



Research Article

AN EXPERIMENTAL STUDY ON THE EFFICACY OF VAGBHATOKTA JALA NIRVISHIKARANA YOGA ON POLLUTED WATER

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ABSTRACT

Background: Increased suffering and death due to impure water consumption, along with the failure of the outreach of advanced techniques to the deprived people with middle socioeconomic status, has directed the evaluation of efficacy of the classical Ayurvedic formulations used in water purification.

Aim: To evaluate the efficacy of *Vagbhatokta Jala Nirvishikarana Yoga* in terms of its physiochemical and antimicrobial qualities for the treatment of polluted river water.

Materials and Methods: The *Jala Nirvishikarana Yoga* (JNY) was prepared according to the classical method, and analytical study was performed. Optimum dose and period for JNY was calculated, and the water sample was treated accordingly. The sample was analysed for various physical, chemical and microbiological parameters before and after treatment. Stability and efficacy of the formulation was determined after 3 months and 1 year. **Results:** JNY is a thick, alkaline and viscous liquid bearing a characteristic taste and pleasant odour. The optimum dose and period was found to be three drops per 100 mL for 30 min of contact time. No significant change in the physiochemical parameters was observed except pH. Apart from the variation in pH, JNY was observed to have antimicrobial activity. Reduction in the coliforms and inhibition of bacterial and fungal colonies in the sample were observed. JNY was observed to be stable for 1 year at room temperature. **Conclusion:** JNY showed efficient antimicrobial activity and was stable and effective for at least 1 year at room temperature. However, in larger doses, its administration resulted in increase of pH of water.

KEYWORDS: *Jala Nirvishikarana Yoga*, Stability, Antimicrobial activity, pH, Dose, Period.

INTRODUCTION

As a result of industrialization, pollution of the natural resources has been one of the major concerns of the modern world. Among them, water pollution poses a huge risk to the well-being of the humans, as it is the most common source of diseases.^[1] Rivers are the most susceptible of contamination, as they are known to be the carriers of agricultural run-offs and wastes from the municipalities and industries.^[2]

Foul smell and presence of toxic substances are often the major concerns of the inhabitants living on the banks of polluted rivers.^[3] Worldwide, approximately 5 million of people or more have perished because of water-borne diseases^[1]. Annually, approximately 37.7 million people in India are known to be affected by water-borne diseases.^[4] Hence, the foremost objective of the environment protection agency should be to minimise the frequency of pollution-oriented problems and

monitor water quality provided for the purpose of drinking, irrigation etc.

The quality of water is usually analysed by considering various parameters, such as temperature, dissolved oxygen level, conventional variables (pH, total dissolved solids, conductivity), and the concentration of nutrients, metals and industrial chemicals. However, microbiological assessment of water source is done by the detection of indicators such as faecal coliforms, faecal enterococci and streptococci, sulphur-reducing clostridia etc.^[5] The faecal contamination of water is usually determined by detecting the presence of faecal coliforms by most probable number (MPN) and membrane filtration method.^[6]

Polluted water is usually treated chemically and biologically before their release in the water bodies. Purification of water by implementing the traditional and ancient method of India, i.e. by use of

herbs and shrubs, helps to cleanse the water while retaining its natural benefits.^[7] As such, environmental toxicology has been known to come under the purview of Agada tantra^[8] and thus, has a wide scope of research.

Therefore, the following study was conducted to evaluate the efficacy of an Ayurvedic formulation, Vagbhatokta's *Jala Nirvishikarna yoga*, in terms of its physiochemical and antimicrobial qualities for the treatment of polluted river water.

MATERIALS AND METHODS

Source of sample

The study was conducted at the Department of Agada Tantra, KLE University's Shri BMK Ayurveda Mahavidyalaya, Belgaum. The water sample was collected from Malaprabha river at Kadrolli, Belgaum (located between 15.6976° North latitude and 74.7352° East) by following standard operating procedures.

Jala Nirvishikarana yoga

Jala Nirvishikarana Yoga (JNY), a novel formulation, was prepared by adding 300 g of each drug: *Ajashrunji* (*Pistacia chinensis*), *Indravaruni* (*Citrullus colocynthis*), *Uttamarini* (*Daemia extensa*), *Guduchi* (*Tinospora cordifolia*), *Phanijjaka* (*Ocimum basilicum*) and *Ativisha* (*Aconitum heterophyllum*) in the form of ash and paste of extra ingredients (*Prativapa dravya*) into distilled water in a fixed ratio as shown in Table 1. The ash was adequately cooled till it turned white and analysed by *Rekhapurnatvum* test. *Prativapa dravya* was prepared by grinding and mixing of *Sarala Niryasa* (*Pinus roxburghii*), *Haridra* (*Curcuma longa*), *Daruharidra* (*Berberi saristata*), *Bruhat Ela* (*Amomum subulatum*), *Manjistha* (*Rubia cordifolia*), *Bakuchi* (*Psoralea corylifolia*), *Rasna* (*Alpinia galanga*) and *Ushira* (*Vetiveria zizanioides*). *Vagbhat's Ksharapakavidhi* was followed for preparing the formulation of JNY. JNY was stored in sealed glass bottle under cold condition for further use.

Table 1: Ratio of ingredients

	Ash	Water	<i>Prativapa Dravya</i>
Quantity	1 <i>Drona</i>	2000 Pala	8 Pala
JNY	100 g	800 g	16 g

Following its preparation, JNY was analysed for organoleptic properties (colour, odour and taste), physical characteristics (weight/mL, refractive index, specific gravity, pH, viscosity and total solids), organic properties (carbohydrates, reducing sugars, hexose sugars, pentose sugars, non-reducing polysaccharides, amino acids, proteins, steroids, tannins and phenolic compounds) and inorganic properties (calcium, magnesium, sodium, potassium, iron, sulphates, phosphate, chloride, carbonate and

nitrate). All the tests were performed as prescribed by the Ayurvedic Pharmacopeia of India.

Optimum dose and period

The optimum dose and optimum period of JNY application was determined by adding JNY to the river sample in required quantity and for requisite time, respectively. Bureau of Indian Standards methodology was followed for conducting the analysis.

The optimum dose of JNY was fixed by taking river water sample (100 mL) in a set of four beakers. One among the sets was kept as control, whereas 0.25 mL (five drops), 0.5 mL (10 drops) and 1 mL (15 drops) of JNY were added to the rest of the beakers, respectively. Similar set of beakers were used for determining the optimum period of JNY. Following the addition of the optimum dose of JNY, the sample in the beakers were incubated for 30 min, 1 h and 2 h, respectively. The samples were then analysed to determine the optimum period of JNY.

Stability study

The stability of JNY was determined by storing the formulation in sterilised air-tight glass bottles for a period of 1 year under aseptic condition. One of the bottles were kept at room temperature whereas the other was stored under cold condition. Periodical testing of the stored JNY was done for above mentioned organoleptic parameters and pH.^[9]

Microbiological parameters

Microbial limit test

1. Most probable number

Under the presumptive test, single strength MacConkey broth (5%) was uniformly distributed in two sets (containing three tubes each). Whereas, double strength MacConkey broth (5%) was dispensed in a set of five tubes. Water samples (0.1 and 1 mL) were inoculated in the sets of single strength MacConkey broth. The tubes containing double strength MacConkey broth were inoculated with 10 mL of the water sample, following which, all the tubes were incubated at what temperature for 24h^[1].

The tubes showing the positive results were further analysed by incubating the tubes for 24 h after treatment with JNY. MPN in each case was calculated by using McCardy's table. The above test was repeated after 1 year.

2. Membrane filtration

The untreated and treated water samples were filtered with a membrane filter (0.45 µ). The filter was then incubated in 100-mL MacConkey broth at 30°C for 24 h. Any change of colour of the broth was the indication of microbial growth.^[10]

3. Standard plate count

The (treated and untreated) water samples were inoculated on soybean casein digest agar^[11] and Sabouraud's dextrose agar.^[12] The bacterial and fungal plates were incubated at $30 \pm 2^\circ\text{C}$ and $25 \pm 2^\circ\text{C}$ for 24h and 5days, respectively. Following incubation, the colonies developed on the plates were enumerated.

Antimicrobial activity of *Jala Nirvishikarana Yoga*

Microbial test cultures (*Salmonella abony*, *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*) were inoculated onto soybean casein digest agar plates. The antimicrobial activity of JNY was evaluated by using various concentrations of JNY (one drop, three drops and five drops) in 100 mL of

distilled water. After serially diluting the respective concentrations of the formulation, each dilution (1 mL) was added into the wells in each of the plates. The plates were incubated at 37°C for 24 h and observed for the zone of inhibition^[13].

RESULTS

Analysis of *Jala Nirvishikarana Yoga*

- 1. Organoleptic test:** The formulation was found to be a dark brown, alkaline, thick and viscous liquid with a pleasant odour and a characteristic slight bitter taste.
- 2. Preliminary test:** The formulation was devoid of any organic compound and had the presence of sodium, nitrates, iron and sulphates. The physical characteristics, as analysed, are listed in Table 2.

Table 2: Physical characteristics of JNY

S.No.	Tests	JNY
1	Weight/mL (mg/mL)	620
2	Refractive index	1.359
3	Specific gravity	1.0978
4	pH	10
5	Viscosity (PaS)	7.27×10^{-4}
6	Total solids (g/10 g)	0.460

Optimum dose and period

The optimum dose was analysed by taking into consideration 0.25, 0.5 and 1mL of JNY, wherein it was found that pH of water sample increased from 7.5 to 10.8 with the increase in concentration of JNY. This pH change was found to be beyond the permissible limits and made the water more alkaline and conductive in nature (Table 3).

Table 3: Estimation of optimum dose of *Jala Nirvishikarana Yoga*

S. No.	Parameters	Permissible limit	Control	0.25 mL JNY	0.5 mL JNY	1 mL JNY
1	pH	6.5–8.5	7.5	9.55	10.5	10.8
2	Conductivity ($\mu\Omega/\text{cm}$)	1000	210	300	420	450
3	Turbidity (NTU)	40	34.8	36.8	39.8	42.5
4	Total hardness (mg/L)	300	32	32	40	32
5	Calcium (mg/L)	75	16	28	28	20
6	Chlorides (mg/L)	250	32	32	44	32
7	Alkalinity (mg/L)	200	36	48	84	120
8	Total dissolved solids (mg/L)	1000	120	122	123	125

Hence, the optimum dose was considered as less than 0.25 mL (or three drops) of JNY. Since none of the tubes showed a considerable change in the parameters considered, 30 min was considered the optimum period for administration of JNY (Table 4).

Table 4: Estimation of optimum period of *Jala Nirvishikarana Yoga*

Sl. No.	Parameters	Permissible limit	Control	30 min	1 h	2h
1	pH	6.5–8.5	7.5	7.99	7.99	7.99
2	Conductivity ($\mu\Omega/\text{cm}$)	1000	210	300	302	300
3	Turbidity (NTU)	40	34.8	36.8	36.9	37.1
4	Total hardness (mg/L)	300	32	32	33	32
5	Calcium (mg/L)	75	16	28	29	29
6	Chlorides (mg/L)	250	32	32	31	31
7	Alkalinity (mg/L)	200	36	48	48	48
8	Total dissolved solids (mg/L)	1000	120	122	128	132

Stability test

The colour remained unchanged even after 1 year of storage in the case of both the samples. The taste and odour was found to slightly decrease in quality after 4 days of storage but thereafter

remained the same till 1 year of storage. pH of JNY was constant (7.99) for approximately 3 months and then decreased to pH 9.0 for both the samples.

Microbial limit test**1. MPN**

The count of coliforms in the river water sample (100 mL) was found to be 180, which reduced considerably to 8 after treatment with JNY. (Table 5, Figures 1 & 2).

Table 5: Analysis of treatment by MPN method

Samples	Before treatment	After treatment
River water	180 MPN	8 MPN
Well water	<1 MPN	-
Borewell water	<1 MPN	-
Tap water	<1 MPN	-

2. Membrane filtration

Prior to the treatment of water sample with JNY, the sample showed the presence of faecal coliforms per 250 mL (yellow-coloured broth) indicating the coliform contamination. However, the broth inoculated with the membrane filter of the treated water sample displayed no change of colour indicating inhibition in the proliferation of coliform by the administration of JNY.

3. Standard plate count

The untreated water sample showed the presence of 267 CFU/mL of bacterial colonies on soybean casein digest agar and 98 CFU/mL of fungal colonies on Sabouraud's dextrose agar. Conversely, upon treatment with JNY, the bacterial and fungal counts were reduced to 83 CFU/mL and 15 CFU/mL, respectively. (Table 6, Figure 3).

Table 6: Analysis of effectiveness of JNY by Standard Plate Count method

Sample	Bacterial count Cfu/ml				Fungal count (Cfu/ml)			
	2012		2013		2012		2013	
River water	267	83	76	53	98	15	45	31
Well water	28	-	35	-	05	-	06	-
Borewell water	32	-	40	-	12	-	11	-
Tap water	105	-	29	-	29	-	08	-

*BT, Before treatment; AT, After treatment

Antibacterial activity

All the concentrations of JNY (one drop, three drops and five drops in 100 mL of distilled water) were found to be effective in inhibiting the growth of the organisms (*Salmonella abony*, *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*).



Figure 1: River water-color change in Double Standard McConkey Broth & Standard McConkey Broth tubes



Figure 2: River water + JNY- No change in Double Standard McConkey Broth & Standard McConkey Broth tubes

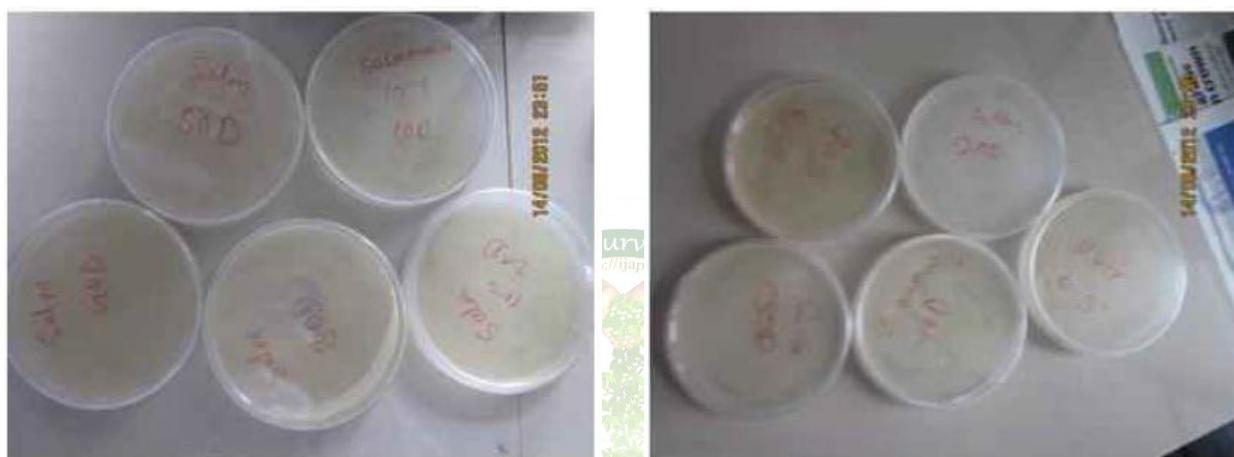


Figure 3: Results of inoculated plates showing no growth

DISCUSSION

The study was conducted to assess the physiochemical and antimicrobial efficacy of JNY on Malaprabha river water, which has been reported to be have high pH and Biological Oxygen Demand (BOD) levels, in the recent years^[14, 15]. As a river can be a used for various activities (e.g. irrigation, washing and drinking), the elevated level of BOD may adversely affect the health of inhabitants living near the banks of the river. Hence, the traditional method of treatment has been attempted to remedy the problem of pollution by heavy metals and microorganisms.

JNY, the Ayurvedic formulation, was found to be alkaline and viscous in nature, which may be due to the presence of *Kshara* and *Sarala Nirayasa*. *Kshara* can lead to increase in the pH of the formulation, thereby giving rise to its alkaline nature^[16] whereas the viscosity may be as a result of the dense and resinous nature of *Sarala Nirayasa*.^[17] The complete oxidation of herbs upon burning resulted in the absence of organic component in JNY. However, the inorganic constituents in JNY such as sulphur,

sodium, nitrates and iron are contributed by ash of the drugs.

Optimum period of administration of JNY was kept to minimum (30 min) even though no significant change in the parameters was observed with prolonged incubation period. Among the parameters analysed for determining the optimum dose of JNY, significant variation was detected in pH of the water probably due to the alkaline nature of JNY. Hence, least dose of JNY (approximately three drops in 100 mL) was considered as the optimum dose to minimise the change in pH. Moreover, at such a low volume, the odour and taste were appreciable, which further helped to ascertain the optimum dose. The characteristic odour may have developed due to the volatile compounds present in *Bruhat Ela*.^[18] *Ushira*^[19] and *Bakuchi*.^[20] However, for highly polluted water, the dosage of JNY should be increased accordingly, as low concentrations of JNY may not be sufficient to reduce the microbial load of the water.

The water sources often vary in their physical properties, especially in pH, due to the presence of

several organic and inorganic substances. The sources of water with low pH may contain higher concentrations of toxic metals^[21], which can become detrimental to the health of the consumers. Malaprabha river has been reported to contain iron beyond permissible levels, which may be as a result of leaching from rocks due to low pH of the river^[14,15]. JNY, due to its alkaline nature, can therefore be used to maintain the pH of the water to some extent leading to decrease in the leaching of metals and limiting the levels of BOD.

Although there was no substantial change in the taste and odour of the formulation, significant post-storage change was observed in case of pH of JNY. *Kshara* contributes to the increase of pH of JNY^[16] and has been stated to possess antibacterial activity against *E. coli* and *S. aureus*^[22]. Small *et al.*^[23] has also stated that the survival rate of *E. coli* (aerobic log-phase cultures) was affected as a result of increase in the pH of the medium. Hence, it can be concluded that at alkaline pH, JNY can have antibacterial activity. Therefore, taking into account the positive effects of variation in pH, JNY can be considered stable for 1 year.

The antimicrobial analysis of JNY revealed that the formulation was effective in limiting the growth of coliforms to a certain level and also inhibited microorganisms such as *S. abony*, *S. aureus*, *E. coli* and *C. albicans*. This activity of JNY can be as a result of the herbs used in formulation. The herbs and shrubs used to prepare the formulation, such as *Uttamarini*, *Ajashruni*, *Indravaruni*, *Guduchi*, *Phanijjaka*, *Ativisha*, *Haridra*, *Daruharidra* and *Rasna* are known to be anti-inflammatory in nature^[24-31]. *Phanijjaka*, *Haridra*, *Bruhat Ela*, *Ushira*, *Daruharidra* have been found to possess antimicrobial activity against fungal and bacterial forms.^[27,30,32-34] In addition to this, *Phanijjaka* and *Ativisha* are also reported to have activity as antioxidants.^[27,35] Therefore, the presence of these substances in the formulation helps to enhance the properties of JNY along with its antimicrobial activity.

Post-evaluation data showed that JNY can be used for reducing the microbial load of polluted water, as it possess antimicrobial activity. This may lead to decrease in the BOD levels of polluted river. JNY, being alkaline in nature, can also aid in maintaining the pH of water resources by limiting the leaching of iron and other metals. JNY has also been known to be stable for more than 1 year at room temperature. However, further studies can be conducted by taking larger samples of polluted water and efficacy of JNY can be evaluated by taking more number of organisms into consideration. Constituents of JNY can be further analysed, which

may result in improved activity of the formulation. *Ativisha* being a costly herb, more cost-efficient herbs can be tested and used to make the formulation more affordable.

CONCLUSION

JNY, a novel Ayurvedic formulation prepared in the present study was observed to have substantial antimicrobial activity with the ability to limit the growth of coliforms, *Salmonella abony*, *Staphylococcus aureus* and *Candida albicans*. This property could help in enabling the purification of water before consumption, especially at the household level. In addition, the formulation was found to be stable for 1 year at room temperature. JNY can therefore be stored and used for a long period of time. However, further research is required to enhance the dose concentration and effectiveness of JNY. Uniformity of the effectiveness of JNY should be further assessed by including samples from different water sources.

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