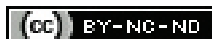


Evaluating the Effect of *Bilvadi Jala Nirvishikaran* Yoga in Different Samples of Polluted Water: A Research Protocol

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ABSTRACT

Introduction: Water is essential for life, and managing water resources is crucial in addressing the global issue of water pollution. Water pollution occurs when substances alter its physical, chemical, or biological properties, harming living organisms. Domestic, agricultural, and industrial activities release pollutants into the environment, contributing to this contamination. Ancient texts, such as those by Acharya Sushruta and Vagbhatta, mention various herbal methods for water purification. Specifically, Acharya Vagbhatta highlights a purification technique involving a decoction of herbs like *Bilva* (Aegle marmelos), *Adhaki* (Cajanus cajan), *Yavakshar* (Hordeum vulgare), *Patola* (Trichosanthes cucumerina), *Balghik* (asafoetida), *Ushan* (Piper nigrum), *Shreeparni* (Gmelina arborea), and *Shalmali* (Bombax ceiba). This herbal combination is believed to neutralise poisons in water and other substances, rendering them safe.

Need of the study: Modern water purification methods often involve chemicals like chlorine, which can produce harmful by-products like Trihalomethanes (THMs) that pose significant health risks, including cancer. To address this, ancient texts, particularly those by Acharya Vagbhatta, suggest natural methods for purifying water. One such method, *Bilvadi Jala Nirvishikarana*

yoga, involves a decoction of plants like *Bilva*, *Adhaki*, *Yavakshar*, *Patola*, and others, which detoxifies contaminated substances. The present study aimed to evaluate the effectiveness of this traditional purification technique through in-vitro experiments. All results will be evaluated at a 95% significance level to assess their statistical relevance and determine the study outcomes.

Aim: To evaluate the effect of *Bilvadi Jala Nirvishikaran* on different samples of polluted water.

Materials and Methods: The experimental in-vitro study will be conducted by the Department of Agadatantra at Mahatma Gandhi Ayurved College, Hospital and Research Centre, Salod (H), Wardha, Maharashtra, India at the National Environmental Engineering Research Institute (NEERI), Nagpur, Maharashtra, India from January 2025 to February 2025. The present study will be completed between January and February 2025. Polluted water samples will be collected from the Pavanar and Dham rivers in the Wardha district. Poisoned water samples will be prepared by adding *Dhatūra* and organophosphorus compound powder to plain tap water. All the samples will be tested for physicochemical parameters before and after the administration of *Bilvadi Jala Nirvishikaran* yoga. The results will be compared among all samples.

Keywords: *Agadatantra*, Organophosphorus compound, *Pavanar* river, Poisoned water, Water pollution, Water purification

INTRODUCTION

The science of life is Ayurveda, which has been demonstrating the optimum lifestyle since the beginning of humanity [1]. *Agadatantra* is one of the branches of *Ashtanga* Ayurveda (the eighth branch of Ayurveda) according to classical texts, which deals with a detailed study of *visha* (poison), its classification, examination, as well as the treatment of poisoning caused by snake bites, insects, worms, scorpions, rodents, and other poisons resulting from inappropriate combinations of substances or drugs. *Visha* (poison) is defined as a substance capable of causing harm to the human body when ingested, inhaled, or consumed [2]. Poison has a long history, as do the symptoms of poisoning. Plant and animal toxins, as well as certain minerals, were among the most common early poisons. Ancient Acharyas have cited the *Agada* as a remedy against the effects of *visha* (poison) [2]. These *Agada* are employed as anti-poisonous treatments for both inanimate and living poisoning cases. Certain *Agada*, according to the ancient literature, have broad-spectrum action that counteracts the effects of both living and inanimate toxins [2].

Water is the most significant factor in forming the environment and regulating the climate. It is one of the most critical substances that have a substantial impact on life. The biological, physical, and chemical characteristics of water are typically used to characterise its quality. However, water quality is decreasing, and aquatic biota is being lost due to significant and diverse pollution in aquatic

ecosystems caused by rapid industrialisation and the negligent use of chemical pesticides and fertilisers in agriculture [3]. At a global level, according to World Health Organisation (WHO), more than 2 billion people consume water contaminated with faeces [4]. In Maharashtra, according to the State Economic Survey 2021-22, 44% of the population has been affected by waterborne diseases, with 8.75 lakh people affected in 2019, 4.9 lakh in 2020, and 4.6 lakh in 2021 [5], according to a central government report. Rivers receive 3,000 million litres of untreated sewage and industrial effluents annually [6]. The human population suffers from waterborne diseases as a result of using contaminated water.

The *Panchmahabhuta* (five elements) theory, of which *Jala* (water) is one, is explained in Ayurvedic literature. These elements are considered crucial to both nature and the human body. Rainwater is referred to in literature as *Divya Jala* (Divine Water), which is bestowed by Lord Indra and is said to be pure and safe to drink. Water is the most abundant and vital component of the environment, making up approximately 70% of the human body. According to currently available data, Earth is the only planet with nearly 70% water, yet only a small portion of it is fresh water. The ease of access to fresh water is a significant issue nowadays. Pesticides, fertilisers, industrial waste, and household sewage are the qualitative causes of water scarcity [7]. Water is considered unfit for consumption if it has abnormal values for criteria related to water pollution, such as high Biological Oxygen Demand (BOD), high Chemical Oxygen

Demand (COD), suspended particles, acidity, alkalinity, and hardness. It can be argued that providing clean, nutrient-rich, and disease-free water requires constant monitoring [8].

In the current scenario, many methods have been established for the purification of water; however, methods that employ chemicals for purification purposes can be more harmful to living beings than contaminated water (e.g., chlorine). Trihalomethanes (THMs) are produced when chlorine reacts with organic materials in water, and these can accumulate in the body over time, increasing the risk of bladder, colon, breast, and skin cancers [9]. Hence, there is a necessity to detoxify and purify contaminated water without adverse effects while improving water quality. Ancient texts describe methods for purifying “*Dushita Jala*” (polluted water) dating back to the Vedic period. Among them, *Acharya Vagbhatta* has mentioned a method for the purification of drinking water. A decoction of *Bilva* (*Aegle marmelos* Carr.), *Adhaki* (*Cajanus cajan* Millsp.), *Yavakshar* (*Hordeum vulgare* Linn. Syn. *H. sativum* Pers.), *Patola* (*Trichosanthes cucumerina* Linn.), *Balvik* (*asafoetida*), *Ushan* (*Piper nigrum* Linn.), *Shreeparni* (*Gmelina arborea* Roxb.), and *Shalmali* (*Bombax ceiba* Linn.) has been found to be effective when poured over poisonous substances such as firewood, fuel, drinking water, clothes, beds, seats, armour, ornaments, umbrellas, and houses; it immediately renders them free of poison [10].

The present study aimed to assess the effectiveness of *Bilvadi Jala Nirvishikarana* yoga through in-vitro experiments.

Aim

Evaluation of the Effect of *Bilvadi Jala Nirvishikaran* yoga in Polluted Water samples.

Primary Objectives:

- To study the physicochemical properties of *Bilvadi Jala Nirvishikarana* yoga.
- To assess the effect of *Bilvadi Jala Nirvishikarana* yoga on two samples of polluted river water (Pavanar and Dham Rivers in Wardha, Maharashtra, India).
- To examine the effect of *Bilvadi Jala Nirvishikarana* yoga on two individual water samples mixed with organophosphorus compounds and *Dhatura* (*Datura metel*) *bija churna*.

Secondary Objectives:

- To determine the optimum dose and duration of *Bilvadi Jala Nirvishikarana* yoga for purifying polluted water samples.
- To compare the purified water samples with distilled water in terms of physicochemical parameters.

Research question: Is *Bilvadi Jala Nirvishikaran* yoga effective in purifying polluted water?

Null hypothesis: *Bilvadi Jala Nirvishikarana* yoga is not effective in purifying polluted water.

Alternate hypothesis: *Bilvadi Jala Nirvishikarana* yoga is effective in purifying polluted water.

REVIEW OF LITERATURE

Bilva:

- Antimicrobial activity:** Significant antimicrobial effects of the ethanolic extract of dried fruit pulp of *Aegle marmelos* have been observed against various intestinal pathogens, such as *Shigella boydii*, *S. sonnei*, and *S. flexneri*, as well as bacteria like *E. coli*, *Salmonella typhi*, and *Pseudomonas aeruginosa*. It has been proposed that certain phytochemicals, including phenols, tannins, and flavonoids, are effective against all of these pathogens [11].
- Antifungal activity:** The antifungal activity of essential oil isolated from the leaves of the bael plant was evaluated using a spore germination assay. It was found to possess variable efficacy against different fungal isolates, as reported by various

scientists. They have documented the antifungal activity of different extracts of the leaves of *Aegle marmelos* [12].

- Anticancer activity:** A study investigating the effects of extracts from Bangladeshi medicinal plants on the in-vitro proliferation of human breast cancer cell lines and the expression of the oestrogen receptor alpha gene found that the extract from *Aegle marmelos* has antiproliferative effects on both the MCF7 and MDA-MB-231 cell lines [13].
- Aadhaki (*Cajanus cajan* Millsp.):** *Acharya Kaidev* has mentioned *Aadhaki* as *krumighna* (anthelmintic) in *Kaidevnighantu*, *Dhanya varga* [14].
- Yavakshar (*Hordeum vulgare* Linn. Syn. *H. sativum* Pers.):** *Yavakshar* is classified as *vishaghna* (anti-poison) in *Dhanvantari Nighantu* within the *Satapuspadi varga* [15]. It is also noted in *Rajnighantu*, *pipalyadi varga* [16].
- Hingu (*Ferula narthex* Vatke):** *Hingu* is classified as *vishaghna* (anti-poison) and *krumihara* (anthelmintic) in *Bhavprakash Nighantu*, within the *Haritakyadi varga* [17]. It is also mentioned in *Dhanvantari Nighantu*, *Shatpushpadi varga* [18].
- Maricha (*Piper nigrum* Linn.):** *Maricha* is considered a *deepana* (appetiser), *krumighna* (anthelmintic), and *vishaghna* (anti-poison) in *Dhanvantari Nighantu*, within the *Shatpushpadi varga* [19], and is also found in the *Haritakyadi varga* of *Bhavprakash Nighantu* [20].
- Shreeparni (*Gambhari*) (*Gmelina arborea* Roxb.):** *Shreeparni* (*Gambhari*) is described as *vishaghna* (anti-poison) in the *Aushadhi varga* of *Kaideva Nighantu* [21].

MATERIALS AND METHODS

The present experimental study (in-vitro study) will be conducted from January 2025 to February 2025. It will be carried out by the Department of Agadatantra at Mahatma Gandhi Ayurved College, Hospital and Research Centre, Salod (H), Wardha, Maharashtra, India at the National Environmental Engineering Research Institute (NEERI) in Nagpur, Maharashtra, India. Institutional Ethics Committee (IEC) approval has been obtained for the experimental in-vitro study, with the reference number MGACHRC/IEC/Jun-2024/814.

Collection of raw materials: Collect plant materials as mentioned in [Table/Fig-1]: *Bilva* (*Aegle marmelos* Carr.) [22], *Adhaki* (*Cajanus cajan* Millsp.) [23], *Yavakshar* (*Hordeum vulgare* Linn. Syn. *H. sativum* Pers.) [24], *Patola* (*Trichosanthes cucumerina* Linn.) [25], *Balvik* (*asafoetida*) [26], *Ushan* (*Piper nigrum* Linn.) [27], *Shreeparni* (*Gmelina arborea* Roxb.) [28], and *Shalmali* (*Bombax ceiba* Linn.) [29]. These ingredients required for the preparation of *Bilvadi Jala Nirvishikaran* yoga will be collected from the vendor cultivating and providing medicines under Good Manufacturing Practice (GMP), Essen Herbs India Pvt. Ltd., Nagpur, Maharashtra, India [22-29].

S. No.	Name of drug	Botanical name	Family	Part used	Quantity
1.	<i>Bilva</i> [22]	<i>Aegle marmelos</i> Carr.	Rutaceae	Root	100 gm
2.	<i>Aadhaki</i> [23]	<i>Cajanus cajan</i> Millsp.	Fabaceae	Seeds	100 gm
3.	<i>Yava</i> [24]	<i>Hordeum vulgare</i> Linn. Syn. <i>H. sativum</i> Pers.	Gramineae	Entire plant for kshar	100 gm
4.	<i>Patola</i> [25]	<i>Trichosanthes cucumerina</i> Linn.	Cucurbitaceae	leaf	100 gm
5.	<i>Hingu</i> [26]	<i>Ferula narthex</i> Vatke.	Apiaceae	Extract	100 gm
6.	<i>Marich</i> [27]	<i>Piper nigrum</i> Linn.	Piperaceae	Fruit	100 gm
7.	<i>Shreeparni</i> [28]	<i>Gmelina arborea</i> Roxb.	Verbenaceae	Root	100 gm
8.	<i>Shalmali</i> [29]	<i>Bombax ceiba</i> Linn.	Bombacaceae	Extract	100 gm

[Table/Fig-1]: Ingredients of *Bilvadi Jala Nirvishikaran* yoga [22-29].

Authentication of raw materials: The drugs will be authenticated based on morphological and organoleptic characteristics at the Department of Dravyaguna, Mahatma Gandhi Ayurvedic College, Hospital and Research Centre, Salod, Wardha, Maharashtra, India.

Details of the Drug:

Classical method of preparation of *Bilvadi Jala Nirvishikaran*:

The preparation of *Bilvadi Jala Nirvishikaran* yoga will take place at Dattatray Rasashala, MGACH and RC, Salod (H), Wardha, as referenced in *Ashtanga Sangraha, Sutrasthan* (8/103-105) [30].

Yavakshar preparation [31]: The entire *Hordeum vulgare* (barley) plant will be harvested, thoroughly cleaned, and sun-dried. Once dried, it will be placed in a large iron pan and completely burned. The resulting ash will be collected after it cools naturally. This ash will then be mixed with four times its volume of water and thoroughly stirred. The mixture will be filtered through cloth and left to stand overnight. The next day, the clear supernatant water will be carefully transferred to another container. This water will then be heated over a mild fire until all the water evaporates, leaving behind a white substance known as *Yava Kshar*. This *Yavakshar* will be used as one of the ingredients to prepare *Bilvadi Jala Nirvishikaran* yoga.

***Bilvadi Jala Nirvishikaran* yoga preparation [10,30]:** The plant materials, including the stem, leaves, fruit, and roots of *Bilva* (*Aegle marmelos* Carr.), *Adhaki* (*Cajanus cajan* Millsp.), *Yavakshar* (*Hordeum vulgare* Linn. Syn. *H.*), *Patola* (*Trichosanthes cucumerina* Linn.), *Balhhik* (*asafoetida*), *Ushan* (*Piper nigrum* Linn.), *Shreeparni* (*Gmelina arborea* Roxb.), and *Shalmali* (*Bombax ceiba* Linn.), will be collected in equal amounts, as mentioned in [Table/Fig-2], and dried in the sunshine. Once dried, the materials will be chopped, crushed, or ground into a coarse powder (*churna*). If the plant materials are fresh (*Ardra dravya*), they will be thoroughly washed before use. The *churna* (powder) will then be added to a vessel containing 16 times its amount in water. The vessel will be placed on a medium flame and heated until the water reduces to one-eighth of its original volume. The vessel will then be removed from the flame, and the contents will be strained. The resulting liquid will be referred to as the prepared *Kwath* (decoction). This prepared *Kwath* will be named *Bilvadi Jala Nirvishikaran* yoga.

according to the Ayurvedic Pharmacopoeia of India (API) guidelines [32], ensuring consistency and efficacy.

The decoction of *Bilvadi Jala Nirvishikaran* yoga will be prepared according to the standard operating procedures outlined in the *Sharangadhar Samhita*, *Madhyam Khand*, 11th Adhyay, Shlokas 102-104 [10], at a GMP-certified pharmacy at Dattatray Rasashala. After preparation, the decoction's physicochemical analysis will be conducted at the analytical laboratory of Mahatma Gandhi Ayurved College Hospital and Research Centre, adhering to Good Laboratory Practices (GLP).

Polluted water samples will be collected from the Pavanar and Dham rivers, and samples of poisoned water will be prepared by adding *Dhatura beej churna* and an organophosphorus compound to plain water. The treatment of these polluted water samples with *Bilvadi Jala Nirvishikaran* yoga will then be carried out to assess its effectiveness. Observations will be made regarding the treatment's impact on water quality, and a conclusion will be drawn based on the results. Finally, the findings established through present study will be documented, confirming the efficacy of the treatment and its potential application in water purification.

Water Samples: 41

Intervention: *Bilvadi Jala Nirvishikaran* yoga

The study will commence with the collection of ingredients: *Bilva* (*Aegle marmelos* Carr.), *Adhaki* (*Cajanus cajan* Millsp.), *Yavakshar* (*Hordeum vulgare* Linn.), *Patlee* (*Trichosanthes cucumerina* Linn.), *Balhhik* (*Ferula narthex* Vatke), *Ushan* (*Piper nigrum* Linn.), *Shreeparni* (*Gmelina arborea* Roxb.), and *Shalmali* (*Bombax ceiba* Linn.) for the preparation of *Bilvadi Jala Nirvishikaran* yoga from authenticated sources, followed by a thorough authentication process of these drugs. Subsequently, *Bilvadi Jala Nirvishikaran* yoga will be prepared as a decoction. Decoction will be prepared by following a GMP.

To prepare *Bilvadi Jala Nirvishikaran* yoga, begin by collecting the necessary plant materials, which may include the stem, leaves, fruit, seeds, and roots. These parts should be thoroughly dried in the sunshine. Once dry, the materials should be chopped, crushed,

S. No.	Drugs	Rasa	Guna	Virya	Vipaka	Karma	Rogadhnta
1.	<i>Bilva</i>	<i>Kashaya Tikta</i>	<i>Ruksha Laghu</i>	<i>Ushna</i>	<i>Katu</i>	<i>Deepan, Balya Pachan, Grahi,</i>	<i>Shotha, Atisar Grahani</i>
2.	<i>Aadhaki</i>	<i>Kashaya, Madhur</i>	<i>Ruksha Laghu</i>	<i>Sheeta</i>	<i>Katu</i>	<i>Vishaghna Grahi, Ruchya</i>	<i>Raktadosha Krimi Roga</i>
3.	<i>Yava</i>	<i>Madhur, Kashay</i>	<i>Guru</i>	<i>Sheeta</i>	<i>Katu</i>	<i>Lekhan, Bala Kara, Vrishya</i>	<i>Pinasa, Kasa Swasa, Prameha</i>
4.	<i>Patola</i>	<i>Tikta, Katu</i>	<i>Snigdha Laghu</i>	<i>Ushna</i>	<i>Katu</i>	<i>Tridoshar Hridya, Varnya</i>	<i>Kasa, Jwar, Krimi, Kushtha</i>
5.	<i>Hingu</i>	<i>Katu</i>	<i>Tikshna Laghu</i>	<i>Ushna</i>	<i>Katu</i>	<i>Pachan, Kaphahar Ruchya, Vatahara</i>	<i>Shoola, Udar Gulma, Krimi</i>
6.	<i>Marich</i>	<i>Katu</i>	<i>Laghu, Ruksha</i>	<i>Ushna</i>	<i>Katu</i>	<i>Kaphahar Vathar Deepana</i>	<i>Kasa, Pravahika Hridrog, Krimi, Vish</i>
7.	<i>Shreeparni</i>	<i>Madhur, Tikta Kashaya</i>	<i>Guru</i>	<i>Ushna</i>	<i>Katu</i>	<i>Bhedana, Deepan, Pachan,</i>	<i>Shotha, Shool Arshas, Vish</i>
8.	<i>Shalmali</i>	<i>Madhur</i>	<i>Laghu, Snigdha</i>	<i>Sheeta</i>	<i>Madhur</i>	<i>Rasayan, Grahi, Balya</i>	<i>Vatrakta, Pravahika, Atisar, Pradara</i>

[Table/Fig-2]: Properties of *Bilvadi Jala Nirvishikaran* yoga.

Parameters of analytical study (Physicochemical analysis) of *Bilvadi Jala Nirvishikaran* yoga:

The following parameters must be measured for the physicochemical analysis of *Bilvadi Jala Nirvishikaran* yoga: Colour, odour, taste, pH in a 5% aqueous solution, viscosity, specific gravity, and total solid content (% w/w).

Experimental design: All these experiments will be conducted at the National Environmental Engineering Research Institute (NEERI), Nagpur, Maharashtra, India.

Study Protocol

The Institutional Ethics Committee has approved the study with reference number MGACHRC/IEC/Jun-2024/814. It will commence with the procurement of the necessary drugs, which will be carefully sourced from authorised suppliers to ensure quality and authenticity. Once acquired, the drugs will undergo authentication to verify their identity, quality, and potency. The drugs will be standardised

or ground into a coarse powder (*churna*). If the plant materials are fresh and wet (*ardra dravya*), they should be washed before use.

After placing the *churna* into a vessel, add water in a proportion of 16 times the amount of the *churna*. Set the vessel on a medium flame and allow it to simmer until the water reaches one-eighth of its original volume. Once the water has reduced sufficiently, remove the vessel from the flame and strain the liquid. This completes the preparation process.

An analytical study involving the physicochemical analysis of the decoction will be conducted by following Good Laboratory Practices (GLP) and as per API, assessing parameters such as colour, odour, taste, pH in a 5% aqueous solution, viscosity, specific gravity, and total solid content.

Concurrently, polluted water samples will be collected from the Pavanar and Dham rivers, as these two rivers are polluted in the

Wardha district of Maharashtra, India [33]. For experimental purposes, a sample of poisoned water will be prepared by adding *Dhatura* seed powder in its fatal dose to plain water, while another poisoned sample will be prepared using organophosphorus compounds, such as Dichlorodiphenyltrichloroethane (DDT), at a fatal dose.

A total of 41 water samples, as mentioned in [Table/Fig-3], including one sample of distilled water and 40 samples of polluted water, will be subjected to various water parameter tests. These tests will measure pH, total hardness, turbidity, bacterial count, alkalinity, chloride levels, BOD, Total Suspended Solids (TSS), light transmission, nitrate concentration, fluoride concentration, iron concentration, dissolved oxygen, total dissolved solids, electrical conductivity, and COD. Samples of poisoned water will contain specified amounts of Dhaturin mixed with *Dhatura* seed powder, as well as the presence of organophosphorus compounds in the poisoned water samples.

To determine the optimal dose and time for the efficacy of *Bilvadi Jala Nirvishikaran* yoga, the decoction will be added to the polluted and poisoned water samples in specified quantities and durations. From the 40 samples (each 100 mL) of polluted and poisoned water, one sample from each of four groups (Pavanar river water, Dham river water, organophosphorus compound poisoned water, and *Dhatura* seed powder poisoned water) will be kept as controls, while the remaining 36 samples will receive *Bilvadi Jala Nirvishikaran* yoga at different doses: 1 mL (20 drops), 1.5 mL (30 drops), and 2 mL (40 drops). These samples will be analysed at three different time intervals-30 minutes, 60 minutes, and 90 minutes-to ascertain the optimal purification time.

Post-treatment, all 36 samples will be compared against the parameters of distilled water, identifying the sample that most closely aligns with distilled water standards, as mentioned in [Table/Fig-3]. The dose and time that yield results nearest to the distilled water parameters will be considered optimal for purifying the polluted and poisoned water samples. This comprehensive methodological approach aims to establish the effective use of *Bilvadi Jala*

water samples will be prepared. In-vitro experiments will be conducted at the National Environmental Engineering Research Institute (NEERI), Nagpur, Maharashtra, India during the last week of January 2025 and the first week of February 2025. Data will be collected, and the analysis of the collected data will be completed in the first week of February 2025. The documentation of the observed data will be carried out during the second and third weeks of February.

Assessment criteria:

Objective parameters: They are explained in [Table/Fig-5].

Subjective parameters: Colour and Odour

Primary Outcomes:

- Bilvadi Jala Nirvishikaran* yoga is expected to significantly reduce chemical and biological contaminants in polluted water.
- The antimicrobial and anti-toxic effects of *Bilvadi Jala Nirvishikaran* yoga will improve the quality of polluted water.
- Bilvadi Jala Nirvishikaran* yoga will lower harmful parameters such as pH, BOD, COD, turbidity, and substances like fluoride and nitrates.
- Purifying water through *Bilvadi Jala Nirvishikaran* yoga will reduce the risks of skeletal fluorosis and gastrointestinal issues.

Secondary Outcomes:

- Bilvadi Jala Nirvishikaran* yoga presents a cost-effective and practical method for rural and disadvantaged communities.
- Bilvadi Jala Nirvishikaran* yoga will help reduce the environmental impact in areas that depend on polluted river water.
- Combining Ayurvedic practices with scientific research could provide sustainable and accessible solutions for water pollution.

STATISTICAL ANALYSIS

In present experimental in-vitro study, statistical analysis will be performed using Statistical Packages of Social Sciences (SPSS) software. Appropriate statistical tests, such as the paired t-test

S. No.	Water samples	Water sample without BJNY	1 mL BJNY/100 mL of polluted river water sample			1.5 mL BJNY/100 mL of polluted river water sample			2 mL BJNY/100 mL of polluted river water sample			Distilled water sample
			30 min.	60 min.	90 min.	30 min.	60 min.	90 min.	30 min.	60 min.	90 min.	
1.	Pavanar river water samples	S-1	S-2	S-3	S-4	S-5	S-6	S-7	S-8	S-9	S-10	S-41
2.	Dham river water	S-11	S-12	S-13	S-14	S-15	S-16	S-17	S-18	S-19	S-20	
3.	<i>Dhatura</i> seed powder mixed water samples	S-21	S-22	S-23	S-24	S-25	S-26	S-27	S-28	S-29	S-30	
4.	Organophosphorus compound mixed water samples	S-31	S-32	S-33	S-34	S-35	S-36	S-37	S-38	S-39	S-40	

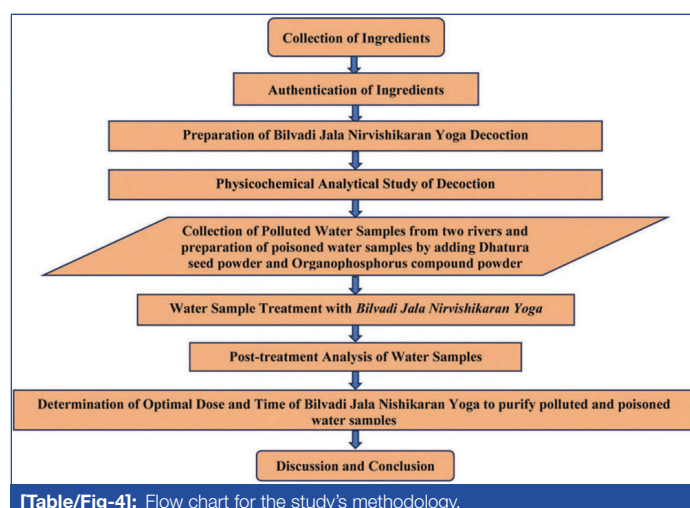
[Table/Fig-3]: Analysis of the river and poisoned water samples, followed by comparison with the distilled water sample.

BJNY: *Bilvadi jala nirvishikaran* yoga; S: Sample

Nirvishikaran yoga in water purification, precisely determining the necessary dose and duration to achieve water quality comparable to distilled water.

The study flow is described in [Table/Fig-4]. The study will begin with the procurement of raw drugs from authenticated sources in the first week of January 2025 and will conclude in the third week of February. Once the drugs are collected, their authentication will be performed at the analytical laboratory of study Institute during the second week of January. In the third week of January, a decoction of all the ingredients will be prepared at Dattatray Rasashala, Mahatma Gandhi Ayurved College Hospital and Research Centre. After preparation, a sample of the decoction will be sent for physicochemical analysis, following GLP, at the analytical laboratory of study Institute.

Simultaneously, in the last week of January 2025, water samples will be collected from the Pavanar and Dham rivers, and poisoned



[Table/Fig-4]: Flow chart for the study's methodology.

For polluted river water		For poisoned water
pH	Light transmission	HPLC of <i>Dhaturin</i>
Turbidity	Nitrate concentration	Organophosphorus compound
Temperature	Fluoride concentration	
Bacteria count	Iron concentration	
Alkalinity	Dissolved oxygen	
Chloride	Total dissolved solids	
Total hardness	Electrical conductivity	
Biological Oxygen Demand (BOD)	COD	
Total Suspended Solids (TSS)		

[Table/Fig-5]: Objective parameters for polluted and poisoned water.

and Analysis of Variance (ANOVA), will be applied based on the characteristics of the data. All results will be evaluated at a 95% significance level to assess their statistical relevance and determine the study outcomes.

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