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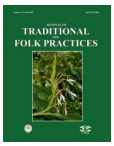

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An *in-vitro* study to analyze the effect of *Nagakesara* (*Mesua ferrea* Linn.) on turbidity and microbial load of drinking water

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Abstract

Acharya Charaka highlights '*Udakam Ashvasakaranam*' to underscore the importance of water in daily life. The 2021 World Water Development Report by UNESCO indicates that over 8,29,000 individuals die annually from diseases linked to poor drinking water and sanitation, with almost 37.7 million people in India impacted by waterborne diseases each year. *Susruta Samhita* states that flowers like *Patala* (*Stereospermum suaveolens* (Roxb.) DC.), *Nagakesara* (*Mesua ferrea* Linn.), *Champaka* (*Michelia champaca* L.), and *Utpala* (*Nelumbo nucifera* Gaertn.) can be utilized for water purification. Studies indicate that *Nagakesara* shown bactericidal action against both gram-positive and gram-negative bacteria. Considering all these factors, the current study was designed to do an *in-vitro* analysis of the impact of *Nagakesara* (*Mesua ferrea* Linn.) on the turbidity and microbiological load of drinking water. The findings of the MPN Presumptive test indicate that, at the measured concentration and duration, *Nagakesara* flowers exhibits potent antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. MIC findings indicated bactericidal impact at the investigated concentrations, with only the *Hima Kalpana* (cold infusion) of the flower exhibiting growth suppression against bacterial strains. The study found that there was no discernible decrease in turbidity. However, it shows the limited impact of *Nagakesara* reducing water turbidity rather than increasing it. These findings suggest that the flowers of *Nagakesara* possess antibacterial properties, primarily bacteriostatic (inhibitory) and bactericidal (bacteria-killing).

Keywords: Antibacterial activity, *Nagakesara*, Public health, Turbidity reduction, Water purification

1. Introduction

Udakam Ashvasakaranam is mentioned by *Acharya Charaka* to highlight the importance of water in daily life (Skandhan *et al*., 2011). Water is utilized for a multitude of functions, including drinking, cleaning, bathing, recreation and a wide range of industrial applications. Water consumption is essential to human health, lifespan and drinking water is a basic human necessity. According to UNESCO's 2021 World Water Development Report, around 8,29,000 people die each year due to diseases caused by poor drinking water and sanitation (Li *et al*., 2022). Waterborne infections afflict around 37.7 million persons in India each year. Nowadays, obtaining adequate access to clean water is one of the biggest issues. 88% of occurrences of diarrheal disease worldwide are believed to be caused by unsafe water (Kulinkina *et al*., 2016). The World Health Organization (WHO) estimates that 1.1 billion people globally consume tainted water. Research indicates that elevated levels of turbidity in drinking water are often associated with higher quantities of harmful microorganisms, such as viruses, parasites, and coliform bacteria. Studies showed that after *Escherichia coli*, *Shigella*, *Staphylococcus* and *Salmonella* species were the most prevalent and prevalent microorganisms found in drinking water samples (Jung *et al*., 2014; Sudip *et al*., 2021).

There are several methods available for domestic water purification, such as boiling, chemical disinfection, ultraviolet light, multi-stage reverse osmosis purification,

etc. But the biggest drawback is that most people are unaware of these methods (Mao, 2016). In order to improve the quality of the water in the present circumstances, a simple home water purifying solution is needed. Despite the fact that water has been cleaned using a variety of traditional filtration methods. There are no relevant scientific studies to back up their efficacy.

The dangers of consuming contaminated and unappealing water were addressed in *Ayurveda*, *Acharya Susruta*'s description of water purification in the *Susruta Samhita* highlights the use of natural ingredients like flowers to purify water. Specifically, the text mentions the following flowers *Utpala* (*Nelumbo nucifera* Gaertn.), *Nagakesara* (*Mesua ferrea* Linn.), *Champaka* (*Michelia champaca* L.) and *Patala* (*Stereospermum suaveolens* (Roxb.) DC.). These flowers are noted for their potential properties in water treatment, reflecting ancient Indian knowledge of natural remedies and water purification methods (Mangalagowri, 2012).

Among these flowers, the *Nagakesara* (*Mesua ferrea* Linn.; Family: Calophyllaceae) is being used in urinary tract disorders, skin diseases, gout, fever, relieves thirst and vomiting and is used in the treatment of female infertility (Prakash, 2015). It is also mentioned among *Eladi gana* and *Priyangvadi gana* (Group of drugs which is described in *Charaka Samhita*. These group of drugs possess features such as anti-inflammatory, antioxidant, antimicrobial, immunomodulatory actions due to their rich phytochemical composition) (Vinayak, 2024). Previous research findings indicate that *Nagakesara* exhibits bactericidal efficacy against a wide range of gram -positive and gram-negative bacterial species (Shifali *et al*., 2021). Although the efficacy of *Nagakesara* flowers in purifying water has not yet been investigated scientifically, combining traditional knowledge with contemporary research may result in creative and long-lasting solutions to the problem of water contamination. The goal of the current study was to analyze the impact of *Nagakesara* flowers on the turbidity and microbial load of drinking water through an *in-vitro* investigation with long term result.

2. Materials and methods

2.1. Collection and preservation of drug

Nagakesara (*Mesua ferrea* Linn.) flowers were collected from the plains of Palode (Latitude 8.7505° N, Longitude 77.0273° E and Altitude 300-350m ASL) in Thiruvananthapuram (Dist.), Kerala, India during the winter season (February 2025). The plant was authenticated by taxonomists at KSCSTE -Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Palode, Thiruvananthapuram, Kerala, India. The flowers were shade dried. After the completion of dehydration (within 5-10 days), the flowers were powdered to suitable size with the help of a multipurpose mixer and passed through mesh no. 60 and made into coarse powder (Plate 1).

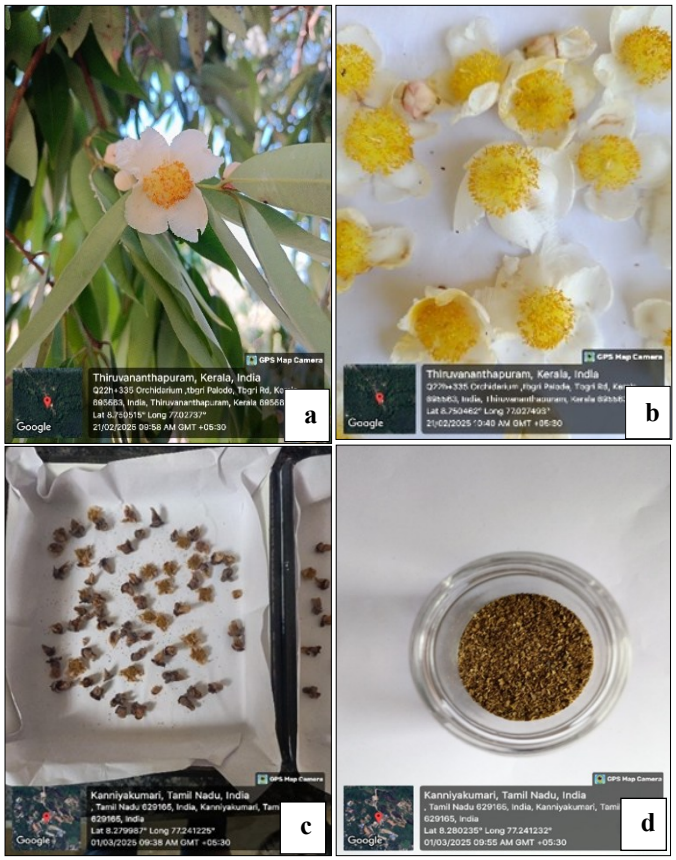

Plate 1. Step wise collection and preservation of *Nagakesara* flowers

2.2. Collection of water samples

Two water samples were collected from Nattalam Panchayat, Kanyakumari District, Tamil Nadu (Latitude 8.274632° N and Longitude 77.238447° E). Sample 1 was river water collected from a river flowing through the panchayat; this water was abstracted by the Kanyakumari District Water Authority, pumped, and supplied to the local population for drinking purposes. Sample 2 was collected from a public well within the same panchayat (Latitude 8.281474° N and Longitude 77.235229° E), which serves as a drinking water source for the local population. The distance between the two sampling sites was approximately 3 km.

2.3. Preparation of *hima kalpana* (cold infusion)

Soaked one part (48 gm or 1 Pala) of coarsely ground powder of *Nagakesara* flower in six parts (288 ml) of water (Sample I: River water, Sample II: Public well water) for the entire night. In the morning, macerate and filter it (Plate 2).

2.4. Turbidity test

The turbidity of the collected water samples was assessed using standardized procedures as per the guidelines of the World Health Organization (WHO) and Bureau of Indian Standards (BIS), Government of India - IS: 3025 (Part 10): 1984 (RA 2002). The turbidity test was performed as part of the water quality assessment to determine the

Plate 2. Process of *Hima Kalpana* (Cold Infusion) preparation

turbidity of the given water samples. This method is based on the principle that turbidity is measured by comparing the intensity of light scattered by suspended particles in the sample under defined conditions with the intensity of light scattered by a standard reference solution under the same conditions. Standard turbidity solutions of 0.02, 10, and 1000 Nephelometric Turbidity Units (NTU) and Millipore water were used as reagents, and the analysis was carried out using a turbidity meter (nephelometer) with appropriate sample cells. Prior to sample analysis, the nephelometer was calibrated according to the manufacturer's operating instructions, and at least one standard was run with each batch within the calibration range to ensure reliable readings across all sensitivity ranges used. For turbidity measurement, the water samples were gently agitated to uniformly disperse suspended solids, allowed to stand until air bubbles dissipated, and then transferred into the sample cells. Turbidity values were read directly from the instrument display and recorded in NTU (Fig. 1). Standard reference for turbidity range (NTU) is given in the Table 1.

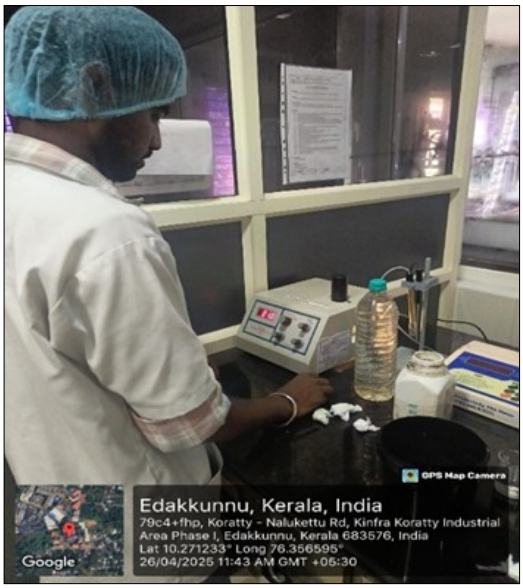

Fig. 1. Turbidity test through the turbidity meter

Table 1. Standard reference for turbidity range (NTU)

Sl. No.	Turbidity Range (NTU)	Report to the nearest (NTU)
1	0 - 1	0.05
2	1 - 10	0.1
3	10 - 40	1
4	40 - 100	5
5	100 - 400	10
6	400 - 1000	50
7	Greater than 1000	100

2.5. *In-vitro* studies

2.5.1 Most Probable Number (MPN) Test: The analysis of total coliforms and *Escherichia coli* in drinking water was carried out using the Most Probable Number (MPN) fermentation technique, in accordance with the procedures described in APHA, Standard Methods for the Examination of Water and Wastewater, 24th Edition (2023). This method is applicable for the detection of total coliforms and *E. coli* in water samples. The analysis was performed using a biosafety cabinet, conical flasks, micropipettes, screw-capped test tubes, an incubator, and culture media including Lauryl Tryptose broth (LST), Brilliant Green Lactose Bile (BGLB) broth, MacConkey agar, and Durham tubes. For the presumptive test, the water sample was shaken vigorously approximately 25 times, after which 10 mL of the sample was inoculated into five tubes containing 10 mL of double-strength LST broth, 1 mL into five tubes of single-strength LST broth, and 0.1 mL into five tubes of single-strength LST broth. The inoculated tubes were incubated at 35 ± 0.5°C and examined after 24 ± 2 hours for growth, gas production, and acid formation indicated by yellow coloration. Tubes showing no gas or acid reaction were re-incubated and re-examined at 48 ± 3 hours, and the absence of both reactions was considered negative; tubes showing growth without gas or acid were subjected to confirmatory testing, along with appropriate controls. For the confirmed test, a loopful of culture from each positive LST tube was transferred to BGLB broth (pH 7.2 ± 0.2)

and incubated at 35 ± 0.5°C for up to 48 ± 3 hours, with gas formation in the Durham tube at any time during incubation confirming the presence of total coliforms. For the completed test, cultures from positive BGLB tubes were streaked onto MacConkey agar plates and incubated at 35 ± 0.5°C for 24 ± 2 hours; typical coliform colonies were further inoculated into single-strength LST broth and nutrient agar slants and incubated under the same conditions. Gas production in LST broth within 48 ± 3 hours, along with Gram-negative bacilli observed from Gram staining of 24-hour nutrient agar slant cultures, confirmed the presence of total coliforms (Plate 3).

2.5.2. Minimum Inhibitory Concentration (MIC) - Macro Broth Dilution Method: The minimum inhibitory concentration (MIC) of the test sample was determined using the macro broth dilution method, following the procedures described in the Manual on Antimicrobial Susceptibility Testing by Dr. M. K. Lalitha. The objective of this assay was to determine the lowest concentration of the sample capable of inhibiting visible bacterial growth. The analysis was performed using Mueller–Hinton broth (MHB) and Mueller–Hinton agar (MHA), with *Streptomycin* (1,000 ppm) used as the standard reference drug, under aseptic conditions in a biosafety cabinet (Class II). Test organisms included *Staphylococcus aureus* (NCIM 2127) and *E. coli* (NCIM 2256). Test tubes were arranged and labelled according to the required concentrations of the sample, along with antibiotic and blank controls. Each tube was filled with 1 mL of MHB, after which 1 mL of the test sample was added to the first tube and serially diluted to obtain the desired concentration range, discarding 1 mL from the final tube to maintain uniform volume. Subsequently, 0.1 mL of an overnight broth culture adjusted to 0.5 McFarland turbidity was inoculated into each tube. All tubes were incubated at 37°C for 24 hours and then examined visually for turbidity. To confirm inhibitory activity, aliquots from each tube were streaked onto MHA plates and further incubated at 37°C for 24 hours. The MIC was defined as the lowest concentration of the sample that showed no visible growth in broth and no bacterial growth on agar plates (Fig. 2).

Plate 3. a. Nutrition broth fills in the test tube; b. Test samples add in the test tubes; c. After the incubation time gas formation in the test tubes

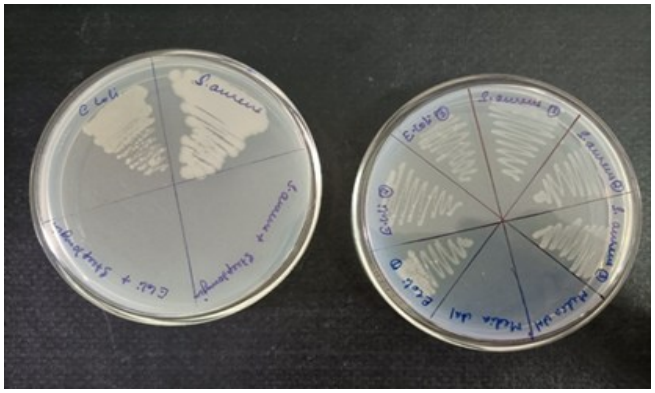

Fig. 2. MHA plates

2.5.3. Analysis for Antibacterial activity - Agar well diffusion Method: The antibacterial activity of the test sample was evaluated using the agar well diffusion method, following the procedure described in Microbiology: An Introduction by Tortora, Funke, and Case, 9th Edition, Chapter 20. The objective of this assay was to assess the antibacterial potential of the given sample against selected bacterial pathogens. The study was carried out under aseptic conditions using Soybean Casein Digest Medium (SCDM) for inoculum preparation and Mueller–Hinton agar (MHA) as the test medium. The test organisms used were *Staphylococcus aureus* (NCIM 2127) and *Escherichia coli* (NCIM 2065). A loopful of bacterial culture from working stock slants was transferred into 5 mL of SCDM and incubated at 37°C until the turbidity matched the 0.5 McFarland standard. Sterile Petri plates were prepared by pouring approximately 25 mL of MHA and allowed to solidify. The standardized bacterial inoculum was uniformly spread over the agar surface using a sterile swab. Wells of 8 mm diameter were aseptically punched into the agar using a sterile cork borer, and 100 µL of the test sample was dispensed into each well. The plates were kept in the biosafety cabinet to allow diffusion of the sample into the agar and then incubated at 37°C for 24 hours. After incubation, the diameter of the zone of inhibition surrounding each well was measured in millimeters using a ruler and recorded as an indicator of antibacterial activity (Plate 4).

Plate 4. a. Addition of sample in MHA plate; b. Zone of inhibition after incubation (Agar well diffusion method)

3. Results and discussion

3.1. Turbidity analysis findings

As presented in Table 2, the turbidity values of the river water and public well water samples were approximately 10 NTU and less than 2 NTU, respectively. Following processing with *Nagakesara* flower using the *Hima Kalpana* (cold infusion) method, the turbidity of the river water sample remained at 10 NTU, while that of the public well water sample continued to be below 2 NTU. When any herbal powders are added to a water sample, the turbidity typically increases because of the particles in the powder, however in this case, the turbidity did not decrease. As a result, the *Nagakesara* exhibits little activity in the turbidity of the water.

The turbidity of water is the reduction of transparency due to the presence of particulate matter such as clay or silt, finely divided organic matter, plankton or other microscopic organisms. These cause light to be scattered and absorbed rather than transmitted in straight lines through the sample. The method is applicable to drinking, surface and saline waters in the range of turbidity O-40 NTU. Higher values may be obtained by dilution of the sample. The values are expressed in Nephelometric Turbidity Units (NTU).

3.2. *In-vitro* Study

Table 3 depicts the MPN test report (MPN/100 mL), indicating the presence of total *coliforms*, fecal *coliforms*, and *E. coli* in river water and public well water samples without *Nagakesara* treatment. Treatment with *Nagakesara* flower resulted in decreased MPN values, suggesting a substantial antibacterial effect against coliforms and *E. coli* at the tested concentration and duration.

The Most Probable Number (MPN) test determines whether *coliforms* are present in the water sample. This

method involves adding measured amounts of water to a number of tubes that contain a liquid growth medium indicator. A distinctive colour shift and growth are observed in the media that are exposed to one or more indicator bacteria. Those that just receive an inoculum of water without indicator bacteria do not experience the colour change. The MPN test consists of three stages: presumptive test, confirmed test, and completeness test.

In presumptive test, a particular enrichment method for *coliform* bacteria, is carried out in fermentation tubes that comprise inverted Durham tubes for the detection of fermentation gas and a selected growth medium (MacConkey lactose broth). Measured amounts of the water sample to be examined are inoculated into a series of lactose broth tubes. Three or four groups of three, five, or more tubes may make up the tube series. Lactose, occasionally a surfactant like Na-lauryl sulfate or Na-taurocholate (bile salt), and frequently a pH indicator colour, like bromcresol purple or brilliant green, are the primary selection variables present in the medium. The reason lactose has a selective effect is that whereas *coliform* bacteria and a few other types of bacteria can ferment it, many other bacteria cannot. Many other bacteria, including spore formers, are inhibited by the surfactant and dye, but *coliform* bacteria are unaffected.

This confirmed test serves to confirm the presence of *coliform* bacteria when either a positive or doubtful presumptive test is obtained. In completed test, the initial turbidity values of public well and river water samples without *Nagakesara* treatment were <2 and 10 NTU, respectively. Following treatment with *Nagakesara* - *Hima Kalpana*, the turbidity values remained <2 and 10 NTU, indicating that *Nagakesara* did not significantly reduce turbidity. Notably, the addition of herbal powders typically increases water turbidity; however, *Nagakesara* treatment did not exhibit this effect, suggesting minimal impact on water turbidity.

The Most Probable Number (MPN) test detected the presence of *coliform* bacteria in well and river water samples. After treatment with *Nagakesara* - *Hima Kalpana*, the *coliform* and *E. coli* counts decreased, indicating the antimicrobial activity of *Nagakesara*.

As shown in Table 4, the growth patterns of *E. coli* and *Staphylococcus aureus* were evaluated in public well water and river water samples, with the following observations: public well water and river water: At the first dilution, both bacteria exhibited a slight increase in growth, as indicated by the streak lines. At the second dilution, growth was observed in both bacteria's streak lines. At the third dilution, both bacteria displayed robust growth in streak lines and the mother inoculum. Control Groups: Negative Control (MHB only): No bacterial growth was observed. Positive Control (Streptomycin + MHB): Both bacteria were inhibited, with no growth observed. MHB-Organism Control: Robust bacterial growth was observed in both the mother inoculum and streak lines, indicating a positive control. The results indicate that *E. coli* and *S. aureus* were present in both river water and public well water samples and were able to grow on MHA plates. The *Nagakesara* treatment had a

Table 2. Turbidity test report

Sl. No.	Parameters	River water		Well water		Unit	Test Method
		Without *Nagakesara* Flower	With *Nagakesara* Flower	Without *Nagakesara* Flower	With *Nagakesara* Flower		
1	Turbidity	10	10	<2	<2	NTU	IS 3025 Part 10

Table 3. MPN test report

Sl. No.	Parameters	River Water		Well Water		Test Method
		Without *Nagakesara* Flower	With *Nagakesara* Flower	Without *Nagakesara* Flower	With *Nagakesara* Flower	
1	Total *Coliforms* (MPN/100 ml)	1600	920	280	150	Standard Methods for the Examination of water & waste water, APHA-24th Edn. 2023, Chap-9221 B
2	Fecal *Coliforms* (MPN/100 ml)	920	540	120	70	Standard Methods for the Examination of water & waste water, APHA 24th Edn. 2023, Chap-9221 E
3	*E.coli* (MPN/100 ml)	540	350	<1.8	<1.8	Standard Methods for the Examination of water & waste water, APHA-24th Edn. 2023, Chap-9221 F

modest impact on both bacteria at the first dilution, with minimal inhibitory effects observed.

Table 5 summarizes the MIC test outcomes assessed by the agar well diffusion method using negative and positive controls, along with *Nagakesara* - treated river water and public well water samples (*Hima Kalpana*), against *S. aureus* and *E. coli*. Following a 24-hour incubation period, measurable zones of inhibition were observed, indicating antimicrobial activity. The results indicate that *Nagakesara* demonstrates moderate to limited antibacterial efficacy against both bacterial strains.

The Macro Broth Dilution Method of MIC test revealed that *Nagakesara* had a modest impact on *S. aureus* and *E. coli* at the initial dilution. In the Agar well diffusion method, *Nagakesara* treated river and public well water samples exhibited zone formation after a 24-hour incubation period, indicating antimicrobial activity against *S. aureus* and *E coli*.

Studies have demonstrated that various parts of *M. ferrea* (*Nagakesara*) possess significant antimicrobial properties. Specifically, coumarins isolated from *Nagakesara* blossoms, including 4-alkyl and 4-phenyl 5, 7-dihydroxycoumarins, have shown selective antibacterial activity against resistant strains of Gram-positive bacteria (Verotta *et al.,* 2004).

The chloroform extract obtained from the stem bark of *M. ferrea* (*Nagakesara*) has exhibited significant antibacterial efficacy against both Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacterial strains. This finding suggests the potential of *Nagakesara* extract as a broad-spectrum antimicrobial agent (Mazumder *et al*., 2003)

The results demonstrate that *Nagakesara* exhibits antimicrobial properties, primarily through bacteriostatic action and warrants further investigation into its potential applications in water treatment. This investigation evaluated the efficacy of *M. ferrea* flower extract prepared *via Hima Kalpana* (cold infusion) in mitigating water turbidity and inhibiting the growth of *E. coli* and *S. aureus*. Building on prior research demonstrating the antimicrobial potential of plant-derived extracts including their capacity to reduce water turbidity, this study provides novel insights into the bioactive properties of *M. ferrea* flowers. The results may inform future research aimed at optimizing the antimicrobial and turbidity-reducing properties of *M. ferrea* cold infusion, potentially enhancing its applications in water treatment.

4. Conclusion

The findings of the present study indicate that *Nagakesara* (*Mesua ferrea* L.) flower - based *Hima Kalpana* has potential utility as a community-level intervention for drinking water treatment. In view of its demonstrated antibacterial activity against *coliform* bacteria and *Escherichia coli*, together with moderate inhibitory

Table 4. MIC test report - Macro broth dilution method

Sl. No.	Parameter	Observation in MHA plates								
		Well water			River water			Media Control (MHB only)	Organism + MHB + Streptomycin	MHB + Organism
		1st Dilution	2nd Dilution	3rd Dilution	1st Dilution	2nd Dilution	3rd Dilution			
1	*Staphylococcus aureus* NCIM 2127	Slight growth in streak lines	Growth in streak lines	Growth in mother inoculum and thick growth in streak lines	Slight growth in streak lines	Growth in streak lines	Growth in mother inoculum and thick growth in streak lines	No Growth	No Growth	Thick growth in mother inoculum and in streak lines
2	*Escherichia coli* NCIM 2065	Slight growth in streak lines	Growth in streak lines	Growth in mother inoculum and thick growth in streak lines	Slight growth in streak lines	Growth in streak lines	Growth in mother inoculum and thick growth in streak lines	No Growth	No Growth	Thick growth in mother inoculum and in streak lines

Table 5. MIC test report - Agar well diffusion method

Sl. No.	Test organism	Test result - (zone of inhibition in mm)		Standard drug	Test method
		River Water	Well water		
1	*Staphylococcus aureus* NCIM 2127	13 mm	13 mm	Streptomycin (1000 ppm)-28 mm	CKL/MB/MOA-044
2	*Escherichia coli* NCIM 2065	15 mm	15 mm	Streptomycin (1000 ppm)-25 mm	CKL/MB/MOA-044

effects on *Staphylococcus aureus*, this method may serve as a cost-effective supplementary disinfection approach to enhance the microbiological safety of drinking water, particularly in rural and resource-constrained settings. The simplicity of the cold infusion process, minimal infrastructural requirements, and use of a culturally familiar medicinal plant support its applicability for decentralized implementation at household and community levels. Incorporation of this traditional Ayurvedic practice into public health initiatives, water safety planning, and community education programs may contribute to improved access to safe drinking water while encouraging sustainable, environmentally friendly, and locally sourced purification practices. Additionally, with appropriate standardization, safety assessment, and regulatory validation, this approach holds promise for application in emergency water treatment, disaster response, and preventive public health strategies.

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