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A Study on Anti-Microbial Effect of Ayurvedic Water Purification Method

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ABSTRACT

Water security and safety is of vital concern in every part of the country. Potable water accessibility and supply is limited due to fluctuating climatic conditions and environmental pollution etc, that lower wholesomeness of most water sources and moreover water is susceptible to contamination with microorganisms. In classics, Acharya Sushruta has mentioned various methods for purifying water by keeping it in vessels made from different materials like Silver, Copper, Clay and by adding different herbs (Kataka beeja). In this experiment, evaluation of water is conducted to assess change in microbial load of water by comparing microbial load of water on storage in vessels prepared from different materials like silver, copper, clay, plastic and steel. Further along with water in these vessels different herbs were added like Kataka beeja, Tulsi patra, Nimba patra individually and separately. They were kept undisturbed for 24 hours and later change in Microbial Load of water sample was evaluated with the help of Serial dilution and Spread Plate Method.

Key Words: *Vessels (silver, copper, clay, plastic, steel), Herbs (Kataka beeja, Tulsi patra, Nimba patra), Microbial Load, Serial Dilution and Spread Plate Method*

INTRODUCTION

Water covers maximum part of the earth surface and is essential for all the living beings. Almost in all biochemical reactions water plays a role. Microbes have been integral part of water that are responsible for nutrient cycling, but also contribute to variety of water borne diseases. Hence there is a necessity to keep water free from harmful microbes.

In ancient era, water was stored in vessels made from different type of materials like *Rajata*[5](Silver), *Tamra*[5] (Copper), and *Mrinmaya*[5](Clay) along with addition of few herbs such as *Kataka Beej*[1,4,5,8] as it is considered to have *Jala Nirmalakara*[4,5,8] property. In *Ayurveda* classics there are references regarding *Jantu-Bhuta-Krimi*[1,5] being the causative factor for water impurity. Solution for this universal constraint was obtained easily with the knowledge of few herbs such as *Tulsi*[1,3,7], *Nimba*[1,2,6] that are said to have *Krimighna*[1,2,3,6,7] property and are easily as well as readily available in day to day life and are not expensive compared to various other measures that require advanced technological support.

Microbial load was determined by using Serial Dilution and Spread Plate Method. A serial dilution is the stepwise dilution of a substance in solution. Usually the dilution factor at each step is constant, resulting in a geometric progression of the concentration in a logarithmic fashion. The spread plate method is a technique to plate a liquid sample containing bacteria so that bacteria are easy to count. In the study this method was used to count the number of bacterial colonies that grow on an agar plate in a given time as it is concentration dependent.

AIMS AND OBJECTIVES

i) To evaluate and compare Microbial Load of water kept for 24 hours in vessels made from different materials like Clay, Plastic, Steel, Copper and Silver.

ii) To evaluate additional effect by comparing Microbial Load of water kept for 24 hours in vessels made from different materials like Clay, Plastic, Steel, Copper and Silver by adding following herbs- *Kataka beeja*, *Tulsi patra*, *Nimba patra.*

MATERIALS AND METHODS

Requirements for study procedures were collected. A sterile glass container was used to collect water sample from the common source (Pond). Five vessels made from different material such as clay, plastic, steel, copper and silver were purchased and sterilized. Crude drugs such as *Kataka*, *Tulsi* and *Nimba* were collected, shade dried and powdered, thereby *Churna* was prepared. Other apparatus like conical flask, test-tube, test-tube stand, measuring cylinder, weighing balance, distilled water, Mullen Hinton agar, Agar, Cotton, spirit, Bunsen burner, marker pen, culture plate were procured.

Method of Serial Dilution of water sample

(a) Preparation of physiological saline: 100ml of distilled water was measured in a sterile measuring cylinder and transferred to a clean sterilized conical flask. 0.98gm of NaCl was weighed using a well calibrated weighing balance and dissolved in 100 ml of distilled water and shook well. Once NaCl completely dissolved in distilled water, it was covered with a cotton plug and kept aside. (Image 1)

Image 1 Test tubes with physiological saline

(b) Serial dilution of collected water sample: Seven test tubes were taken and kept in an autoclave for 20-30 minutes to sterilize it. Once the test tubes were sterilized, it was labeled as 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} and kept aside in a test tube stand. Prepared physiological saline, 9ml was measured using a well calibrated glass pipette and transferred to each test tube and was further covered with a cotton plug.

Freshly collected water sample, 1ml from pond was pipetted with a glass pipette and was transferred to test tube labeled as 10^{-1}, contents in test tube was mixed well and left undisturbed for 2-3 minutes. From the test tube labeled as 10^{-1}, 1ml was pipetted in a glass pipette and transferred to test tube labelled as 10^{-2} mixed well and left undisturbed for 2-3 minutes. This procedure was repeated to each of the successive test tubes till test tube labeled as 10^{-7} to achieve serial dilution.

Next, the water sample collected from pond and kept for 24 hours undisturbed in five different vessels made from clay, plastic, steel, copper and silver were also subjected to serial dilution as per above procedure individually and separately.

Next, serial dilution procedure explained above was followed for 100ml of pond water sample mixed separately with 10 grams of drug *Nimba*, *Tulsi* and *Kataka* and kept separately in five different vessels made from clay, plastic, steel, copper and silver respectively for 24 hours undisturbed.

Estimation of Microbial load of above water sample subjected to serial dilution

a) Preparation of culture media: A Conical flask was sterilized by keeping in autoclave for 20- 30 minutes. 250 ml of distilled water was measured in a measuring cylinder and transferred to a sterile conical flask. 4.25gm of agar and 9.5gm of Mullen Hinton agar was measured and mixed with the distilled water and covered with a cotton plug further sealed with paper to avoid air entry. This conical flask containing 250 ml of distilled water and 4.25gm of agar, 9.5gm of Mullen Hinton agar was kept in autoclave at 121^0c for 20-30 minutes.

b) Pour plate culture: One ml of serial dilution test tube content marked as 10^{-4}, 10^{-5}, 10^{-6} were poured in a sterile petri plate labeled as 10^{-4}, 10^{-5}, 10^{-6} respectively and was added with 15ml of melted agar at 45-50 ^{0}C and allowed to set after mixing it by rotating clockwise, anti clockwise, up and down. It was kept overnight (12 hours) for incubation at 37^0C, the bacterial colonies distributed throughout the depth of the media were counted. The number gives the viable number of bacterial colonies. (Image 2)

This above method of preparation of culture media and pour plate culture was separately followed for estimation of microbial load in i) Freshly collected water sample, ii) water sample kept for 24 hours in different vessels namely clay, plastic, steel, copper and silver, iii) water sample mixed with three different drugs namely *Nimba*, *Tulsi*, *Kataka* separately in 5 different vessels made of clay, plastic, steel, copper and silver and kept undisturbed for 24 hours.

RESULTS

Result of microbial load assessment of freshly collected water sample, comparison of microbial

load of pond water sample kept in different vessel (silver, copper, clay, steel, and plastic) for 24 hrs and comparison of microbial load of pond water sample kept in different vessels (clay, copper, silver, plastic and steel) along with addition of different herbs (*nimba, tulsi and kataka* separately) for 24 hours are mentioned in Table 1, Table 2 and table 3 respectively.

Table 1 Estimation of microbial load of freshly collected pond water sample

Number of bacterial colonies in pond water sample at different concentrations		
10^{-4}	10^{-5}	10^{-6}
40 (Image 3)	31 (Image 4)	30 (Image 5)

DISCUSSION

On analysis of table 3 on microbial load assessment at 10^{-4} concentration, pond water sample shows minimum bacterial load when kept separately in silver vessel followed by plastic, steel, copper and clay vessel in successive order.

Table 2 Comparison of microbial load of pond water sample kept in different vessels (silver, copper, clay, steel, plastic) for 24 hours

Type of vessels	Number of bacterial colonies at different concentrations		
	10^{-4}	10^{-5}	10^{-6}
Silver vessel	16 (Image 6)	15 (Image 7)	15 (Image 8)
Copper vessel	28 (Image 9)	19 (Image 10)	16 (Image 11)
Clay vessel	40 (Image 12)	26 (Image 13)	26 (Image 14)
Steel vessel	20 (Image 15)	17 (Image 16)	13 (Image 17)
Plastic vessel	20 (Image 18)	18 (Image19)	19 (Image 20)

Table 3 Comparison of microbial load of pond water sample kept in different vessels (clay, copper, silver, plastic and steel) along with addition of different herbs (*nimba, tulsi and kataka* separately) for 24 hours-

Kept in following vessel	Number of colonies at 10^{-4}concentration (Image 21-40)				Number of colonies at 10^{-5}concentration (Image 41-60)				Number of colonies at 10^{-6}concentration (Image 61-80)			
	PW	PW +N	PW +T	PW +K	PW	PW +N	PW +T	PW +K	PW	PW+N	PW +T	PW +K
Clay	40	4	6	2	26	1	4	1	26	Nil	5	1
Copper	28	4	5	10	19	3	3	10	16	1	2	9
Silver	16	4	7	11	15	4	4	12	15	2	1	7
Plastic	20	5	8	3	18	4	4	1	19	2	2	4
Steel	20	3	8	15	17	2	5	14	13	4	3	7

PW: Pond water PW+N: Pond water + *Nimba* PW+T: Pond water + *Tulsi* PW+K: Pond water + *Kataka*

The same pond water sample when mixed with *Nimba* and kept for 24 hours minimum bacterial growth was observed in steel followed by silver, copper, clay and plastic. On addition of *Tulsi* with pond water sample minimum bacterial load was observed in copper followed by clay, silver, plastic and steel. With the addition of *Kataka Beeja* with pond water sample minimum microbial load is noted in clay vessel followed by plastic, copper, silver and steel respectively.

Hence at 10^{-4} concentration- Microbial load

Nimba- steel<silver, copper, clay<plastic

Tulsi- copper<clay<silver<plastic, steel

Kataka-clay<plastic<copper<silver<steel

On assessment of microbial load at 10^{-5} concentration of Pond water, minimum microbial

Image 3:Bacterial colonies at 10^{-4}

Image 4:Bacterial colonies at 10^{-5}

Image 5:Bacterial colonies at 10^{-6}

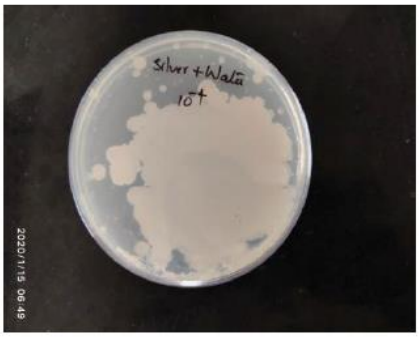

Image 6:Bacterial colonies at 10^{-4}

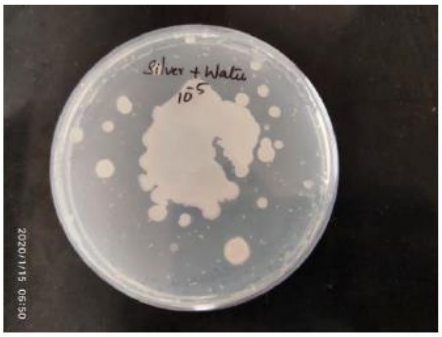

Image 7:Bacterial colonies at 10^{-5}

Image 8:Bacterial colonies at 10^{-6}

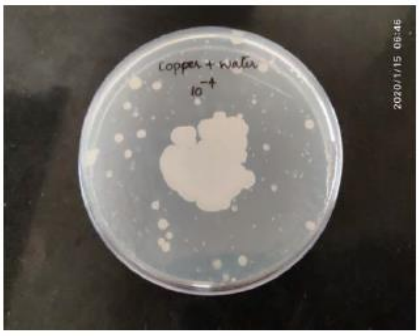

Image 9:Bacterial colonies at 10^{-4}

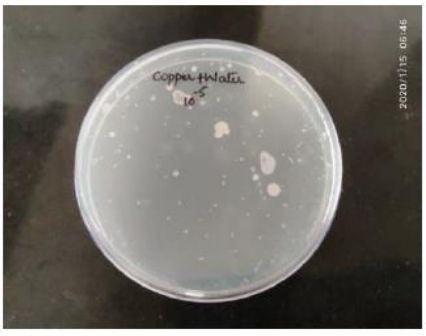

Image 10:Bacterial colonies at 10^{-5}

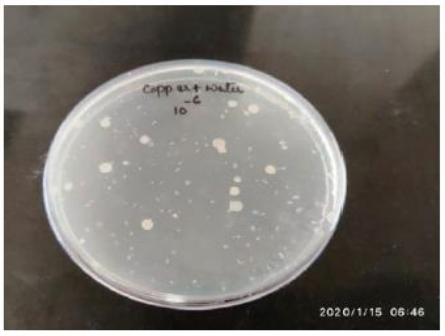

Image 11: Bacterial colonies at 10^{-6}

Image 12:Bacterial colonies at 10^{-4}

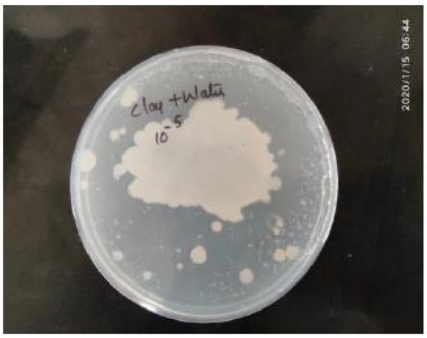

Image 13:Bacterial colonies at 10^{-5}

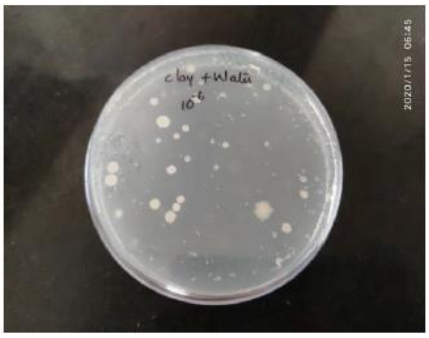

Image 14: Bacterial colonies at 10^{-6}

load was observed in silver followed by steel, plastic, copper and then clay respectively.

With addition of drug such as *Nimba* to pond water sample minimum microbial load is seen in clay

Image 15:Bacterial colonies at 10^{-4}

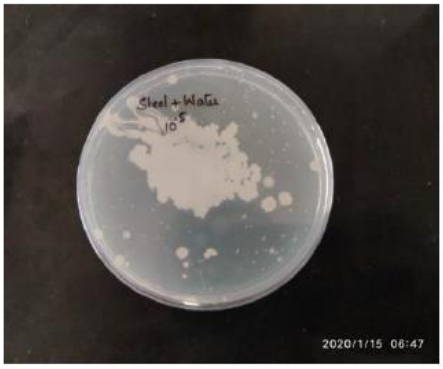

Image 16:Bacterial colonies at 10^{-5}

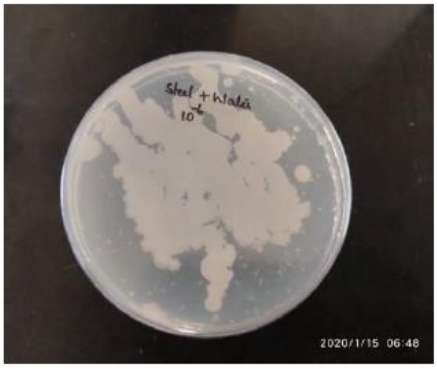

Image 17:Bacterial colonies at 10^{-6}

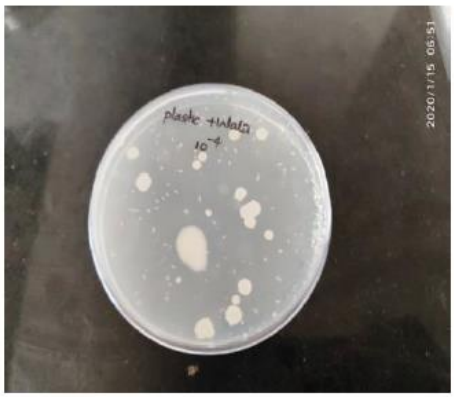

Image 18:Bacterial colonies at 10^{-4}

Image 19:Bacterial colonies at 10^{-5}

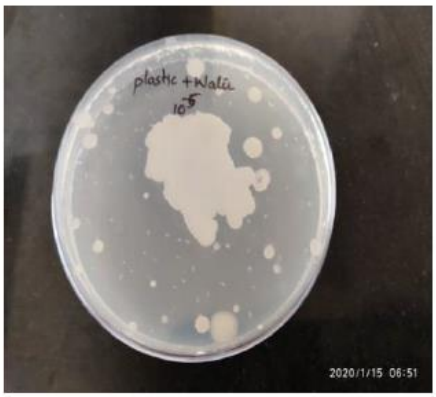

Image 20:Bacterial colonies at 10^{-6}

Image21:Colonies at 10^{-4}

Image22:Colonies at 10^{-4}

Image23:Colonies at 10^{-4}

Image24:Colonies at 10^{-4}

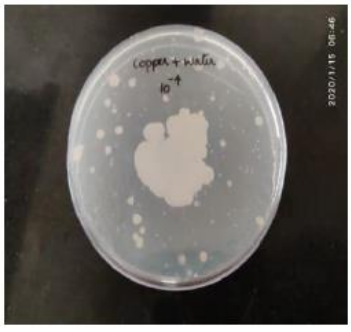

Image25:Colonies at 10^{-4}

Image26:Colonies at 10^{-4}

Image27:Colonies at 10^{-4}

Image28:Colonies at 10^{-4}




Image29:Colonies at 10^{-4} Image30:Colonies at 10^{-4} Image31:Colonies at 10^{-4} Image32:Colonies at 10^{-4}

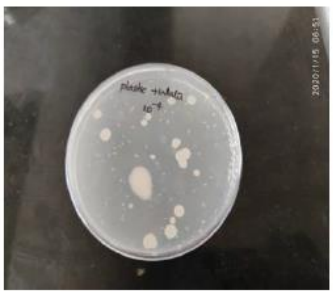


Image33:Colonies at 10^{-4} Image34:Colonies at 10^{-4} Image35:Colonies at 10^{-4} Image 36:Colonies at 10^{-4}




Image37:Colonies at 10^{-4} Image38:Colonies at 10^{-4} Image39:Colonies at 10^{-4} Image40: Colonies at 10^{-4}

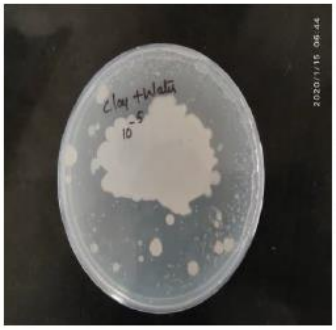


Image41:Colonies at 10^{-5} Image42:Colonies at 10^{-5} Image43:Colonies at 10^{-5} Image44:Colonies at 10^{-5}




Image45:Colonies at 10^{-5} Image46:Colonies at 10^{-5} Image47:Colonies at 10^{-5} Image48: Colonies at 10^{-5}




Image49:Colonies at 10^{-5} Image50:Colonies at 10^{-5} Image51:Colonies at 10^{-5} Image52:Colonies at 10^{-5}




Image53:Colonies at 10^{-5} Image54:Colonies at 10^{-5} Image55:Colonies at 10^{-5} Image56:Colonies at 10^{-5}




Image57:Colonies at 10^{-5} Image58:Colonies at 10^{-5} Image59:Colonies at 10^{-5} Image60:Colonies at 10^{-5}

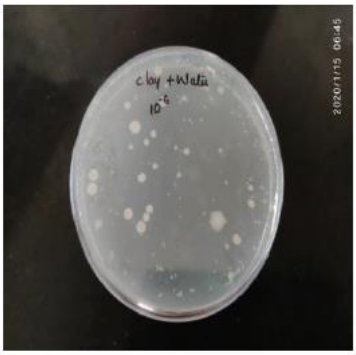

Image61:Colonies at 10^{-6}

Image62:Colonies at 10^{-6}

Image63:Colonies at 10^{-6}

Image64:Colonies at 10^{-6}

Image65:Colonies at 10^{-6}

Image66:Colonies at 10^{-6}

Image67:Colonies at 10^{-6}

Image68:Colonies at 10^{-6}

Image69:Colonies at 10^{-6}

Image70:Colonies at 10^{-6}

Image71:Colonies at 10^{-6}

Image72:Colonies at 10^{-6}

Image73:Colonies at 10^{-6}

Image74:Colonies at 10^{-6}

Image75:Colonies at 10^{-6}

Image76:Colonies at 10^{-6}

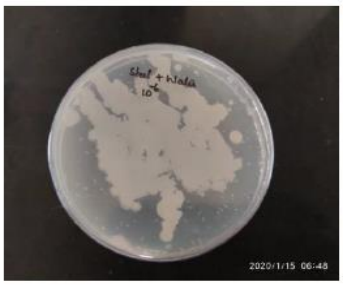


Image77:Colonies at 10^{-6} Image78:Colonies at 10^{-6} Image79:Colonies at 10^{-6} Image80:Colonies at 10^{-6}

followed by steel, copper, silver, plastic respectively. On addition of *Tulsi*, copper showed minimum microbial load followed by silver, clay, plastic, and steel. On addition of *Kataka* microbial load was noted minimum in clay and plastic followed by copper, silver and steel in successive order.

Hence at 10^{-5} concentration- Microbial load

Nimba- clay< steel< copper<silver, plastic

Tulsi- copper< silver, clay, plastic<steel

Kataka- clay, plastic<copper<silver<steel

Assessment of microbial load at 10^{-6} concentration of pond water sample shows that water kept in five different vessels namely clay, plastic, silver, steel and copper undisturbed for 24 hours shows minimum microbial load noted in steel, silver, copper, plastic and clay in respective order. On adding drugs such as *Nimba* and kept undisturbed for 24 hours in five different vessels separately, minimum microbial load is seen in clay followed by copper, silver then plastic and steel. On addition of *Tulsi* the minimum microbial load was noted in silver, copper, plastic, steel and clay. With *Kataka* decreasing microbial load was observed in clay, plastic, silver, steel and copper respectively.

Hence at 10^{-6} concentration-Microbial load

Nimba-clay<copper<silver,plastic<steel

Tulsi-silver<copper, plastic<steel<clay

Kataka-clay<plastic<silver,steel<copper

On decreasing the concentration of inoculum and assessing the microbial load at different concentration namely 10^{-4}, 10^{-5}, 10^{-6}, the pond water sample kept in five different vessels separately and with addition of drugs such as *Nimba, Tulsi* and *Kataka* individually; shows that Clay acts better at lesser concentration in decreasing the microbial load and best with addition of *Nimba* followed by *Kataka* and then *Tulsi*. In Copper vessels microbial load is noted minimum at lower concentration along with the addition of *Nimba, Tulsi* and *Kataka* in respective order. In silver, minimum microbial load was noticed at 10^{-6} concentration and with addition of *Tulsi*, *Nimba* and *Kataka* in successive order, where as in plastic the best action is seen at lower concentration 10^{-6} with addition of *Nimba* and at 10^{-5} with the addition of *Kataka*. In steel vessel minimum microbial load is seen at higher concentration such as 10^{-5}, and 10^{-6} with addition of *Nimba* followed by *Tulsi.*

CONCLUSION

Assessment of Microbial load of pond water kept for 24 hours in vessels made from clay, copper,

silver, plastic and steel at serial dilution concentration of 10^{-4}, 10^{-5} and 10^{-6} showed least microbial load for silver, steel and plastic respectively. On addition of *Nimba, Tulsi* and *Kataka* separately and individually, pond water kept for 24 hours with serial dilution 10^{-4}, 10^{-5} and 10^{-6} showed least microbial load on assessment for 10^{-4} dilution *Kataka* in clay vessel, at 10^{-5} dilution *Nimba* and *Kataka* in clay vessel along with *Kataka* in plastic vessel and 10^{-6} dilution *Nimba* with clay vessel.

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