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Antimicrobial Potential of *Rhus semialata* Murr. against Bacterial Diarrhoea

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Diarrhoeal diseases are a leading cause of childhood morbidity and mortality in developing countries. Many new microbial causes of diarrhoea have been discovered during the past three decades. The present study was undertaken to evaluate the effect of methanol extract of the fruits of *Rhus semialata* Murr for its antimicrobial potential against bacteria causing diarrhoea. Methanol extract was tested for its minimum inhibitory concentration (MIC) against both Gram-positive and Gram-negative bacteria causing diarrhoea. Further, the zones of inhibition produced by the crude extract against few sensitive strains was measured and compared with those of standard antibiotic ciprofloxacin. It is evident that the methanol extract is very active against the bacteria causing diarrhoea at low concentrations. The antibacterial efficacy of the fruit extract was found to decrease in the following order against different tested bacterial strains like, *Escherichia coli, Vibrio cholerae, Shigella boydii, Shigella dysenteriae, Shigella sonnei and Salmonella typhimurium.*

Key words: *Rhus semialata* Murr; antibacterial; diarrhoea; methanol extract.

In developing countries, the majority of people living in rural areas almost exclusively use traditional medicines in treating all sorts of disease including diarrhoea. There are large numbers of epidemiological and experimental evidence pertaining to worldwide acute-diarrhoeal disease, which is one of the principal causes of death in the infants, particularly in the malnourished, in developing countries[1]. *Rhus semialata* Murr (Anacardiaceae) is a small tree found in the outer Himalayan ranges, the hills of Assam and Naga hills. The tree is important because of its fruit. The sour tasting fruits are harvested during winter. The fruits are dried and extract is produced. It is taken orally by the rural people for diarrhoea and dysentery[2-7]. In present investigation the methanol extract of the fruits of *Rhus semialata* Murr was subjected for its effectiveness against both Gram-positive and Gram-negative bacteria causing diarrhoea. Fruits contain tannin, gallic acid and the potassium acid salts, together with small amount of aluminium, calcium, magnesium and iron acid salts of malic, tartaric and citric acid[8]. Various phytochemicals isolated from the fruits of *Rhus semialata* Murr are tannins and carbohydrates. The

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present investigations were undertaken to find out the antibacterial potentiality of the methanol extract of the fruits of *Rhus semialata* Murr against some Gram-positive and Gram-negative bacteria causing diarrhoea.

MATERIAL AND METHODS

The fruits of *Rhus semialata* Murr were collected from local area of Pandam (East Sikkim) and authenticated by Botanical Survey of India, Shibpur, Howrah, West Bengal, India. The voucher specimen has been preserved in our laboratory for future reference. The fruits were shed dried, reduced to coarse powder and extracted in a soxhlet apparatus with methanol and solvent was totally removed under vacuum. A semisolid viscous crude extract of the fruits so obtained was tested for the antimicrobial activity against various bacterial strains. These bacterial strains were clinical isolates collected from the Department of Pharmaceutical Technology, Jadavpur University, Kolkata and Institute of Microbial Technology, Chandigarh. All strains used were pure cultures and preserved as slant agar culture at 4°C.

Determination of Minimum Inhibitory Concentration (MIC)

The molten nutrient agar media containing various concentrations of the extract (50, 100, 250, 500, 1000 and 2000 µg/ml) were poured and solidified into sterile 100 mm petridishes to give sterile nutrient agar plates with varying dilutions of extract. Then these plates were kept in a refrigerator (4°C) for 24 hours for uniform diffusion of the extract into the nutrient agar media. The plates were then dried at 37°C for 2 hours before spot inoculation[9]. One loopful (diameter: 3 mm) of the overnight grown peptone water culture of each test organism was placed in petridish marked by checker board technique[10]. The back of each test plate was marked by checker board technique for the location of each inoculum and the test organisms were spot inoculated accordingly. The spot inoculated plates were incubated at 37°C for 24 hours and the MIC values were obtained.

Determination of Zones of inhibition by Disc Diffusion method

Here we have taken pure ciprofloxacin as a standard antibiotic for comparison of the results. Two sets of four dilutions each of fruit extract of *Rhus semialata* Murr (250, 500, 1000 and 2000 µg/ml) and ciprofloxacin (25, 50, 100 and 200 µg/ml) were prepared in sterile Mc Cartney bottles. The extracts and standard drugs were dissolved in DMSO. Sterile nutrient agar plates were prepared and incubated at 37°C for 24 hours to check for any sort of contamination. Four sterile filter paper discs (Whatman No.1) of 6 mm diameter were soaked in four different dilutions of crude extract and placed in appropriate position on the surface of the flooded plate, marked as quadrants at the back of the Petridishes. The Petridishes were incubated at 37°C for 24 hours and the diameters of zone of inhibition measured in mm[11]. Similar procedure was adopted for the pure ciprofloxacin and the corresponding zone of diameters was compared accordingly.

Determination of mode of antimicrobial action of extract

Two of the highest sensitive strains (*Escherichia coli* 748 and *Vibrio cholerae* 865) to the extract were grown in sterile nutrient broth medium overnight, 2 ml of which were added to 4 ml of sterile nutrient broth and incubated for 2 hours at 37°C, so that the culture attained logarithmic phase of growth. After 2 hours of incubation, the extracts were added at a higher concentration than its MIC value for that particular strain. The number of colony forming units (CFU/ml) was determined at an interval of 2 hours up to 6 hrs and then after 18 hours starting from 0 hr.

RESULTS AND DISCUSSION

The results of determination of MIC values of the methanol extract of the fruits of *R. semialata* Murr were tabulated in Table 1. It was evident that the methanol extract is very active against the bacteria causing diarrhoea at low concentrations. The results of inhibition of the crude fruit extract and its comparison with standard antibiotic ciprofloxacin were recorded in Table 2. The antibacterial efficacy of the fruit extract was found to decrease in the following order against different tested bacterial strains *Escherichia coli, Vibrio cholerae, Shigella boydii, S. dysenteriae, S. sonnei* and *Salmonella typhimurium.*

The extract also proved to be bactericidal in nature as shown in Table 3. This antimicrobial property against bacteria causing diarrhoea may be due to presence of some antimicrobial substances present in the fruit. Different tannin substances are producing good anti-diarrhoeal activity have already been reported[12]. So, the anti-diarrhoeal activity of aforementioned plant may be due to the presence of tannins. Now our study is directed to find out the lead antidiarrhoeal compound from the same plant.

Table 1. Determination of MIC of methanol extract of the fruits of *Rhus semialata* Murr against different microbes causing diarrhoea

Name of the bacteria	Growth in nutrient agar containing different concentration of extract in µg/ml						
	0*	50	100	250	500	1000	2000
Shigella dysenteriae 6	+	+	±	-	-	-	-
Shigella dysenteriae 7	+	+	+	-	-	-	-
Shigella sonnei 1	+	+	+	-	-	-	-
Shigella sonnei 2	+	+	+	±	-	-	-
Shigella boydii 2	+	+	+	-	-	-	-
Shigella boydii 8	+	+	-	-	-	-	-
Salmonella typhimurium NCTC74	+	+	+	-	-	-	-
Escherichia coli TG1	+	+	-	-	-	-	-
Escherichia coli 10Hd	+	+	±	-	-	-	-
Escherichia coli 748	+	+	-	-	-	-	-
Escherichia coli 3	+	+	-	-	-	-	-
Escherichia coli C-22	+	+	+	±	-	-	-
Escherichia coli 798	+	+	-	-	-	-	-
Escherichia coli ABB12	+	+	-	-	-	-	-
Escherichia coli 741	+	+	-	-	-	-	-
Escherichia coli 871	+	+	+	±	-	-	-
Vibrio cholerae 14033	+	+	-	-	-	-	-
Vibrio cholerae 10	+	+	±	-	-	-	-
Vibrio cholerae 865	+	+	-	-	-	-	-
Vibrio cholerae 71	+	+	-	-	-	-	-
Vibrio cholerae 937	+	+	±	-	-	-	-
Vibrio cholerae 5	+	+	-	-	-	-	-

* Control, '+' growth, '-' no growth, '±'inhibited growth

Table 2. Determination of zone of inhibition (mm) produced by the methanol extract of the fruits of *Rhus semialata* Murr and its comparison with ciprofloxacin

Name of bacteria	Fruit extract (µg/ml)				Ciprofloxacin (µg/ml)			
	250	500	1000	2000	25	50	100	200
Shigella dysenteriae 6	8.0	9.0	10.5	11.5	20.0	25.5	31.0	36.0
Shigella boydii 2	7.5	8.5	10.0	11.0	22.0	26.0	32.5	38.0
Escherichia coli 10HD	8.5	9.5	10.5	11.0	16.0	18.0	19.5	21.0
Vibrio cholerae 865	9.0	10.0	11.5	13.0	21.0	23.5	28.0	34.0
Salmonella typhimuriumNCTC74	7.0	8.0	9.5	10.0	15.5	18.0	20.0	22.5

Tests in Triplicate.

Table 3. Mode of antibacterial activity of methanol extract of the fruits of *Rhus semialata* Murr against two most sensitive bacteria causing diarrhoea

Time	CFU/ml against *Escherichia coli* 798	CFU/ml against *Vibrio cholerae* 865
0	88×10^7	9.6×10^7
2	86×10^5	7.5×10^5
4	85×10^3	6.6×10^4
6	7.8×10^2	5.8×10^2
18	0	0

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