Journal of Chemical Health Risks

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JCHR (2026) 16(2), 4453-4462 | ISSN:2251-6727

Herbal Extracts as Alternative Antimicrobials against Waterborne Colistin-Resistant *Escherichia coli* from the Narmada River

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(Received: 16 February 2026 Revised: 14 March 2026 Accepted: 25 April 2026)

KEYWORDS

Escherichia coli, colistin resistance, Narmada River, medicinal plants, phytochemicals, antibacterial activity

ABSTRACT:

The presence of colistin-resistant *Escherichia coli* in freshwater ecosystems pose a growing environmental and public health risk. This study evaluated the occurrence of colistin-resistant *E. coli* in the Narmada River and assessed the antibacterial potential of 14 locally available medicinal plants. Out of 250 water samples collected over two years, 40 *E. coli* isolates were recovered, of which 14 were colistin-resistant. Frequency-based analysis revealed that *Cyperus rotundus* (Nagarmotha) and *Acorus calamus* (Bach) inhibited 71.4% of resistant isolates. Chi-square analysis confirmed significant variation in inhibition frequency among extracts ($\chi^2 = 54.79$, df = 13, $p < 0.001$). These findings, consistent with previous work on flavonoid-rich *C. rotundus* extracts, highlight the potential of herbal phytochemicals as alternative antibacterial agents to combat colistin-resistant bacteria in freshwater environments.

1. Introduction

Freshwater is essential for human survival, with rivers providing drinking water, irrigation, and other domestic uses. The Narmada River, flowing through central India, serves as a lifeline for millions of people and considered holy river by Hindus (Sharma et al., 2012, Israni et al., 2024a). However, rivers in India are increasingly polluted due to untreated sewage, unhygienic practices, and fecal contamination, which contribute to the prevalence of waterborne diseases such as diarrhea, cholera, and typhoid (Anastasi et al., 2012; Odonkor and Mahami, 2021). Among waterborne pathogens, *Escherichia coli* is a key indicator of fecal contamination due to its thermotolerant nature and prevalence in human and animal feces (Israni et al., 2024b).

The rapid emergence of antibiotic-resistant bacteria, including colistin-resistant strains, poses a global public health challenge. Colistin is considered a last-resort antibiotic for multidrug-resistant Gram-negative infections, and the *mcr*-1 gene mediates plasmid-borne colistin resistance (Teng et al., 2021). Environmental dissemination of such resistant bacteria underscores the need for alternative antibacterial strategies.

Plant-derived phytochemicals have regained attention as potential agents to combat antibiotic resistance (El-Khatib and Basyony, 2024). Previous work from our group demonstrated that flavonoid-rich extracts of *Cyperus rotundus* effectively inhibited colistin-resistant bacteria from the Narmada River (Sharma et al., 2025). Building on this, the present study evaluates the occurrence of colistin-resistant *E. coli* in the river and screens 14 local medicinal plants for antibacterial activity, aiming to identify potential alternatives to conventional antibiotics.Narmada, part of major riverine systems in India represents critical interface where environmental, human, and agricultural antibiotic-resistant microorganisms converge and circulate.

2. Objectives

Study has been planned to investigate the effectiveness of different phytochemicals of the medicinal plants on the

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Colistin-resistant pathogenic bacteria through their phytochemical investigation and *in vitro* antibacterial activity. This study will deliver baseline information to further investigate these plants both *In vitro* and *In vivo* in order to scrutinize new biologically active compounds for the production of novel drugs. This research will provide scientific data about the prevalence of colistin resistant bacteria in Narmada River.

The state of microbial resistance is increasing rapidly and the use of antibacterial agents is uncertain. Therefore, it is important to take strict measures to reduce the impact of this issue. As a result of the increased prevalence of multidrug-resistant Gram-negative Enterobacteriaceae, colistin has regained worldwide interest. In parallel, colistin-resistant strains emerged in response to the chaotic use of this antibiotic. The main purpose of this work is to study colistin-resistant strains in the Narmada River and to develop appropriate surveillance data and systems to report the growth rates of these resistant strains. Widely used as a remedy for many infectious diseases, it is necessary to look for plants that contain antibacterial substances. Therefore, this study seeks to isolate a practical plant lead molecule as an alternative to colistin-resistant strains.

3. Methods

Collection of Water Samples

Water samples (1 L each) were collected from major ghats along the Narmada River in Madhya Pradesh, India, thrice annually over two years. Samples were collected in sterile bottles and transported under refrigeration.

4. Isolation and Identification of *Escherichia coli*

Samples were filtered through 0.45 µm gridded filter papers, which were then plated on Chromogenic Coliform Agar (HiMedia, India) and incubated at 37 ± 1 °C for 48 h. Blue colonies were identified as *E. coli* and confirmed via Gram staining, biochemical tests, Eosin Methylene Blue agar, and MacConkey agar.

5. Colistin Sensitivity Testing

Colistin resistance was determined using the disc diffusion method (50 µg discs, HiMedia, India) as described by Bauer et al. (1966). After 48 h incubation, the absence of a clear inhibitory zone indicated colistin resistance.

6. Collection of Plants and Phytochemical Extraction

Fourteen medicinal plants were collected locally (Table 1). Dried plant parts (10 g) were extracted with ethanol using a Soxhlet extractor and concentrated to 500 mg mL^{-1}. Extracts (50 µL) were tested against colistin-resistant *E. coli* using the well diffusion method on Müller Hinton Agar (HiMedia, India). Zone of inhibition (mm) was recorded for each isolate.

Table 1. Medicinal plants selected for antibacterial activity against colistin-resistant *Escherichia coli*

S. No.	Vernacular name	Scientific name	Family	Plant part used
1	Nagarmotha	*Cyperus rotundus*	Cyperaceae	Rhizome
2	Gudmar	*Gymnemasylvestre*	Apocynaceae	Leaves
3	Sarpgandha	*Rauwolfia serpentina*	Apocynaceae	Root
4	Bach	*Acorus calamus*	Acoraceae	Rhizome
5	Safed Musli	*Chlorophytum borivilianum*	Asparagaceae	Tubers
6	Keokand	*Curculigoorchioides*	Hypoxidaceae	Rhizome
7	Shatavari	*Asparagus racemosus*	Asparagaceae	Root
8	Kalmegh	*Andrographis paniculata*	Acanthaceae	Stem
9	Chandrashur	*Lepidium sativum*	Brassicaceae	Seeds
10	Isabgol	*Plantago ovata*	Plantaginaceae	Seeds
11	Ashwagandha	*Withania somnifera*	Solanaceae	Root
12	Bael	*Aegle marmelos*	Rutaceae	Fruit

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				pulp
13	Aonla	*Phyllanthus emblica*	Phyllanthaceae	Fruit
14	Giloy	*Tinospora cordifolia*	Menispermaceae	Stem

1. Although minimum inhibitory concentration (MIC) estimation provides quantitative insight, diffusion-based assays remain suitable for comparative screening of multiple botanical extracts against environmental isolates, as widely applied in exploratory antimicrobial studies.

2. Quality control

All culture media and reagents used in the study were prepared according to the manufacturers' instructions and sterilized prior to use. Sterility controls were included for each batch of media. A reference strain of *Escherichia coli* (ATCC 25922) was used as a quality-control organism for culture characteristics and antimicrobial susceptibility testing. All experiments were performed in triplicate to ensure reproducibility.

3. Statistical analysis

Frequency-based analysis was performed to determine the proportion of colistin-resistant isolates inhibited by each plant extract. Differences in inhibition frequency were evaluated using the Chi-square test (χ^2, df = 13), with significance set at $p< 0.05$. Since, CLSI interpretive criteria are not available for plant-derived extracts; statistical analysis was limited to comparative screening evaluation.

7. Results

Isolation and Identification of *E. coli*

Out of 250 water samples, 40 *E. coli* isolates were recovered, of which 14 exhibited colistin resistance. Colonies appeared blue on Chromogenic Coliform Agar, green with a metallic sheen on EMB agar, and pink on MacConkey agar (Figure 1).

Antibacterial Activity of Herbal Extracts

Frequency-based analysis revealed heterogeneity in antibacterial activity (Table 2). *Cyperus rotundus* (Nagarmotha) and *Acorus calamus* (Bach) inhibited the highest proportion of isolates (71.4%), followed by *Gymnemasylvestre*, *Aegle marmelos*, and *Phyllanthus emblica* (64.3%). Extracts such as *Plantago ovata*, *Withaniasomnifera*, and *Curculigoorchioides* showed no activity. Chi-square analysis confirmed a significant association between extract type and inhibition frequency ($\chi^2 = 54.79$, df = 13, $p< 0.001$), demonstrating significant variation among extracts.

Figure 1: Representative plates of *E. coli* on Chromogenic Coliform Agar, EMB agar, and MacConkey agar

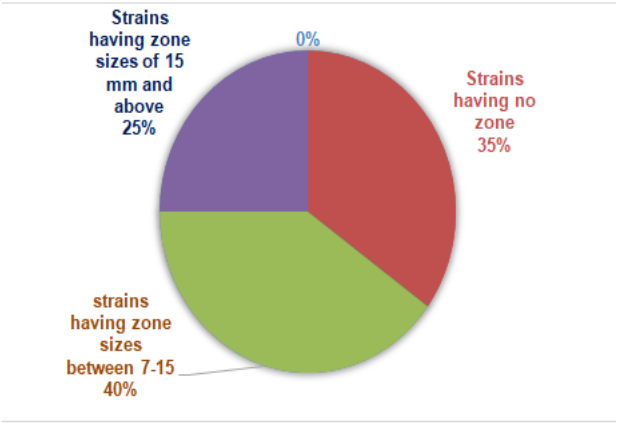

Figure 2: Pie chart showing % inhibition of colistin-resistant isolates by each plant extract with significance annotation (χ^2 $p < 0.001$).

Name of the States	Rural		
	2015-16	2019-21	% Change
Andhra Pradesh	33.12	31.4	-1.72
Arunachal Pradesh	20.91	15.8	-5.11

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Assam	30.82	33.6	2.78
Bihar	44.62	41.8	-2.82
Chhattisgarh	39.61	32.7	-6.91
Goa	21.22	26.6	5.38
Gujarat	44.23	43.5	-0.73
Haryana	29.91	21.8	-8.11
Himachal Pradesh	21.62	25.6	3.98
Jharkhand	49.71	41.4	-8.31
Karnataka	37.72	34.9	-2.82
Kerala	16.74	19.9	3.16
Madhya Pradesh	45.1	34.2	-10.9
Maharashtra	40.05	38	-2.05
Manipur	14.21	13.5	-0.71
Meghalaya	29.91	27.3	-2.61
Mizoram	15.72	15.8	0.08
Nagaland	17.9	27.7	9.8
Odisha	35.81	31	-4.81
Punjab	21.1	16.4	-4.7
Rajasthan	38.4	28.1	-10.3
Sikkim	15.4	14.9	-0.5
Tamil Nadu	25.7	23.5	-2.2
Telangana	33.1	35	1.9
Tripura	25	28.3	3.3
Uttarakhand	27.1	20.9	-6.2
Uttar Pradesh	41	33.1	-7.9
West Bengal	33.6	33.5	-0.1

Age Group
5–6
7–8
9–10
11–12

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Table 2. Frequency-based antibacterial activity of ethanolic plant extracts against colistin-resistant *Escherichia coli*

Plant extract	**Isolates tested (N)**	**Isolates inhibited (n)**	**Inhibition (%)**
Tinospora cordifolia (Giloy)	14	5	35.7
Asparagus racemosus (Shatavari)	14	6	42.9
Gymnemasylvestre (Gudmar)	14	9	64.3
Andrographis paniculata (Kalmegh)	14	6	42.9
Lepidium sativum (Chandrashur)	14	3	21.4
Aegle marmelos (Bael)	14	9	64.3
Acorus calamus (Bach)	14	10	71.4
Phyllanthus emblica (Amla)	14	9	64.3
Plantago ovata (Isabgol)	14	0	0.0
Withania somnifera (Ashwagandha)	14	0	0.0
Chlorophytum borivilianum (Safed Musli)	14	3	21.4
Curculigoorchioides (Keokand)	14	0	0.0
Cyperus rotundus (Nagarmotha)	14	10	71.4
Rauvolfia serpentina (Sarpagandha)	14	5	35.7

Frequency-based analysis showed that *Cyperus rotundus* and *Acorus calamus* extracts inhibited the highest proportion of colistin-resistant *Escherichia coli* isolates (71.4%), followed by *Gymnemasylvestre*, *Aegle marmelos* and *Phyllanthus emblica* (64.3%). Chi-square analysis confirmed that inhibition frequency varied significantly among the extracts ($\chi^2 = 54.79$, df = 13, $p < 0.001$). The significant chi-square association confirms that observed differences in inhibition frequency were not random but attributable to extract-specific antibacterial potential

8. Discussion

The present study confirms the presence of colistin-resistant *Escherichia coli* in the Narmada River, reinforcing concerns that freshwater ecosystems act as reservoirs and dissemination pathways for resistance to last-resort antibiotics. Rivers receiving anthropogenic inputs are increasingly recognized as hotspots for antimicrobial resistance, facilitating exchange of resistance determinants among environmental, animal, and human bacterial populations (Berendonk*et al.*, 2015; Larsson *et al.*, 2018). Reports of colistin resistance in aquatic environments worldwide further support the environmental persistence and spread of both chromosomal and plasmid-mediated resistance mechanisms (Poirel*et al.*, 2017; Rhouma*et al.*, 2016).

Screening of fourteen medicinal plant extracts revealed significant heterogeneity in antibacterial activity, with *Cyperus rotundus* and *Acorus calamus* demonstrating the highest inhibition frequency (71.4%). The statistically significant association between plant extract type and inhibition frequency ($\chi^2 = 54.79$, df = 13, $p < 0.001$) confirms that antibacterial efficacy is strongly dependent on plant-specific metabolite profiles (Borges et al., 2016). Similar variability among medicinal plants has been widely attributed to differences in phytochemical composition, particularly phenolics, flavonoids, and terpenoids, which target bacterial membranes and metabolic pathways with differing efficiencies (Cowan, 1999; Cushnie and Lamb, 2011).

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The pronounced activity of *C. rotundus* aligns with previous studies describing its richness in flavonoids and phenolic compounds with established antibacterial properties (Nagulendran*et al.*, 2007; Kilani *et al.*, 2008). These findings are consistent with our earlier work demonstrating effective inhibition of colistin-resistant bacteria from the same river using flavonoid-rich *C. rotundus* extracts (Sharma*et al.*, 2025), as well as other studies (Agbo et al., 2021). Flavonoids are known to compromise bacterial membrane integrity, inhibit nucleic acid synthesis, and modulate efflux pump activity, thereby enhancing susceptibility even in resistant strains (Daglia, 2012; Farhadi *et al.*, 2019).

The observed isolate-to-isolate variability in susceptibility likely reflects multiple colistin resistance mechanisms, including lipid A modification mediated by chromosomal regulatory systems (*pmrA/pmrB*, *phoP/phoQ*) in addition to plasmid-borne *mcr* genes (Olaitan *et al.*, 2014; Sun *et al.*, 2018). Such mechanistic diversity may influence membrane permeability and intracellular target access, resulting in differential responses to plant-derived compounds.

Although no single extract inhibited all isolates, the results highlight the potential of locally available medicinal plants as supplementary antibacterial agents in environments impacted by antimicrobial resistance. The findings support further bioassay-guided fractionation, chemical characterization of active constituents, and evaluation of synergistic combinations, which may provide environmentally sustainable strategies to mitigate the spread of antibiotic-resistant bacteria in freshwater systems (Hemaiswarya*et al.*, 2008; Karkman*et al.*, 2018).

The present study is limited by its reliance on phenotypic screening of colistin resistance and antibacterial activity. Molecular confirmation of resistance determinants such as *mcr* genes and phytochemical profiling of active extracts were beyond the scope of this work. In addition, inhibition frequency was used as a comparative indicator rather than minimum inhibitory concentration values. Nevertheless, the applied approach is appropriate for environmental surveillance and preliminary screening studies, and provides a foundation for targeted molecular and chemical investigations in future work

Conclusion

The present study demonstrates the occurrence of colistin-resistant *Escherichia coli* in the Narmada River, indicating environmental dissemination of resistance to a last-resort antibiotic in a major freshwater system of central India. The detection of resistant isolates underscores potential public health risks associated with the use of contaminated surface waters. Among the tested medicinal plants, *Cyperus rotundus* exhibited notable antibacterial activity against colistin-resistant isolates, while several other plants showed moderate or weak effects. The variable susceptibility patterns observed suggest heterogeneity in resistance mechanisms and differential responses to phytochemicals. Although no single plant extract was effective against all resistant isolates, the results highlight the potential of plant-derived compounds as complementary antimicrobial agents. Further studies focusing on molecular characterization of resistance determinants and isolation of active phytoconstituents are necessary to explore their application in combating antimicrobial resistance.

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