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Antibacterial activity of methanol extract of roots of Heracleum nepalense D Don. on bacteria causing diarrhoea

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SUMMARY

Heracleum nepalense D Don. (Umbelliferae) is a small shrub having high glabrescent stem found in stream banks in Sikkim. Various medicinal properties which include antidiarrhoeal, antiseptic, anti-influenzal etc. have been attributed for this plant in the traditional system of medicine in Sikkim. In present investigation the methanol extract of roots of *Heracleum nepalense* was subjected for its effectiveness against both Gram-positive and Gram-negative bacteria causing diarrhoea. The roots extract was tested for its minimum inhibitory concentration (MIC) against both Gram-positive and Gram-negative organisms 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 root extract was found to decrease in the following order against different tested bacterial strains like *Shigella dysenteriae, Escherichia coli, Shigella boydii, Vibrio cholerae, Salmonella typhimurium.*

Key words: *Heracleum nepalense*; Antibacterial; Diarrhoea; Methanol extract

INTRODUCTION

Heracleum nepalense D Don. (Umbelliferae) is a small shrub having high glabrescent stem found in stream banks in Sikkim. Various medicinal properties have been attributed to the plants of same genera in the traditional system of the tribal people of Sikkim mainly *Heracleum wallichii* is known for its antidiarrhoeal, tonic and aphrodisiac activity (Rai *et al.*, 1994; Tsarong, 1994; Pal *et al.*, 1998; Gurung, 2002). The plant *Heracleum nepalense* itself is known for its antidiarrhoeal, antiseptic, anti-influenzal and antispasmodic activity (Dhar *et al.*, 1968). Various phytochemicals have been isolated from the root of the plant *Heracleum nepalense* are furocoumarins namely bergapten, byakangelicin, allo-imperatorin etc; comrarins, steroids namely β-sitosterol and alkaloids (Anonymous, 1959; Asolkar *et al.*, 1992).

Various medicinal plants possess significant antibacterial activity and are used in different alternative systems of medicine successfully. The methanol extract of *Solanum trilobatum, Andrographis paniculata, Psoralea coryfolia,* (Citarasu *et al.*, 2003) *Allium vineale, Chaerophyllum macropodum* and *Prangos ferulacea* (Durmaz *et al.*, 2006) possess significant antibacterial activity. The present investigations were undertaken to find out the antibacterial potentiality of the methanol extract of the roots

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against some Gram-positive and Gram-negative bacteria causing diarrhoea to lengthen the queue of antibacterial herbs.

MATERIALS AND METHODS

Plant materials

The roots of *Heracleum nepalense* were collected from local area of Pandam (Sikkim) and authenticated by Botanical Survey of India, Gangtok. The voucher specimen has been preserved in our laboratory for future reference. The roots were shed dried, reduced to coarse powder and extracted in a soxhlet apparatus with methanol and solvent was removed under vacuum. A semisolid viscous crude extract of the root 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. All strains used were pure cultures 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, 1,000 and 2,000 μg/ml) were poured and solidified onto 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 h for uniform diffusion of the extract into the nutrient agar media. The plates were then dried at 37°C for 2 h before spot inoculation (Mandal *et al.*, 2000). 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 (Mazumder *et al.*, 2000). The spot inoculated plates were incubated at 37°C for 4 h and the MIC values were obtained.

Determination of zone 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 root extract of *Heracleum nepalense* (250, 500, 1,000 and 2,000 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 h, 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 Petri dishes. The Petri dishes were incubated at 37°C for 24 h, and the diameters of zone of inhibition measured in mm (Mandal *et al.*, 2000). 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 (*Shigella dysenteriae 6 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 h at 37°C, so that the culture attained logarithmic phase of growth. After 2 h of incubation, the extracts were added at a higher concentration than its MIC value for that particular strain. The number of colony forming unit (CFU/ml) was determined at an interval of 2 h up to 6 hours and then after 18 h starting from zero h.

RESULTS AND DISCUSSION

The results of determination of minimum inhibitory concentration (MIC) values of the methanol extract of the root of *Heracleum nepalense* have been tabulated in Table 1. It is evident that the methanol extract is very active against the bacteria causing diarrhoea at low concentrations. The results of inhibition of the crude root extract and its comparison with standard antibiotic ciprofloxacin

Table 1. Determination of MIC of methanol extract of the root of *Heracleum nepalense* 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	1,000	2,000
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	+	+	+	-	-	-	-

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

Table 2. Determination of zone of inhibition (mm) produced by the root extract and its comparison with ciprofloxacin

Name of bacteria	Root extract (μg/ml)				Ciprofloxacin (μg/ml)			
	250	500	1,000	2,000	25	50	100	200
Shigella dysenteriae 6	11.0	12.0	13.5	17.0	20.0	25.5	31.0	36.0
Shigella boydii 2	13.0	15.0	16.5	18.0	22.0	26.0	32.5	38.0
Escherichia coli 10HD	9.0	11.0	12.5	15.0	16.0	18.0	19.5	21.0
Vibrio cholerae 865	12.0	14.0	15.5	17.0	21.0	23.5	28.0	34.0
Salmonella typhimurium NCTC74	10.0	12.0	13.5	15.0	15.5	18.0	20.0	22.5

Tests in triplicate.

is recorded in Table 2. The antibacterial efficacy of the root extract was found to decrease in the following order against different tested bacterial strains like *Shigella dysenteriae, Escherichia coli, Shigella boydii, Vibrio cholerae* 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

Table 3. Mode of antibacterial activity of methanol extract of the root of *Heracleum nepalense* against two most sensitive microorganisms causing diarrhoea

Time	CFU/ml against *Shigella dysenteriae* 6	CFU/ml against *Vibrio cholerae* 865
0	9.6×10^7	7.6×10^6
2	9.4×10^5	5.3×10^4
4	9.2×10^3	6.0×10^3
6	8.7×10^2	3.6×10^2
18	0	0

in the root. Different tannin substances are producing good anti-diarrhoeal activity have already been reported (Kokate *et al.*, 2003). 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.

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