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RESEARCH ARTICLE

Effect of *Rosa indica* on *E. coli* Isolated from Groundwater Samples

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Abstract

E.coli strains have the ability to develop resistance against various antibiotics thus extracts from natural products may serve as a better alternative towards their control. In the present study effect of flower and leaf extracts of *Rosa indica* were tested on *E.coli* isolated from 20 ground water samples of Dayalbagh region, Agra (U.P). The isolates were confirmed by various biochemical tests including the Nitrate, Catalase, Oxidase and IMViC tests. Aqueous and ethanolic extracts of *Rosa indica* flowers and leaves were evaluated for their potential bacteriostatic activity against the standard (MTCC 723) and one isolate of *E.coli*. Both ethanolic and aqueous flower and leaf extracts of *Rosa indica* were found to be highly effective in controlling the growth of the standard as well as the isolate of *E.coli*. The findings of this study suggest that *Rosa indica* exhibits a high antibacterial property and can be used as an additive in various drinks and food preparations to check *E.coli* contamination.

Keywords

Antibiotics, *E.coli*, groundwater, *Rosa indica*.

Introduction

Escherichia coli is a Gram negative bacteria that normally inhabits the gastrointestinal tract of mammals. *E.coli* was first described as *Bacterium coli communis* by the German pediatrician and bacteriologist Theodor Escherich in 1885, but later it was named as *Escherichia coli* (Escherich, 1885). *E. coli is c*ommonly found in the lower part of the intestine of endotherms and the pathogenic strains have been associated with several diseases including diarrhea, urinary tract infections and meningitis (Russo and Johnson, 2003). In developing countries, diarrheal diseases are often associated with infant and child deaths (Sobel *et al,* 2004). The presence of *E. coli* in water is an indication of fecal contamination and represents a high risk of disease (Leclerc *et al,* 2001). According to Hunter *et al,* (2001) a fraction of the world's population (20%) has no access to safe drinking water. This fact, in conjunction with inadequate sanitation, leads to millions of deaths every year. In developing countries, infantile diarrhea is associated with typical *E. coli* EPEC strains (Trabulsi *et al,* 2002). Multidrugresistant *E. coli* that produce extended-spectrum β lactamases (ESBLs), such as the CTX-M enzymes, have emerged within the community setting as an important cause of bloodstream infections (Laupland *et al,* 2008). Development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances, chemotherapeutic agents and agrochemicals that combine antimicrobial efficacy with low toxicity and have a minor environmental impact. Natural products offer an untold diversity of chemical structures therefore interest in exploiting medicinal values of plants has increased in recent years. In the present study presence of *E. coli* was examined and it was isolated from various groundwater samples. The antibacterial activity of flower and leaf extracts of *Rosa indica* was investigated against *E. coli.*

Materials and Method

Standard strain and chemicals used

The standard culture of *E.coli* (MTCC 723) was procured from MTCC (Microbial type culture collection) Chandigarh, India. The chemicals and reagents used were obtained from HIMEDIA, MERCK and SRL.

Isolation and Identification of *E.coli*

E.coli was isolated and confirmed from groundwater samples collected from various areas of Dayalbagh region in Agra city (U.P.) India in three steps:- the presumptive test, the confirmed test and the completed test. (Baheerathi *et al,* 2011)

Presumptive Test

Most probable number was determined by inoculating three dilutions of the water sample (10ml, 1 ml and 0.1

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ml) in lactose broth containing inverted Durham tubes. Five replicas of each dilution were prepared using 10ml lactose broth in each of the test tube. After 24-48 hours of incubation at 37°C the results were noted based on gas production in the tubes. The medium in the inverted Durham's tubes within the test tubes was replaced by the gas produced, thus enabling the observation and the values were compared with MPN standard chart.

Confirmed Test

Inoculums from the lactose broth tubes showing fermentation were streaked on Eosin Methylene Blue (EMB) agar. Streaked plates were incubated at 37^0C for 24 hours. After incubation, round colonies with green metallic sheen were picked up and inoculated in fresh nutrient broth. These isolated colonies were then subjected to the completed test (Thenmozhi, 2010).

Completed Test

Isolated colonies of *E.coli* from EMB agar were inoculated in nutrient broth and were further confirmed by performing Gram's staining and various biochemical tests like IMVIC test and sugar fermentation test.

Preparation of Extracts

Flowers and leaves of *Rosa indica* were used to prepare aqueous and ethanolic extracts. For aqueous extracts 1g of powdered samples were dissolved in 10ml of hot water and kept on rotary shaker for 6 hours whereas for ethanolic extracts 1g of powdered samples were soaked in 10ml of ethanol (95%) for 24 hours. The extracts were then filtered using autoclaved muslin cloth. The filtrate was centrifuged at 3200rpm for 10 minutes. The supernatant was then concentrated by evaporating the solvent. The supernatants of the concentrated aqueous and ethanolic extracts were dissolved in distilled water and 5 % DMSO to yield a concentration of 50 mg/ml. (Kumar *et al,* 2010; Hena, 2010)

Determination of Antibacterial Activity of *Rosa indica* Extracts Against *E.coli*

Antibacterial activity of aqueous and ethanolic flower and leaf extracts of *Rosa indica* against the standard and isolate of *E.coli* (MTCC-723) was determined using agar well diffusion method on Muller Hinton Agar (MHA) plates. 150 µl of the culture was added to 15ml of MHA and poured on sterile petriplates. After solidification, wells of 6mm diameter were bored using sterile borer and 40µl of extracts was introduced in wells. The plates were then incubated without inverting at 37^0C for 24hrs and then the zone of inhibition was measured (Sahoo *et al,* 2011).

Positive and negative control

Distilled water was used as the negative control for aqueous extracts and 5% DMSO was used as the negative control for ethanolic extracts (Ramya *et al,* 2008). Streptomycin disc was used as the positive control (Kirby-Bauer disk diffusion method) (Hudzicki, 2009).

Results and Discussion

20 samples of groundwater collected from various areas of Dayalbagh region of Agra city were analyzed for the presence of *E.coli* of which 14 samples were found to be contaminated with *E.coli*. MPN count of each of the water samples was also determined as indicated in table 1. MPN count helped in determining the microbial population in various water samples and in assessing the microbial quality of water samples. The main cause of such a high contamination could be leakage in some sewer lines near the groundwater sources. The isolates of *E.coli* were further confirmed using Gram staining, various biochemical and sugar fermentation tests (Table 2, 3).

Table 1. MPN count of collected water samples

S.no	Area	Samples collected		MPN Count
1.	Area 1	2	S1	17
			S2	900
2.	Area 2	2	S3	22
			S4	14
3.	Area 3	4	S5	22
			S6	30
			S7	17
			S8	17
4.	Area 4	2	S9	8
			S10	60
5.	Area 5	1	S11	14
6.	Area 6	1	S12	≥1600
7.	Area 7	1	S13	900
8.	Area 8	3	S14	4
			S15	4
			S16	70
9.	Area 9	4	S17	240
			S18	2
			S19	13
			S20	≥1600
	Total	20		

In order to check the antimicrobial activity of extracts of *Rosa indica* against *E.coli*, agar well diffusion method of Kirby Bauer was used. Table 4, Fig.2 and 3 shows the results of zone of inhibitions observed for the extracts and the standard antibiotic streptomycin used throughout the study. Antimicrobial properties of *Rosa*

Table 2. Gram staining and biochemical characterization of various *E.coli* isolates

S.No	*E.coli* isolates	Gram staining	Indole test	MR test	VP test	Citrate test	Nitrate test	Catalase test	Oxidase test	TSI Agar test
1.	S1	-	+	+	-	-	+	+	-	+
2.	S2	-	+	+	-	-	+	+	-	+
3.	S5	-	+	+	-	-	+	+	-	+
4.	S6	-	+	+	-	-	+	+	-	+
5.	S7	-	+	+	-	-	+	+	-	+
6.	S8	-	+	+	-	-	+	+	-	+
7.	S12	-	+	+	-	-	+	+	-	+
8.	S13	-	+	+	-	-	+	+	-	-
9.	S14	-	+	+	-	-	+	+	-	+
10.	S15	-	+	+	-	-	+	+	-	+
11.	S16	-	+	+	-	-	+	+	-	+
12.	S17	-	+	+	-	-	+	+	-	+
13.	S19	-	+	+	-	-	+	+	-	+
14.	S20	-	+	+	-	-	+	+	-	+
15.	Standard	-	+	+	-	-	+	+	-	+

Table 3. Sugar fermentation tests of *E.coli* isolates

S.No	*E.coli* Isolates	Sugars				
		Glucose	Lactose	Sucrose	Maltose	Raffinose
1.	S1	+	+	+	+	+
2.	S2	+	+	+	+	+
3.	S5	+	+	-	+	-
4.	S6	+	+	-	+	-
5.	S7	+	+	-	+	-
6.	S8	+	+	-	+	-
7.	S12	+	+	+	+	+
8.	S13	+	+	-	+	-
9.	S14	+	+	+	+	+
10.	S15	+	+	+	+	+
11.	S16	+	+	+	+	+
12.	S17	+	+	+	+	+
13.	S19	+	+	+	+	+
14.	S20	+	+	+	+	+
15.	Standard	+	+	+	+	+

Table 4. Observed zones of inhibition against the standard *E.coli* (MTCC-723) and its isolate

S.No	Material Used			Averaged diameter of zone of inhibition (mm) against	
				E.coli standard (MTCC-723)	*E.coli* isolate
1.	*Rosa indica*	Flowers	Aqueous	16.5	14
			Ethanolic	17.8	16
		Leaves	Aqueous	15.5	15.4
			Ethanolic	16.5	16.6
2.	Streptomycin (Positive control)			11.0	12
3.	DMSO (5%) (Negative control)			0	0
4.	Autoclaved distilled water (Negative control)			0	0

Indica have been reported earlier by Koday *et al,* 2010. From the present study it can be seen that the ethanolic extracts of flowers and leaves of *Rosa indica* were found to be more effective than aqueous extracts while the aqueous extracts are more effective than the standard antibiotic in controlling the growth of *E.coli* (Fig. 1). Hence, *Rosa indica* can be used as an effective antimicrobial agent against *E.coli* infections. *Rosa indica* is edible and has been used since time immemorial in our country as 'Gulkand' and 'Gulabjal'. The present study strongly recommends the use of aqueous extract of *Rosa indica* which can be incorporated in drinking water and various drinks and food preparations to inhibit the pathogenic *E.coli*.

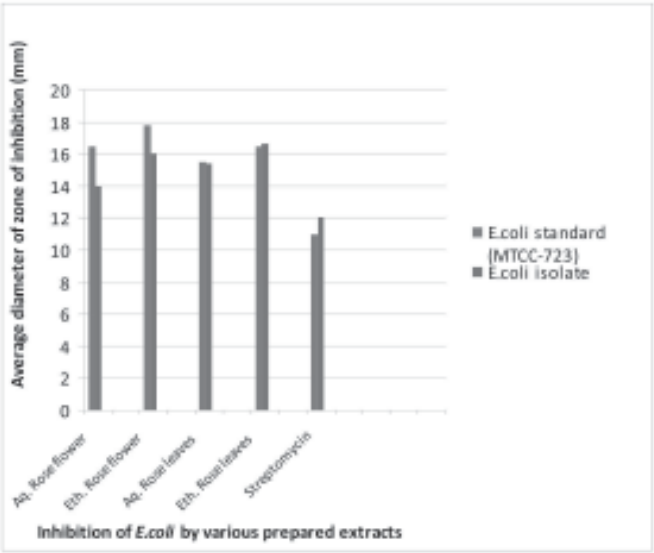

Fig.1. Inhibitory effect of extracts of *Rosa indica* against *E. coli*

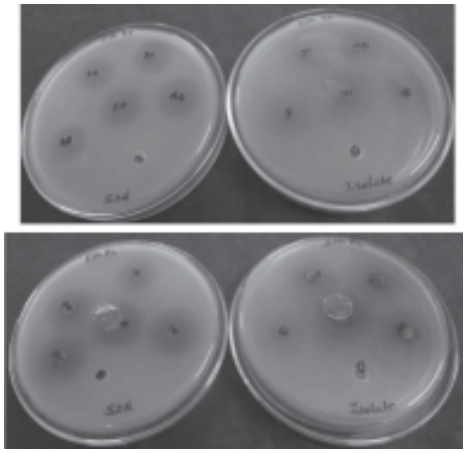

Fig.2. Zone of inhibition produced by ethanolic flowers and leaves extracts of *Rosa indica* against *E.coli.*

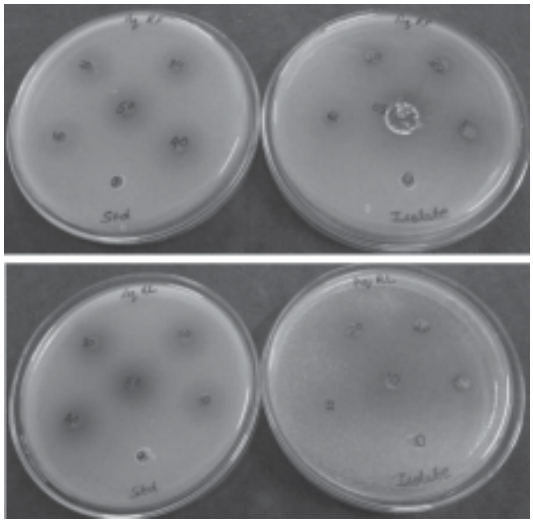

Fig.3. Zone of inhibition produced by aqueous flowers and leaves extracts of *Rosa indica* against *E.coli.*

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