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AN IN-VITRO STUDY ON ANTI-BACTERIAL ACTION OF *DHAVĀDI BHASMA* IN WATER PURIFICATION: A POTENTIAL NATURAL HERBAL COAGULANT FOR WATER TREATMENT

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ABSTRACT:

Background: Herbal plants are being used for the treatment of various ailments in the Ayurvedic system of Medicine. Ayurveda mentioned various plants and their compound formulations for the treatment of polluted and contaminated water. It has been estimated that upto 80% of all diseases and sicknesses in many developing countries are caused by inadequate sanitation, polluted and contaminated water or unavailability of water. The present study aims to analyze the phytochemical and antibacterial activity of *Dhavādi Bhasma* and its applications in water purification as a natural herbal coagulant.

Methods: *Dhavādi Bhasma* was analysed for antibacterial activity against six different microbes of standard bacterial strains by using MIC & MBC methods. Three different water samples such as Ghataprabha River (WS-1), Muchakhandi Kere /Lake (WS-2) and Pond (WS-3) were collected from Bagalkot city. All the samples were treated with *Dhavādi Bhasma* at the optimum dosage of 3g/l by jar test method to assess the coagulation potential. The untreated and treated water samples were analysed for physicochemical and microbiological parameters by using the standard methods.

Results: *Dhavādi Bhasma* showed antibacterial activity against tested six different microbes of standard bacterial strains i.e., *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Treatment of 3gm/l of *Dhavādi Bhasma* significantly reduced the water turbidity thereby achieving a turbidity reduction of 91.26%, 90.57% and 91.90% for the Ghataprabha River (WS-1), Muchakhandi Kere (Lake) (WS-2) and Pond water (WS-3) samples respectively. However, the resultant pH, conductivity, alkalinity, total dissolved solids and total hardness were partially increased but remained within the permissible limit as per WHO/USPH standards. A significant reduction in the microbial load (coliform counts) was found with the water of the Ghataprabha River and Pond water (71.42%) in each and Muchakhandi Kere (66.66%).

Conclusion: *Dhavādi Bhasma* had strong antibacterial activity against tested six different microbes of standard bacterial strains. It acts as an efficient natural herbal coagulant and anti-bacterial agent which makes the *Dhavādi Bhasma* a good source for water purification.

KEYWORDS: *Dhavādi Bhasma*, River water treatment, Antibacterial activity, Natural coagulant

INTRODUCTION:

It has been estimated that up to 80% of all diseases and sicknesses in many developing countries are caused by inadequate sanitation, polluted and contaminated water or unavailability of water. So, it's become more and more difficult to supply high-quality potable water from surface and groundwater stocks and removal of harmful bacteria, viruses and protozoa assume greater significance. Generally, many chemical inorganic coagulants and flocculants (e.g., aluminium and ferric salts) and synthetic organic polymers (e.g., polyacrylamide derivatives and polyethylene amine) are widely used in conventional water purification processes. This excess use of the amount of chemical inorganic and synthetic organic coagulants can affect human health e.g. Aluminium has also been indicated to be a causative agent in neurological diseases such as pre-senile dementia[1] and synthetic coagulants, especially concerning Alzheimer's disease.[2,3] So, in recent years several studies have been developed towards water treatment to replace chemical

coagulants with alternative coagulants and flocculants preferably from natural and renewable resources to prevent the detrimental effects of these coagulants on human health.

Herbal plants are being used for the treatment of various ailments in the Ayurvedic system of Medicine. Ayurveda mentioned various plants and their compound formulations for the treatment of polluted and contaminated water. There are many methods of water treatment in Ayurveda that mainly emphasized health-promoting benefits. Sushruta Samhita (the Ayurvedic text) says about the purification method of *Vishajushta Jala* (contaminated water) in the *Jangamavisha Vidhivijnāniya* chapter. The cold ashes (*Bhasma*) of *Dhava (Anogissus latifolia), Ashwakarna (Diptocarpus turbinatus), Asana (Pterocarpus marsupium), Paribhadra (Erythrena variegata), Patala (Stereospermum souvelens), Rajadruma (Cassia fistula), Sinduvāra (Vitex negundo Linn)* and *Somavallaka (Acacia catechu)* burnt together and it should be cast into the poisoned pool or Pond, whereby its water would be purified; as an alternative, an Anjali measure (half a seer) of the said ashes cast in a Ghata measure (sixty-four seers) of required water would lead to its purification.[4] A few recent investigations report that natural coagulants from *Strychnos potatorum* (*Nirmali* or *Kathaka*) seeds, which has received a significant degree of attention for the water treatment process.[5]

Now-a-days, with the advancement in science and technology, there has been growing interest in alternative medicine, especially resulting from plant-derived products. This interest in drugs of plant origin is due to several reasons, namely, conventional medicine can be inefficient (e.g., side effects and ineffective therapy), abusive or incorrect use of synthetic drugs results in side effects and other problems.[6]

By keeping this idea, the present research study aims to evaluate the effectiveness of an in-vitro study on the Anti-bacterial action of *Dhavādi Bhasma* in water purification: A potential natural herbal coagulant for water treatment and thereby protect and promote human health.

AIMS AND OBJECTIVES:

The present study was aimed to analyze the phytochemical and antibacterial activity of *Dhavādi Bhasma* and its applications in water purification as a natural herbal coagulant.

The specific objectives of the study were

1. To screen the presence of phytochemical compounds in *Dhavādi Bhasma.*
2. To analyse the anti-bacterial activity of *Dhavādi Bhasma.*
3. To analyse the microbial load of the water samples before and after the addition of *Dhavādi Bhasma.*
4. To study the physicochemical parameters of water samples before and after the addition of *Dhavādi Bhasma.*

MATERIALS AND METHODS:

Dhavādi Bhasma: The ingredients of *Dhavādi Bhasma* were procured from the KLE Ayurveda Pharmacy, Belgaum, Karnataka, Local drug suppliers and C.L. KURIAN & BROS, Indian Drugs Merchants & Commission Agents, Thrissur, Kerala. They were dried under shade and used for the studies.

Table – 1 Ingredient of *Dhavādi Bhasma*

SN	***Name of the Plant***	***Parts Used***
1.	Dhava (Anogissus latifolia)	*Bark*
2.	Ashwakarna (Sāla) (Shorea robusta)	*Bark*
3.	Asana (Pterocarpus marsupium)	*Bark*
4.	Pāribhadra (Erythrena variegata)	*Bark*
5.	Pātalā (Stereospermum souvelens)	*Bark*
6.	Rājadruma (Cassia fistula)	*Bark*
7.	Sinduvāra (Vitex negundo Linn)	*Bark*
8.	Somavallaka (Acacia catechu)	*Bark*

Preparation of Dhavādi Bhasma: A *Bhasma* means ash obtained through incineration. The ingredients of *Dhavādi Bhasma* (Table – 1) were put into a container and burnt. The collected ash was sieved, stored in a sterile at room temperature in a dark place and used for the study. The ingredients were authenticated by experts of Dravya Guna department (Pharmacognosy in Ayurveda). The *Bhasma* was prepared in BVVS Ayurvedic Pharmacy under the experts of Rasashastra and Bhaishajya Kalpana (Iatrochemistry & Pharmaceuticals sciences in Ayurveda) and authenticated by BVVS Central Research Laboratory.

Fig. – 1 Preparation of *Dhavādi Bhasma*

Water Samples: Three different water samples such as Ghataprabha River (WS-1), Muchakhandi Kere (Lake) (WS-2) and Pond (WS-3) were collected from Bagalkot city. The samples were collected in pre-cleaned 10 litres of polyethylene containers. They will be kept in the deep freezer and used for further study.

Fig. – 2 Collection of water samples

(1) Ghataprabha River Water (2) Muchakhandi Kere Water (3) Storage Pond Water

Preliminary phytochemical screening of Dhavādi Bhasma: A preliminary phytochemical study was screened for the presence of organic and inorganic constituents by using standard methods.

Table – 2 Standard Methods of preliminary phytochemical constituents (PPC)

SN	PPC	Standard Method
A.	**Organic constituents:**	
1.	Alkaloids	Mayer's Test
2.	Carbohydrates	Molisch's test
3.	Anthraquinones	Bontrager's test
4.	Tannins	Lead acetate test
5.	Saponins	Foam test
6.	Flavonoids	Ferric chloride test
7.	Glycosides	Legal's test
8.	Terpenoids	Salkowski test
9.	Phenols	ferric chloride solution (5%)
10.	Steroids	Liebermann burchard reaction
11.	Proteins	Biuret test
B.	**Inorganic constituents:**	
1.	Carbonates	Chemical test with HCL
2.	Sodium	Flame test
3.	Potassium	Flame test
4.	Chloride	Chemical test with $AgNO_3$
5.	Sulphate	Barium Chloride ($BaCl_2$) test
6.	Aluminum	Chemical test with Ammonium Acetate
7.	Magnesium	Chemical test with NH_3 Solution
8.	Calcium	Chemical test with Acetic Acid & Potassium Ferrocyanide
9.	Phosphate	Chemical test with $FeCl_3$
10.	Iron	Chemical test with Potassium Ferrocyanide
11.	Iodine	Silver Nitrate ($AgNO_3$) test
12.	Lead	Chemical test with Sodium Acetate
13.	Ammonium Salts	Chemical test with NaOH Solution
14.	Barium Salts	Chemical test with HCL & H_2SO_4

Analysis of the antibacterial activity of Dhavādi Bhasma: The *Dhavādi Bhasma* was tested against six different microbes standard bacterial strains, of which two were gram-positive bacterial strains i.e., *Staphylococcus aureus* (ATCC25923), *Staphylococcus epidermidis* and four strains were gram-negative bacterial strains i.e., *Escherichia coli* (ATCC25922), *Proteus mirabilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* (ATCC27853). The bacterial strains were obtained from the Dept. of Microbiology, BVVS SN Medical College & HSK Hospital, Bagalkot. The MIC and MBC methodology was used to assess the antibacterial activity.

Minimum Inhibitory Concentration (MIC) Method: The antimicrobial agent was dissolved in sterile normal saline water and dilutions were made in decreasing concentrations viz. 5.12 mg, 2.56 mg, 1.28 mg, 0.64 mg, 0.32 mg, 0.16 mg, 0.08 mg, 0.04 mg and 0.02 mg. To each dilution, an equal volume of 0.5 MacFarland turbidity (10^5 CFU/ml) standard of *S. aureus* (ATCC25923) was added in Mueller Hinton broth. All the respective tubes were incubated at 37^0C for 24 hours. The control tubes contained all the ingredients except the antimicrobial agent. The lowest concentration at which there was no visible turbidity was considered as the MIC of the antimicrobial agent for that organism.

Minimum Bactericidal Concentration (MBC) Method: The MBC test determines the lowest concentration at which an antibacterial agent will kill a particular bacterium. It is determined using a series of steps, undertaken after a broth dilution minimum inhibitory concentration (MIC) tests by subculturing to agar plates. A loopful of broth from each tube was sub-cultured on nutrient agar and incubated at 37^0C for 24 hours. The lowest concentration at which there is no visible bacterial growth was considered as the MBC of the antimicrobial agent for that organism.

The test was repeated for remaining gram-positive and gram-negative bacterial strains.

Jar test method: A jar test is a test used to find out the optimum dosage of coagulants like Alum etc. Varying dosages of coagulants are added to the jars and the jars are operated at more speed for the coagulation process and less speed for the flocculation process. A 1000 ml of raw water sample (first beaker) with no *Dhavādi Bhasma* were kept as control. After evaluation, four different concentrations of coagulant for the loading dose were prepared by weighing 2.5gm, 3gm, 3.5gm and 4gm of *Dhavādi Bhasma* each separately and added into the remaining four beakers containing 1000 ml of raw water respectively. The jars were put into the jar test apparatus in which they underwent flash mixing at 100 rpm for 20 minutes followed by 10 minutes of slow mixing at 25 rpm. The suspensions were left to stand without disturbance for 30 minutes. After sedimentation, the supernatants treated water samples were withdrawn using a pipette from a height of 4-5cm below the surface of each beaker and it was used for further analysis of physicochemical and microbiological parameters of water quality as per standard methods before and after the treatment. The optimal dosage (efficiency dosage) for *Dhavādi Bhasma* was determined by calculating the best turbidity removal percentage for the coagulant.

Analysis of microbial load of the water samples:

Most Probable Number (MPN) method: It was used to detect the coliform bacteria from all the collected raw water samples and after the addition of *Dhavādi Bhasma* (treated water). 50 ml and 10 ml of double strength MacConkey broth (Hi-Media, Mumbai) and 5 ml of single strength MacConkey broth were dispensed in suitable glass containers or test tubes with inverted Durhams tubes and sterilized. After sterilization, air from the Durhams tube was removed. The bottle containing the water to be tested was inverted several times to mix and redistribute any deposit. One 50 ml volume was aseptically pipetted in 50 ml double strength MacConkey broth, five 10 ml volumes were pipetted into five 10 ml double strength MacConkey broth and five 1 ml volumes were pipetted aseptically into single five 5 ml strength MacConkey broth. All the seeded media were incubated at 37^0C. At the end of 48 hours, the media was inspected for colour change from red to yellow (i.e., production of acid) and production of gas (collection of gas in Durhams tube) and the most probable number was estimated. An estimate of the number of coliforms (MPN) was done in the presumptive test. A loopful of samples from each positive tube was streaked on MacConkey agar and incubated at 37^0C for 24 hours. Following this, all the plates were examined for the presence of typical coliform colonies.

Analysis of physicochemical and microbiological parameters of water samples: The raw water samples and treated water samples (supernatants formed at the optimum dosage of *Dhavādi Bhasma*) was used for the analysis of physical parameters of water such as colour, odour, turbidity and conductivity; chemical parameters of water such as pH, alkalinity, TDS, and Total hardness; and microbiological parameters such as Total Coliform count by standard methods accordingly and were analysed as per WHO/ USPH standards.[7]

Table – 5 Standard Methods of Physico-chemical & bacteriological parameters

SN	Parameters	Standard Methods
A	**Physical:**	
	1. Colour	Visual/ Colour Comparator
	2. Odour	Organoleptic/ Physiological sense
	3. Turbidity	Digital Turbidity meter
	4. Conductivity	Conductivity meter
B	**Chemical:**	
	1. pH	Digital pH meter
	2. Alkalinity (as $CaCO_3$)	Acid-Base titration Method

	3. TDS	Gravimetric Method
	4. Total Hardness	Titration Method
C	**Bacteriological:**	
	1. Total Bacteria (coliform count)	MPN Method

RESULTS AND DISCUSSIONS:

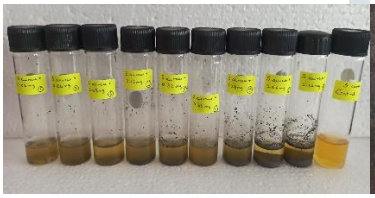
Preliminary phytochemicals of Dhavādi Bhasma: *Dhavādi Bhasma* is the ash prepared from plants that usually represents the inorganic part of the plant because the ash destroys all the organic material present in the plant sample. Hence, all the organic compounds were absent. It was found that however, the main chemical component of the sample *Dhavādi Bhasma* was carbon, it contained varying amounts of other inorganic elements such carbonates, sodium, potassium and a trace amount of chloride and sulphate.

Antibacterial activity of Dhavādi Bhasma: The MIC value of *Dhavādi Bhasma* with an optimum dosage of 3.0mg/l against tested *Staphylococcus aureus* was 2.56 mg/ml, for *Staphylococcus epidermidis* was 1.28mg/ml and 0.64mg/ml for *Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* in each. The MBC value against tested *Staphylococcus aureus* was 1.28 mg/ml, for *Staphylococcus epidermidis, Escherichia coli, Proteus mirabilis* and *Klebsiella pneumoniae* was 0.64mg/ml in each and 0.32mg/ml for *Pseudomonas aeruginosa* (Table – 2).

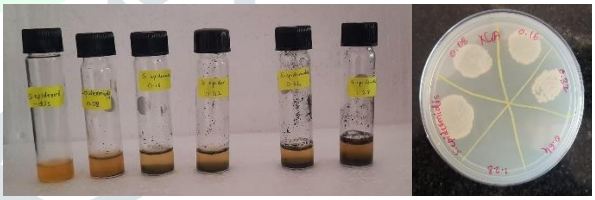
Table – 6 MIC & MBC values of *Dhavādi Bhasma*

SN	Organisms	MIC Value (mg/ml)	MBC Value (mg/ml)
A.	Gram-positive		
1.	*Staphylococcus aureus*	2.56	1.28
2.	*Staphylococcus epidermidis*	1.28	0.64
B.	Gram-negative		
1.	*Escherichia coli*	0.64	0.64
2.	*Proteus mirabilis*	0.64	0.64
3.	*Klebsiella pneumoniae*	0.64	0.64
4.	*Pseudomonas aeruginosa*	0.64	0.32

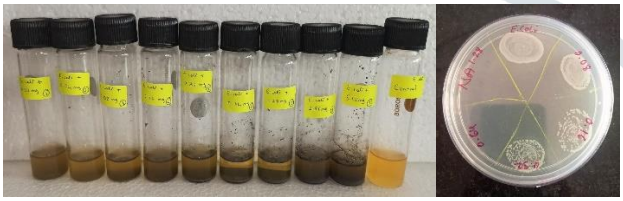
Fig. – 3 Antibacterial Activity of *Dhavādi Bhasma*



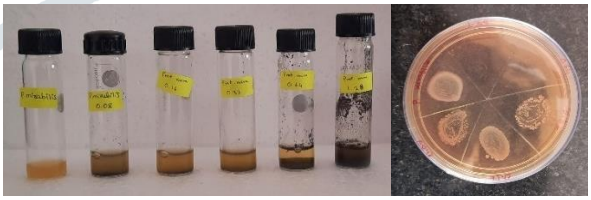
(1) *S. aureus*: MIC 2.56mg/ml, MBC 1.28mg/ml



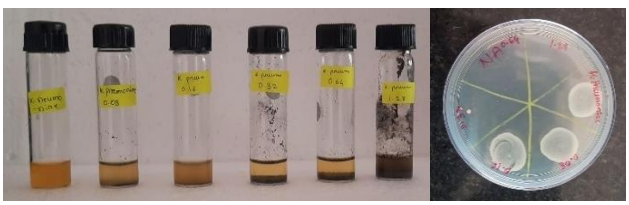
(2) *S. epidermidis*: MIC 1.28mg/ml, MBC 0.64mg/ml



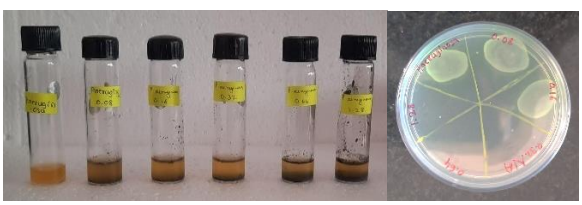
(3) *E. coli* : MIC 0.64mg/ml, MBC 0.64mg/ml



(4) *P. mirabilis* : MIC 0.64mg/ml, MBC 0.64mg/ml



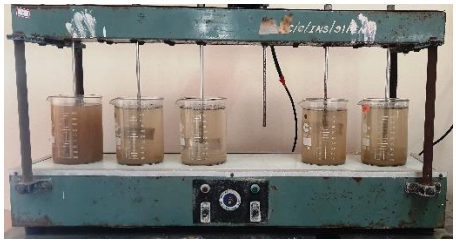
(5) *K. pneumoniae*: MIC 0.64mg/ml, MBC 0.64mg/ml



(6) *P. aeruginosa*: MIC 0.64mg/ml, MBC 0.32mg/ml

Optimization of dosage of Dhavādi Bhasma: The optimum dosage for *Dhavādi Bhasma* was 3g/l with a maximum percentage of reduction in turbidity of all treated water samples and used for further experimental procedures. It was found that at this dosage, within 30 minutes an effective result was observed regarding sedimentation of inorganic and organic matter in raw water samples (Fig. –4).

Fig. – 4 Optimization of dose of *Dhavādi Bhasma* by Jar test method



Sample - 01 (GRW)
(RW– 2.5g/l – **3g/l** – 3.5g/l – 4g/l)

Sample - 02 (GKW)
(RW– 2.5g/l – **3g/l** – 3.5g/l – 4g/l)

Sample - 03 (PW)
(RW– 2.5g/l – **3g/l** – 3.5g/l – 4g/l)

Physical Parameters of the water samples:

Table – 7 Physical parameters of water samples Before and After addition of *Dhavādi Bhasma*

SN	Physical parameters		Sample - 1	Sample - 2	Sample - 3	WHO/USPH Standards
1	Colour	BF	Dark Brown	Greyish black	Faint Brown	Colourless 5 Hazen
		AF +DB	Colourless	Colourless	Colourless	
2	Odour	BF	Present	Present	Present	Odourless/ Unobjectionable
		AF+DB	Reduced Odour	Reduced Odour	Reduced Odour	
3	Turbidity (NTU)	BF	138.5	145.3	126	DL: 5 NTU PL: 10 NTU
		AF+DB	12.1	13.7	10.2	
4	Conductivity (mho/cm)	BF	968	864	940	DL: 2500 µS/cm
		AF+DB	1052	980	1040	

BF: Before (the addition of DB); AF +DB: After the addition of *Dhavādi Bhasma* (3 g/l)

1. **Colour:** During the study, the dark brown, greyish black and faint brown colour were observed in raw water sample-1, 2 and 3 respectively. After the addition of Dhavādi Bhasma, the colour of all three water samples were removed. This suggests that the *Dhavādi Bhasma* shows absorbent properties. However, All the treated water samples showed the colour of the water samples within 5 Hazen.

2. **Odour**: In the present study, all three raw water samples have an odour. After the addition of *Dhavādi Bhasma* as a coagulant, the odour of all three water samples was reduced.

3. **Turbidity:** It was observed in the present study that the turbidity of the raw water sample-1, 2 and 3 was 138.5 NTU, 145.3 NTU and 126 NTU respectively and were beyond the limit as per WHO/USPH standards. After the addition of *Dhavādi Bhasma* at the dosage of 3g/l, the turbidity was decreased to 12.1 NTU, 13.7 NTU and 10.2 NTU (Fig – 5.1 to 5.3) on the turbidity meter thereby achieving a turbidity reduction of 91.26%, 90.57% and 91.90% in water sample -1, 2 and 3 respectively and were again still beyond the limit as per WHO/USPH standards. The maximum decrease of the turbidity was observed in water sample-3 (91.90%) & the minimum in water sample -2 (90.57%).

   Turbidity Reduction with Percentage-wise: Further, the study also revealed that when the dose of *Dhavādi Bhasma* was increased from 3g/l to 3.5g/l, 4g/l, the turbidity tends to increase (Fig. – 5.1 to 5.3). This is probably due to charge reversal in all three water samples and it could be explained by the presence of free positively charged molecules of the flocculants repelling, leading to the flocs floating or suspended in the water. However, the results of the present study revealed that the *Dhavādi Bhasma* significantly reduce the turbidity of the water at the optimum dose of 3g/l and acts as a natural herbal coagulant.

Fig. –5.1 Turbidity reduction of water sample-1 at a different dosage of *Dhavādi Bhasma*

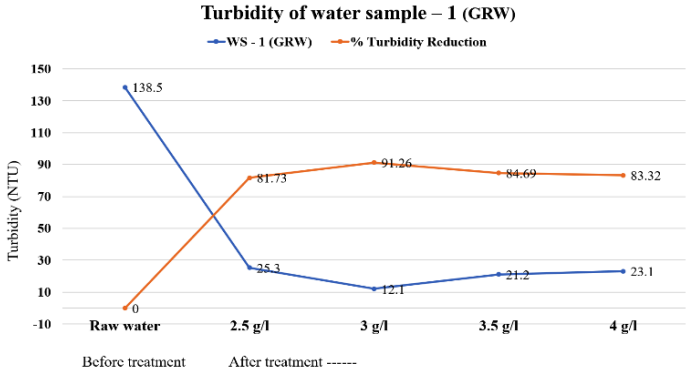


Fig. – 5.2 Turbidity reduction of water sample-2 at a different dosage of *Dhavādi Bhasma*

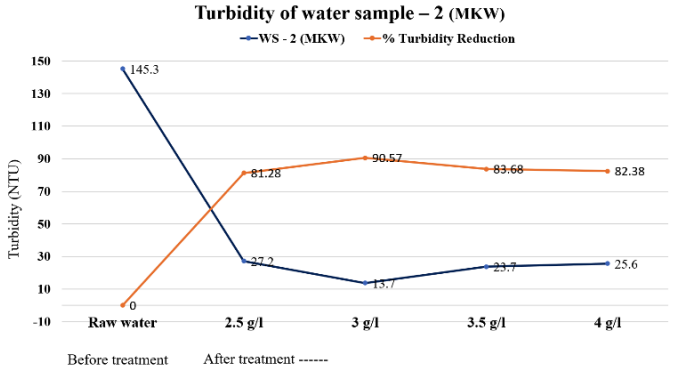
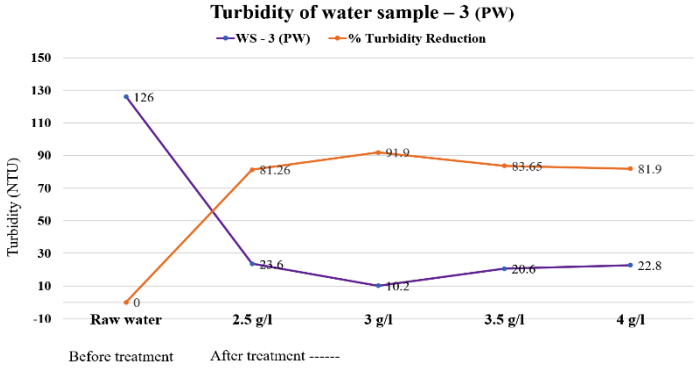


Fig. – 3.3 Turbidity reduction of water sample-3 at a different dosage of *Dhavādi Bhasma*



4. **Conductivity:** It was observed in the current study that the conductivity of the raw water sample-1, 2 and 3 was 968 mho/cm, 864 mho/cm and 940 mho/cm respectively. After the addition of *Dhavādi Bhasma* at the dosage of 3 g/l, the conductivity of the water sample-1, 2 and 3 was increased to 1052 mho/cm, 980 mho/cm and 1040 mho/cm respectively and indicates that treated water was considerably ionized probably due to the dissociation of *Dhavādi Bhasma* in water which raises the electrical conductivity. The maximum increase of the conductivity was observed in water sample-2 (13.42%) and the minimum in water sample -1 (8.67%). However, the results of the current study showed that the *Dhavādi Bhasma* increases the conductivity of the water at the optimum dosage.

Chemical Parameters of the water samples:

Table – 8 Chemical parameters of water samples Before and After addition of *Dhavādi Bhasma*

SN	Physical parameters		Sample - 1	Sample - 2	Sample - 3	WHO/USPH Standards
1	pH	BF	7.86	7.70	7.66	6.5 to 8.5
		AF +DB	7.93	7.83	7.76	
2	Alkalinity (as $CaCO_3$) (mg/ml)	BF	140	120	128	DL: 200mg/l PL: 600mg/l
		AF+DB	196	216	176	
3	TDS (mg/l)	BF	484	432	470	DL: 500mg/l PL: 2000mg/l
		AF+DB	526	490	520	
4	Total Hardness (mg/l)	BF	292	260	284	DL: 300mg/l PL: 600mg/l
		AF+DB	304	276	296	

BF: Before (the addition of DB); AF +DB: After the addition of *Dhavādi Bhasma* (3 g/l)

5. **pH:** It was observed in the present study that the pH of the raw water sample-1, 2 and 3 was 7.86, 7.70 and 7.66 respectively and were within the desirable limit of pH as per WHO/USPH standards. After the addition of *Dhavādi Bhasma* at the dosage of 3g/l, the pH of water sample-1, 2 and 3 was slightly increased to 7.93, 7.83 and 7.76 and were within the desirable limit of pH as per WHO/USPH standards. It was however noted in the current study that the resultant pH of all treated water samples were slightly increased with the optimum dose of *Dhavādi Bhasma*. This could be probably because of the alkaline nature (Kshāra - alkali) of coagulant *Dhavādi Bhasma*. Generally, the plant ashes are almost as alkaline when dissolved in water, with a pH level varying from about 9 to 11. Water with a pH value of more than 9 or less than 4.5 become unsuitable for most life forms and also for other uses. The maximum increase of pH was observed in water sample -2 (1.68%) and the minimum in water sample -1 (0.89%). However, the current study results showed that the *Dhavādi Bhasma* at 3g/l dosage slightly increases the pH of all the treated water samples.

6. **Alkalinity:** In the present study, the total Alkalinity as $CaCO_3$ of raw water sample-1, 2 & 3 was observed to be 140 mg/l, 120 mg/l & 128 mg/l respectively which were within the desirable limit as per WHO/USPH standards. After the addition of *Dhavādi Bhasma* at the dosage of 3g/l, the Total Alkalinity as $CaCO_3$ of water sample-1, 2 and 3 was increased to 196 mg/l, 216 mg/l and 176 mg/l respectively which were within the desirable limit except for water sample -2 but all were within the permissible limit as per WHO/USPH standards. The current study revealed that the resultant alkalinity of all treated water partially increased with the optimum dose of *Dhavādi Bhasma*. This could be probably because of the alkaline nature (Kshāra - alkali) of coagulant *Dhavādi Bhasma*. The maximum increase of alkalinity was observed in water sample -2 (80%) and the minimum in water sample -3 (37.50%). However, the results of the present study showed that the *Dhavādi Bhasma* at 3g/l dosage partially increases the total alkalinity of all the treated water samples which were within the permissible limit as per WHO/USPH standards.

7. **Total dissolved solids:** In the present study, the concentration of TDS of raw water sample-1, 2 and 3 was 484 mg/l, 432 mg/l and 470 mg/l respectively which were within the desirable limit of TDS as per WHO/USPH standards. After the addition of *Dhavādi Bhasma* at the dosage of 3g/l, the TDS of water sample-1, 2 and 3 was increased to 526 mg/l, 490 mg/l and 520 mg/l respectively and were beyond the desirable limit except for water sample -2 but were within the permissible limit of TDS as per WHO/USPH standards. The present study revealed that the resultant TDS of all treated water partially increased. This could be probably because, the *Dhavādi Bhasma* contains a certain substantial amount of inorganic chemicals such as carbonates, sodium, potassium, sulfates may contribute to an increase in the TDS of the water samples. The maximum increase of TDS was observed in water sample -2 (13.42%) and the minimum in water sample -1 (8.67%). However, the results of the present study showed that the *Dhavādi Bhasma* at 3g/l dosage partially increases the TDS of all the treated water samples which were within the permissible limit as per WHO/USPH standards.

8. **Total Hardness:** In the present study, the Total Hardness of raw water sample-1, 2 and 3 was 292 mg/l, 260 mg/l and 284mg/l respectively which are within the desirable limit of TH as per WHO/USPH standards. After the addition of *Dhavādi Bhasma* at the dosage of 3g/l, the Total Hardness of water sample-1, 2 and 3 was slightly increased to 304 mg/l, 276 mg/l and 296 mg/l respectively which were within desirable limit except for water sample -1 but all were within the permissible limit of TH as per WHO/USPH standards. This could be probably due to the presence of a certain substantial amount of inorganic chemicals of *Dhavādi Bhasma* may contribute to an increase in the TH of the water samples. The maximum increase of Total Hardness was observed in water sample -2 (6.15%) and the minimum in water sample -1 (4.10%). However, the results of the present study showed that the *Dhavādi Bhasma* at 3g/l dosage partially increases the Total Hardness of all the treated water samples which were within the permissible limit as per WHO/USPH standards.

Microbiological Parameters of the water samples:

(The microbial load of the water samples)

Table – 9 Microbiological parameters of water samples Before and After addition of *Dhavādi Bhasma*

Water Samples	No. of tubes giving a positive reaction			MPN INDEX /100ml	
	1 x 50ml DS	5 x 10ml DS	5 x 1ml SS	CFU/ 100ml	% of reduction
WS -1	0	1	2	7	-
WS-1 + DB	0	0	0	<1	71.42
WS -2	0	2	1	6	-
WS-2 + DB	0	0	0	<1	66.66
WS -3	0	1	2	7	-
WS-2 + DB	0	0	0	<1	71.42

BF: Before (the addition of DB); AF+DB After the addition of Dhavādi Bhasma (3 g/l)

It was observed in the present study that the initial MPN of all three raw water samples was present and it was beyond the limits of WHO/USPH standards indicating that water was contaminated with coliforms. After the addition of *Dhavādi Bhasma* with an optimum dosage of 3g/l, MPN/100ml coliform was greatly decreased in all the three water samples thereby achieving 71.42% of reduction of (bacterial load) coliform/100ml in water sample 1 and 3 each, 66.66% of reduction in water sample -2. Hence it can be revealed from the above data that *Dhavādi Bhasma* acts as an efficient antibacterial agent in water treatment. However, the MPN was present in all the three water samples was less than 1 coliform/100ml after treatment indicating that it is above the limits of WHO/ USPH standards.

Supernants formed water samples before and after the addition of Dhavādi Bhasma:

Fig. – 4 Supernatants formed water samples Before & After the addition of *Dhavādi Bhasma* (3g/l)

water sample-1 (GRW)

water sample- 2 (MKW)

water sample-3 (PW)

CONCLUSION:

The preliminary phytochemical analysis of *Dhavādi Bhasma* revealed that the inorganic compounds such carbonates, sodium and trace amounts of potassium, chlorides and sulphates were present. However, the organic compounds were not present. It has shown a potential antibacterial activity against all the six test micro-organisms i.e., *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

Dhavādi Bhasma treated water samples at 3g/l concentrations have shown a significant reduction of turbidity with a sedimentation time of 30 mins and thereby achieving a turbidity reduction of 91.26 %, 90.52% and 91.90% in the water samples of Ghataprabha River, Muchakhandi Kere (lake) and Pond respectively. However, the resultant pH, conductivity, alkalinity, TDS and Total hardness of all the treated water samples were partially increased and were within the permissible limit as per WHO/USPH standards. The microbial load (coliform counts) has also reduced significantly. All the resultant water samples have shown a clear appearance indicating their absorbent properties.

Hence, the overall data of the present study indicate that the assorted coagulant *Dhavādi Bhasma* has antibacterial activity. It acts as a potential natural herbal coagulant, flocculent, absorbent and anti-bacterial agent in water treatment. This study has also suggested that the plant's ash have potentially antibacterial compounds against pathogens and their ability to either inhibit or circumvent resistance mechanisms could improve the treatment and eradication of microbial strains and their potential applications could help control bacteria that cause waterborne bacterial diseases.

Scope for Future Work:

1. A study on the different types of herbs as coagulants and coagulant aid for different water samples such as drinking water, sewage water, wastewater etc. for water purification.
2. Comparative study of the effect of herbal and chemical coagulants on water quality parameters of different water samples in the purification of raw water.
3. There is a need to carry out toxicity tests on the *Dhavādi Bhasma*.

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