladesh: Chemical constituents and Uses, Asiatic Society of Bangladesh, Dhaka, 18.

Goldstein. 1974. Principles of drug action-the basis of pharmacology, 2nd edition: New York. 736, 752, 755.

Harborne, J.B. 1998. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, 3rd Ed. London: Chapman & Hall.

J. F. Morton. Country Borage (*Coleus aromaticus* Lour.): A Potent Flavouring and Medicinal Plant. Journal of Herbs, Spices and Medicinal Plants, 1992; 1(1-2): 77.

Malaya Gupta, Upal Kanti Mazumder and P. Gomathi. Antioxidant and Antimicrobial Properties of *Galega purpurea* Root. Asian Journal of Plant Sciences, 2007; 6:533-537

M. S. Blois. Antioxidant Determinations by the Use of a Stable Free Radical. Nature, 1958; 181(4617): 1199-1200.

Pelczar M.J. Chan J.R, Noel E.C.S, Krieg R. 1986. Microbiology, New Delhi: Tata McGraw-Hill Publishing Company Ltd. 38-39, 99,103-104,106.

R. Kahl and H. Kappus. Toxicology of the Synthetic Antioxidants BHA and BHT in Comparison with the Natural Antioxidant Vitamin E. Zeitschrift für Lebensmitteluntersuchung und-Forschung A, 1993; 196(4): 329-338.

Ranolds, James EF and Martindale 1982. The Extra Pharmacopoeia, 28th ed., England: The Pharmaceutical press, 245.

S. K. Jain and S. Lata. Amazonian Uses of Some Plants Growing in India. Indigenous Knowledge and Development Monitor, 1996; 4(3): 21-23.

Sunil B, Bedi K, Singla A, Johri R. Antidiarrhoeal activity of piperine in mice. Planta Medica. 2001; 67:284–287

Tripathi KD. 2001. Essentials of Medical Pharmacology, Updated Reprint 4th ed. New Delhi 432: Jaypee Brothers, Medical Publishers (pvt.) Ltd.

Tripathi, K. D. 2008. Essentials of Medical Pharmacology, 6th ed. India: Jaypee Brothers Medical Publishers.

Yoshio K, Kazuko S, Bunsyo M, Kazunori H, Atsushi I, Yasuhiro K. Relationship between antidiarrhoeal effects of Hange-Shashin-To and Its active components. Phytother Res. 1999; 13:468–473

Zavala M, Perez C, Vargas R, Perez M. Antidiarrhoeal activity of Waltharia americana, Commelina coelestis and Althernan repens. J. Ethnopharmacol. 1998; 61:41–47.

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