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MADHUCA INDICA – A HIGHLY POTENT REMEDY TO TACKLE WATER BORNE INFECTIONS

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Abstract: **Water is the basic necessity of life. Ingestion of water contaminated by human or animal excrement, containing pathogenic microorganisms can lead to Water borne infections. Cholera, typhoid and other diarrheal diseases are examples of such diseases. Contaminated drinking water used in the preparation of food or washing of hands and utensils with contaminated water can also lead to water borne disease. In India, over one lakh people die of water-borne diseases annually. The most common water borne pathogenic bacteria include *Escherichia coli,* Salmonella spp., Shigella spp. and *Vibrio cholerae*.**

A major section of the world population still relies on natural remedies like medicinal plants for their primary treatment against infections. Medicinal plants either as pure compounds or as standardized extracts provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. *Madhuca indica*, majorly found in central parts of India, also called as Mahua, is known to possess various medicinal properties.

In the present study, the leaves and bark of *Madhuca indica* were screened for their antimicrobial potential against the above mentioned water borne pathogenic bacteria. The primary screening of the extracts was done by Agar Ditch Method. The MIC determination was carried out using Agar Cup Method. The best extracts showed activity in the range of 5mg/ml to 7.5 mg/ml. The antioxidant potential of the extracts was determined using DPPH method which gave around 96 - 97 % activity. The primary phytochemical screening of the extracts was done by Thin Layer Chromatography.

Keywords: **Antibacterial, Antioxidant, *Madhuca indica*, Medicinal plants, Phytochemical screening, Water, Water borne infections.**

I. INTRODUCTION

Water is a tasteless and odourless substance composed of the chemical elements hydrogen and oxygen and which exists in all three states – solid, liquid and gaseous. It is one of the most abundant and essential compound. It is in liquid state at room temperature which has the ability to dissolve many other substances. It is believed that life originated in the aqueous solutions of the world's oceans, and living organisms depend on aqueous solutions, such as blood and digestive juices, for biological processes [1].

A safe, reliable, affordable, and easily accessible water supply is essential for good health. Yet, for several decades, about a billion people in developing countries have not had a safe and sustainable water supply. It has been estimated that a minimum of 7.5 litres of water per person per day is required in the home for drinking, preparing food, and personal hygiene [2]. Water is the basis of all life. It is fundamental for human existence, ecological balance and for the very future of our planet. Safe drinking water is a basic need and a right for every human being. Clean, safe and adequate fresh water is vital to the survival of all organisms and the smooth functioning of key systems, entities and economies [3].

A major reason for inadequacy of safe drinking water is the existence of water pollution. Water Pollution can be defined

as the presence of excessive amounts of a hazard (pollutants) in water in such a way that it is no longer suitable for drinking, bathing, cooking or other uses. It is a result of industrial and commercial waste, agricultural practices, everyday human activities and most notably, models of transportation [4].

The water quality of the rivers has a considerable importance for the reason that these water resources are generally used for multiple matters such as: drinking domestic and residential water supplies, agriculture (irrigation), hydroelectric power plants, transportation and infrastructure, tourism, recreation, and other human or economic ways to use water [5].

Pollution poses a serious risk to life especially when the water is a source of drinking and for domestic purposes for humans polluted waters are potent agents of diseases such as cholera, typhoid and tuberculosis [4].

Waterborne diseases arise from the contamination of water, either by pathogenic viruses, bacteria or protozoa or by chemical substances. These agents are directly transmitted to people when the water is used for drinking, preparing food, recreation or other domestic purposes [6].

Contaminated drinking-water is a frequent cause of diseases such as cholera, typhoid, viral hepatitis A and dysentery [6]. Bacteria, which are the most common pathogens in water, gain entrance into water mostly through fecal contamination. Some of the bacteria pathogens include Salmonella, Shigella, pathogenic strain of *Escherichia coli*, Yersinia, Campylobacter, Vibrio spps [7]. More than 4 million people die of illnesses contacted through microorganisms, and most cases are caused by water contaminated by microorganisms [8].

Water Borne Diseases in India

In India, almost 70 per cent of its surface water resources and a growing percentage of its groundwater reserves are contaminated by biological, toxic, organic, and inorganic pollutants. In many cases, these sources have been rendered unsafe for human consumption as well as for other activities, such as irrigation and industrial needs [9]. The UN reported that India's water quality is poor - it ranks 120th among the 122 nations in terms of quality of water available to its citizens. Waterborne diseases infection commonly results during bathing, washing, drinking, preparation of food, or the consumption of food thus infected. According to the World Health Organization, such diseases account for an estimated 4.1% of the total global burden of disease, and cause about 1.8 million human deaths annually.

Importance of Medicinal plants in treatment of infections

The dependence of human beings on plants dates back to the start of the human race. Solid evidences can be cited in favor of herbs being used for the treatment of diseases and for restoring and fortifying body systems in ancient systems of medicine such as Ayurvedic, Unani, and Chinese traditional medicine. According to an estimate, 70-80% of the developing world is dependent on conventional plant-obtained remedies, as pharmaceuticals are high priced [10].

Medicinal plants are frequently used as raw materials for extraction of active ingredients which are used in the synthesis of different drugs [11]. Moreover, the increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the spectra of untreatable bacterial infection and adds urgency to the search for new infection fighting strategies [12].

Natural products from medicinal plants, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. Botanicals and herbal preparations for medicinal usage contain various types of bioactive compounds [13].

Madhuca indica (Mahua) is a medium sized tree and belongs to the family Sapotaceae [14]. *Madhuca indica* is ever green or semi ever green tree. The various parts of *Madhuca indica* such as flowers, leaves, bark, seeds, and seed oil have great medicinal value. *Madhuca indica* leaves and stem extract are used for Wound healing activity, Antioxidant activity, Antimicrobial activity, Astringent, Rheumatism, Piles, Nutritive, hemorrhoids, Antihyperglycemic activity and chronic bronchitis. The stem extracts is also used for treatment of snake bite poisoning [15]. Mahua leaf extracts show antibacterial activity against human pathogenic bacteria [14].

Previous studies on *Madhuca indica* bark showed excellent antibacterial, antidiabetic and antioxidant properties [16,17].

For the current study, the leaves and bark of *Madhuca indica* were collected and screened. The study has proved the antibacterial and antioxidant properties of the selected plant parts.

II. MATERIALS AND METHODS

2.1 Collection of water samples [18,19]

Water samples were collected from selective water bodies across Mumbai, Thane and Raigad districts of Maharashtra, India. The seven water bodies selected for the study were: -

1. Barwi Dam, Badlapur, Dist - Thane
2. Khadavli River, Khadavli, Dist - Thane
3. Ulhas River, Vangani, Dist - Raigad
4. Charlotte Lake, Matheran – Neral, Dist - Raigad
5. Kalu River, Titwala, Dist - Thane
6. Tulsi Lake, Bhandup Complex – Sanjay Gandhi National Park, Dist - Mumbai
7. Vihar Lake, Bhandup Complex – Sanjay Gandhi National Park Dist – Mumbai

The sampling was carried out in all three seasons (Summer, Rainy and Winter season). The water samples were collected in sterile 100 ml plastic containers (Tarson) aseptically. The samples were carried to laboratory and processed within 24 hrs.

2.2 Isolation of Pathogenic Bacteria [20-23]

All the collected water samples were processed by direct streaking on various selective and differential media using the T-streak method. The plates were incubated at 37˚C for 24 hrs and observed for their colony characteristics which were noted down. The media used for sample processing and isolation of water borne pathogens were:

- Sterile Thiosulphate citrate bile salts sucrose agar - for *Vibrio cholerae*
- Sterile MacConkey's agar - for *Escherichia coli*
- Sterile Salmonella Shigella agar - for Shigella spps and Salmonella spps

2.3 Identification of Isolates

The identification of the isolates was carried out on the basis of Gram's character, colony characters and biochemical properties according to Bergey's Manual of Determinative Bacteriology, 8th Edition.

2.4 Antibiotic Sensitivity Testing of Isolates

The antibiotic sensitivity testing of the isolates was carried out by the **Agar Disc Diffusion Method** [24]**.**

Commercially prepared filter paper discs, impregnated with specific amounts of antibiotics were used for the testing of isolates. Twelve different antibiotics were used for the antibiotic sensitivity testing, based on their use in treatment by clinicians. The antibiotics used for testing were Ampicillin, Cephalothin, Chloramphenical, Clindamycin, Erythromycin, Gentamicin, Oxacillin, Vancomycin, Tetracycline, Ciprofloxacin, Metranidazole, Doxycycline.

2.5 Collection of Plant Material

The leaves and bark of *Madhuca indica* were collected from **Lok Swastha Parampara Vanaushadhi Udyan, Kashele, Karjat, Raigad district, Maharashtra**. The plant parts were authenticated and voucher specimens were preserved. The plant parts were cleaned, dried in sunlight and powdered.

2.6 Preparation of Extracts

Water (HWE and CWE), Ethanol (HEE and CEE), Ethyl acetate (HEaE and CEaE) and Hexane (HHE and CHE) were used for hot and cold extraction procedures along with powdered plant parts [25-27]. The extracts were recovered and stored at 4°C.

2.7 Antibacterial Screening

The antibacterial screening of the extracts was carried out using the following techniques:

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❖ **Primary Screening**

The primary screening of the extracts was carried out using **Agar Ditch Method** [28]. The extract to be tested was mixed with sterile agar in a concentration of 5 % and introduced in the ditch. Test organisms were streaked across the ditch and after incubation their inhibition was observed. Appropriate solvent controls were maintained

❖ **Determination Of Inhibitory Concentrations**

The Minimum Inhibitory Concentration (MIC) of the extracts was determined by **Agar well diffusion** method [29,30].

The lowest concentration of the compound which caused complete inhibition of the test organisms was considered as the minimum inhibitory concentration (MIC) of that compound. Appropriate solvent controls were maintained.

2.8 Antioxidant Screening

All the extracts were subjected to antioxidant screening or free radical scavenging using the DPPH method [31,32].

The reaction mixture consisted of 1.8 ml of 0.5 mM DPPH and 0.2 ml of different dilutions of extracts. The reaction mixture was allowed to incubate for 5 minutes at room temperature in dark and the antioxidant activity of each extract was quantified by decolourization at 515 nm.

$$\text{Percentage inhibition of DPPH radical} = \frac{\text{Abs. of control} - \text{Abs. of sample}}{\text{Abs. of control}} \times 100$$

2.9 Phytochemical Screening

The phytochemical screening of the powders and extracts was done for compounds like alkaloids, tannins, flavonoids, cardiac glycosides, saponins and steroids [33 - 37]. For powders, tube methods were used. Thin layer Chromatography was used for the screening of extracts.

III. RESULTS

3.1 Collection of water samples

A total of 465 water samples were collected across three seasons. The number of samples from every location was kept constant in all three seasons. The details of sampling are represented in Table 3.1.

Table 3.1, Distribution of samples

Sr. No.	Location	Samples collected per season	Total samples collected
1.	**Barwi Dam**, Badlapur, Thane	30	90
2.	**Khadavli River**, Khadavli, Thane	20	60
3.	**Ulhas River**, Vangani, Raigad	25	75
4.	**Charlotte Lake**, Matheran, Raigad	14	42
5.	**Kalu River**, Titwala, Thane	11	33
6.	**Tulsi lake**, SGNP, Mumbai	30	90
7.	**Vihar Lake**, SGNP, Mumbai	25	75
	TOTAL SAMPLES		**465**

3.2 Isolation of Pathogenic Bacteria

A total of 516 colonies were selected from selective and differential plates. 171 colonies from MacConkey's agar plates, 223 colonies from Salmonella Shigella agar plates and 122 colonies from TCBS agar plates were selected.

From the selected 516 colonies, only 215 isolates were obtained on selective culturing (MacConkey's Agar – 67, Salmonella Shigella Agar – 83 and TCBS Agar – 65).

3.3 Identification of Isolates

Using the Bergey's Manual of Determinative Bacteriology, all the 215 isolates were identified. The isolates were

identified as *Escherichia coli* (10.23 %), *Salmonella typhi* (12.56 %), *Salmonella paratyphi* A (5.12 %), *Salmonella paratyphi* B (4.19%), Shigella spps. (5.58 %) and *Vibrio cholerae* (13.02 %).

3.4 Antibiotic Sensitivity Testing of Isolates

The antibiotic sensitivity testing of the isolates was carried out by the **Agar Disc Diffusion Method** [15]. Most of the isolates showed resistance to 5 – 7 antibiotics employed. Their respective Multiple Antibiotic resistance Indices (MAR Index) were calculated. Isolates showing MAR Index in excess of 0.4 were selected for further studies.

3.5 Preparation of Extracts

A total of 16 extracts were prepared using the 2 powders and 4 solvents. Water and ethanol extraction procedure provided higher yields of extract as compared to ethyl acetate and hexane. In case of *Madhuca indica* leaves, HWE and CWE showed highest percentage yields with 10.2 % and 11.06 % yield respectively. Similarly in case of *Madhuca indica* bark, HEE and CEE showed best percentage yields with 10.4 % and 13.2 % yield respectively. All the recovered extracts were labeled and stored at 4°C.

3.6 Antibacterial Screening

The screening of all the extracts was carried out against the isolated and selected water borne pathogens. Standard cultures of *Escherichia coli* MTCC 1885, *Salmonella enterica* (typhi) MTCC 734, *Shigella flexneri* MTCC 1457 and *Vibrio cholerae* MTCC 3906 were also used for screening purpose.

The antibacterial screening of the extracts was carried out in the following steps:

❖ **Primary Screening**

All the 16 extracts showed antibacterial activity against all the test organisms at 5 %. The extracts were further screened for determination of Minimum Inhibitory Concentrations.

❖ **Determination of Inhibitory Concentrations**

The Minimum Inhibitory Concentration (MIC) of the extracts was determined by **Agar well diffusion** method. The test organisms were same as used in primary screening. The concentrations of extracts used for MIC determination were 10, 20, 30, 40 and 50 mg/ml. In addition, extracts showing antibacterial activity at 10 mg/ml were further screened at 2.5 mg, 5 mg and 7.5 mg/ml concentration.

Table 3.2, MIC values for *Madhuca indica* leaf extracts (in mg/ml)

Test Organisms	HWE	HEE	HHE	HEaE	CWE	CEE	CHE	CEaE
Standard Cultures								
E.coli	40	10	50	2.5	2.5	5	50	5
S.typhi	40	7.5	50	2.5	7.5	7.5	50	5
S. paraA	50	7.5	50	2.5	10	5	50	5
S. para B	40	10	50	2.5	5	7.5	50	5
Shigella	50	7.5	50	2.5	10	7.5	50	7.5
V. cholerae	50	7.5	50	2.5	10	10	50	7.5
Isolated cultures								
E.coli	40	10	50	2.5	2.5	2.5	50	5
S.typhi	40	10	50	2.5	7.5	7.5	50	7.5
S. paraA	40	10	50	2.5	7.5	7.5	50	7.5
S. para B	50	7.5	50	2.5	7.5	7.5	50	7.5
Shigella	40	10	50	2.5	7.5	7.5	50	7.5
V. cholerae	40	10	50	2.5	7.5	7.5	50	5

In case of *Madhuca indica* leaves, HWE, HHE and CHE showed MIC values in the range of 40 mg/ml to 50 mg/ml. HEE, HEaE, CWE, CEE and CEaE showed MIC values in the range of 2.5 mg/ml to 10 mg/ml. Best activity was shown by HEaE and CEE.

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Table 3.3, MIC values for *Madhuca indica* bark extracts (in mg/ml)

Test Organisms	HWE	HEE	HHE	HEaE	CWE	CEE	CHE	CEaE
Standard Cultures								
E.coli	10	5	50	5	7.5	5	50	5
S.typhi	10	5	50	5	7.5	5	50	5
S. paraA	10	5	50	7.5	7.5	5	50	10
S. para B	10	5	50	7.5	7.5	5	50	10
Shigella	10	5	50	7.5	7.5	5	50	10
V. cholerae	10	5	50	5	7.5	5	50	10
Isolated cultures								
E.coli	2.5	5	50	5	5	5	50	5
S.typhi	7.5	5	50	5	7.5	5	50	7.5
S. paraA	10	5	50	7.5	7.5	5	50	10
S. para B	7.5	5	50	5	7.5	5	50	10
Shigella	10	5	50	7.5	7.5	5	50	7.5
V. cholerae	7.5	5	50	7.5	7.5	5	50	7.5

In case of *Madhuca indica* bark, HHE and CHE showed activity at 50 mg/ml only. HWE, HEE, HEaE, CWE, CEE and CEaE showed MIC values in the range of 5 mg/ml to 10 mg/ml. Best activity was shown by HEE and CEE.

3.7 Antioxidant Screening

All the extracts were subjected to antioxidant screening or free radical scavenging using the DPPH method. All the extracts showed antioxidant activity at 0.1% concentration. The ethanolic extracts showed best antioxidant activity.

Table 3.4, Antioxidant activity of *Madhuca indica* – Leaf (0.1 % Concentration)

EXTRACT	Percentage inhibition of DPPH
HWE	**66.47 %**
HEE	**97.60 %**
HHE	**31.03 %**
HEaE	**75.08 %**
CWE	**53.10 %**
CEE	**96.64 %**
CHE	**75.43 %**
CEaE	**82.44 %**

Table 3.5, Antioxidant activity of *Madhuca indica* – Bark (0.1 % Concentration)

EXTRACT	Percentage inhibition of DPPH
HWE	**48.58 %**
HEE	**96.90 %**
HHE	**25.72 %**
HEaE	**48.58 %**
CWE	**97.23 %**
CEE	**97.23 %**
CHE	**26.71 %**
CEaE	**46.77 %**

3.8 Phytochemical Screening

The phytochemical screening of the powders and extracts was done. For powders, tube methods were used. Thin layer Chromatography was used for the screening of extracts.

The powders showed the presence of tannins, cardiac glycosides, alkaloids and steroids.

The *Madhuca indica* leaf extracts showed presence of terpenes and alkaloids followed by flavanoids. CHE and CEaE showed the presence cardiac glycosides and tannins respectively.

The *Madhuca indica* bark extracts showed presence of terpenes followed by saponins and cardiac glycosides. HEaE showed the presence of flavonoids and tannins. HWE showed the presence of flavonoids only. Tannins were present in HEaE and CEE.

4. SUMMARY & CONCLUSION

The current study provides leads and evidences in view of using *Madhuca indica* (Mahua) as a remedy for water borne or gastro intestinal tract infections. The antibacterial properties of the extracts can be wisely used to tackle bacterial pathogens. The antioxidant potentials of the extracts are suggestive of the possibility of their applications in anticancer studies.

The multi-utility nature of the *Madhuca indica* plant and extracts can be of great help to medicinal studies and further research can lead us to even more potent possibilities beneficial to mankind.

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