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Potential use of low-cost herbs against contaminated water for health issues

Shikha Rangra Chandel

Assistant Professor, Division of Microbiology, School of Pharmaceutical and Health Sciences, Career Point University, Hamirpur - 176041, Himachal Pradesh, India

Correspondence author email: shikha777rangra@gmail.com

Ashish Jaiswal

Research Scholar, Division of Microbiology, School of Pharmaceutical and Health Sciences, Career Point University, Hamirpur - 176041, Himachal Pradesh, India
Email: ashishjaiswal12492@gmail.com

Simpy

Master of Science, Division of Microbiology, School of Pharmaceutical and Health Sciences, Career Point University, Hamirpur - 176041, Himachal Pradesh, India

Shriyanshu Thakur

Master of Science, Division of Microbiology, School of Pharmaceutical and Health Sciences, Career Point University, Hamirpur - 176041, Himachal Pradesh, India

Abstract--The main objective of this study is to identify the most suitable herbal plants for removing contaminants to reduce the use of chemicals for water contamination. The microbial studies were performed in the contaminated kitchen and household wastewater treated (with various medicinal plants, i.e., *Azardichta Indica*, *Ocimum tenuiflorum*, and *Pinus roxburghii*) using the serial dilution method. Aqueous extracts of the dried powdered plant material were used directly to treat the collected water samples. We examined that the enumeration of bacterial load in the treated water was reduced as compared to contaminated water. Enumeration of Bacterial load in the kitchen wastewater were ranging from 80×10^4 to 17×10^{10} , However, the Cfu (colony forming unit) in treated kitchen water with *Ocimum tenuiflorum* were ranging from 13×10^3 to 2×10^{10} . Bacterial load in the household sewage wastewater were ranging from 105×10^7 to 19×10^{10} . However, the Cfu in treated household sewage wastewater with *Pinus roxburghii* were ranging from 75×10^4 to 4×10^{10} . We found that *Ocimum tenuiflorum* was more effective against kitchen runoff compared to other selected medicinal plants. Whereas the *Pinus roxburghii* showed more effectiveness in household wastewater to

overcome the burden of micro-organisms, which includes some viruses, fungi, archaea, protozoa, algae, and bacteria (Jaiswal et al., 2022). The study also revealed a possible interaction between plant extracts and water sources. Studies have been carried out globally to verify their efficacy and some of the findings have led to the production of plant-based medicines. This paper discusses the role, contribution, and benefits of medicinal plants in controlling diseases of public health importance, with a particular focus on current strategic approaches to disease prevention. These approaches offer interesting and new perspectives in the field of medicinal plants. Recommendations are proposed for the strategic design of medicinal plants' future role and position in disease prevention.

Keywords---medicinal plants, microbial load, contaminated water, bacterial colonies.

Introduction

Water is an important resource and is used in a variety of ways. All living things need water to survive. Humans cannot survive without fresh water for more than five to seven days. Water is an inorganic, transparent, tasteless, odorless, and nearly colorless chemical substance that is the main component of the Earth's hydrosphere and the fluids of all known living organisms. It is essential to all known forms of life, although it does not provide food, energy, or organic micronutrients. It is one of the most plentiful and important compounds. At room temperature, it is a tasteless and odorless liquid and has the important ability to dissolve many other substances (WHO/UNICEF, 1996). Wastewater reuse or water recycling is the use of treated wastewater (or untreated wastewater) for a beneficial purpose. One of the main benefits of water recycling is the protection of water resources by reducing the discharge of water pollution and the need to remove water from natural habits and later recycle it (Raquel Gaviln Prraga, 2014). More than half of all illnesses and deaths among children are caused by germs, which get into the mouth via water and food. The World Health Organization has estimated that up to 80% of all disease and sickness in the World is caused by inadequate sanitation, polluted water, or unavailability of water. Approximately three out of every five persons in developing countries do not have access to drinking water and only one in four has any kind of sanitary facility (Cheesbrough, 1984,). Many diseases spread from contaminated water. Waterborne disease is also one the disease all. Waterborne diseases are diseases transmitted through drinking water contaminated with pathogenic microorganisms such as protozoa, viruses, bacteria, and intestinal parasites (Gary R. Brenniman 1999) Most waterborne diseases are characterized by diarrhea and can result in dehydration and even death in very serious cases. (Montgomery.M., 1985).

Poor personal hygiene practices like preparing, eating, or handling food and water without washing hands well after defecation can lead to contamination due to fecal matter that contains disease-causing microorganisms. (APHA, 1992). Contamination can also occur at the source or while transmitting water through

pipes that are located close to sewage lines. This can lead to the mixing of sewage with drinking water, contaminating it with disease-causing microorganisms. Other factors such as inadequate water supply, poor sanitary conditions, uncovered sources of drinking water, and defecation in the open near sources of drinking water can increase the chances of contamination. (Williams, R. B., and Culp, G. L., 1986.). The pathogenic microorganisms, their toxic exudates, and other contaminants together, cause serious conditions such as cholera, diarrhea, typhoid, amebiasis, hepatitis, gastroenteritis, giardiasis, campylobacteriosis, scabies, and worm infections, to name a few water-related diseases are grouped according to their transmission mechanism: water bore, water hygiene, water-based, water-related. The main transmission mode for waterborne diseases is the ingestion of contaminated water. (M Pięłowski 2019). Waterborne diseases contribute to the death of about 4 million children in developing countries every year and the situation in Nigeria rises steadily. Access to water is a basic human necessity unavailable to hundreds of millions of the World's poorest people. Yet, the search for water and the effects of unsafe water and poor sanitation is an enormous burden on their daily lives, especially for most rural communities in Nigeria where the situation is rather increasing, thus little few prospects for development (UNICEF, 1993). Some medicinal plants can also be used for the purification of water. some of these medicinal plants are Neem, Moringa Oleifera, Vetiver, Nirmali, Luffa cylindrica, orange peel, tulsi, pinus, coriander, etc.

The term medicinal plants include various types of plants used in herbalism and some of these plants have medicinal activities. Medicinal plants are known as the "backbone" of traditional medicine, which means regularly more than 3.3 billion people in less developed countries utilize medicinal plants (Davidson, 2000). Plants with medicinal properties enjoyed the highest reputation in the indigenous systems of medicine all over the world and still constitute one of the major sources of drugs in modern as well as traditional systems of medicine despite tremendous development in the field of synthetic drugs and antibiotics (Chandel et al, 2019). Different types of ingredients are present in medicinal plants which can be used in the Purification of wastewater. Besides that, these plants also play an important role in the development of human cultures around the whole world. Medicinal plants, also called medicinal herbs, have been discovered and used in traditional medicine practices since prehistoric. Plants synthesize hundreds of chemical compounds for functions including defense against insects, fungi, diseases, and herbivorous mammals. Medicinal plants such as Aloe, Tulsi, Neem, Turmeric, and Ginger cure several common ailments. These are considered home remedies in many parts of the country. It is a known fact that lots of consumers use Basil (Tulsi) for making medicines, black tea pooja, and other activities in their day-to-day life. Many medicinal plants like tulsi, neem, pinus, etc. are used for water purifications (M Fitzgerald 2020). Keeping in view the above perspective, the study focuses on the medicinal plants used against contaminated water.

Material and Method

In the present study three medicinal plants i.e., *Azadirachta indica*, *Ocimum tenuiflorum*, and *Pinus roxburghii* collected from the Bhoranj block of Hamirpur, Himachal Pradesh for the treatment of contaminated water

Selection of sampling sites

The leaves of *Azadirachta indica*, *Ocimum tenuiflorum*, and *Pinus roxburghii* were collected from different locations in Hamirpur, Himachal Pradesh. All three locations were randomly picked for sampling.

Surface Sterilization of Plant Materials

The collected plant material was systematically washed under running tap water to remove dust particle, soil, and their unwanted substances. After that leave samples were sterilized with 70% ethanol and then dried in a hot air oven at 37°C for a week. Then the samples were powdered with the help of an electric grinder and stored in airtight bottles in the dark till further use.

Preparation of leaves extract

The Aqueous extract of each leaf *Azadirachta indica*, *Ocimum tenuiflorum*, and *Pinus roxburghii* was prepared by using the maceration process. Then each left sample was dissolved in distilled water (50 ml) in a conical flask, plugged with cotton wool, and kept on a rotary shaker at 120rpm for two days to ensure complete extraction. The extracts were filtered through the Whatman No.1 filter paper. The filtrate was collected and stored in airtight bottles till further use (Chandel et al., 2020).

Treatment of Contaminated Water with Medicinal Plants

For the treatment purpose, we take the 5gm of filtered water of *Azadirachta indica*, *Ocimum tenuiflorum*, and *pine tree* extract. After that dissolve these extracts into 15gm of the contaminated water (Kitchen, Household Sewage). After the incubation of two days filter the treated water and use it for the further studies

Bacterial enumeration

For serial dilution we take 9 test tubes (10^{-1} – 10^{-9}), start by filling several test tubes with 9 milliliters of a dilution liquid, like water. A sterile pipette is taken and add 1ml Of properly mixed sample is drawn into the pipette. The sample is added to the 1st test tube to make the volume of 10ml to make a dilution of 10^{-1} . The dilution is thoroughly mixed by emptying and filling the pipette several times. As nine Tubes are Used, the final dilution for the bacteria cells will be 10^{-9} .

Results and Discussion

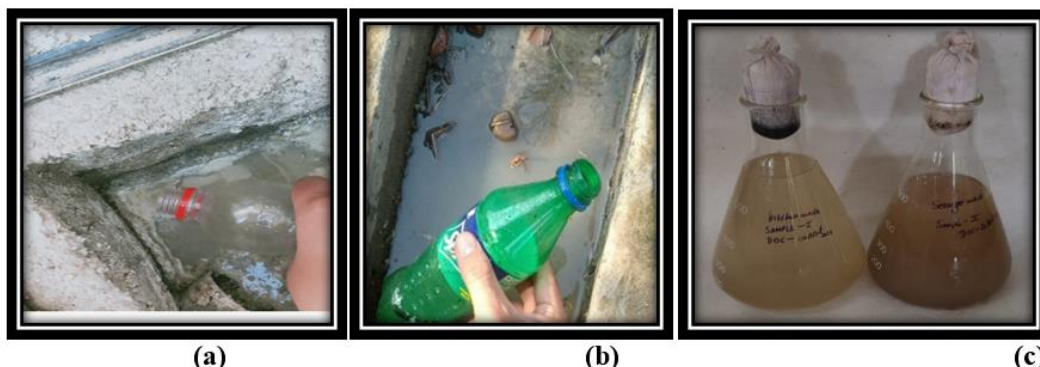
In the present study, we selected three medicinal plants i.e., *Azardichte Indica*, *Ocimum tenuiflorum*, and *Pinus roxburghii* increase the bacterial load present in contaminated water.

Collection of medicinal plants

Contaminated water collected from the Bhoranj block of Hamirpur: Himachal Pradesh

Location: Kharwar

Source: Wastewater (Kitchen, Household Sewage)



(a) Collection of kitchen wastewater (b) Collection of household sewage water (c) Sample of contaminated water stored and transferred in the flask

Collection of medicinal plants

Medicinal plants were collected from the Bhoranj block of Hamirpur, Himachal Pradesh.

Location: Kharwar, Jahu

Parts used: Leaves of *Azadirachta Indica*, *Ocimum tenuiflorum* *Pinus roxburghii* were collected



(a) *Azadirachta indica*

(b) *Ocimum Tenuiflorum*

(c) *Pinus roxburghii*

Figure: Showing the medicinal plants (a) *Azadirachta India* (b) *Ocimum Tenuiflorum* (c) *Pinus roxburghii*

Assessment of bacterial load in contaminated water

In the present study, we checked the bacterial load present in kitchen wastewater. We observed that different dilutions show different numbers of colonies. As we observed that the dilution 10^{-1} and 10^{-2} were showing non-countable colonies. Whereas the dilution 10^{-6} , 10^{-7} , and 10^{-8} showed the number of colonies of 44, 24, and 20 respectively in the Kitchen wastewater. However, the bacterial load present in treated kitchen wastewater with *Azadirachta indica* showed the number of colonies 6, 4, and 3 respectively. The bacterial load present in treated kitchen waste water *Ocimum tenuiflorum*. We observed the different dilutions show the different CFU (colony forming unit). As shown in table 5 the dilution 10^{-1} were showing the non-countable cfu. Whereas the dilution 10^{-6} , 10^{-7} , and 10^{-8} showed different CFU i.e., 30×10^6 , 30×10^8 , and 20×10^{10} respectively. In the current study, we checked the bacterial load present in treated kitchen waste water *Pinus roxburghii*. We observed the different dilutions show the different CFU (colony forming unit). As shown in table 7 the dilution 10^{-1} were showing the non-countable cfu. Whereas the dilution 10^{-6} , 10^{-7} and 10^{-8} and 10^{-9} showed the different CFU i.e., 1.40×10^8 , 2.20×10^9 , 50×10^8 and 20×10^9 respectively.

Table 2: Showing the bacterial load in kitchen wastewater

Sr. No.	DILUTION	UNTREATED WATER		TREATED WATER					
		KITCHEN		<i>Azadirachta indica</i>		<i>Ocimum tenuiflorum</i>		<i>Pinus roxburghii</i>	
		No. of colonies	Cfu/ml water	No. of colonies	Cfu/ml water	No. of colonies	Cfu/ml water	No. of colonies	Cfu/ml water
1.	10^{-1}	NC	-	123	12.3×10^3	NC	-	NC	-
2.	10^{-2}	NC	-	10	1.0×10^4	13	1.30×10^4	70	7.0×10^4
3.	10^{-3}	80	8.00×10^5	8	8×10^3	9	90×10^3	27	2.70×10^3
4.	10^{-4}	78	7.80×10^6	8	80×10^4	6	60×10^4	23	2.30×10^6
5.	10^{-5}	72	7.20×10^7	6	60×10^5	5	50×10^5	21	2.10×10^7
6.	10^{-6}	44	4.40×10^8	6	60×10^6	3	30×10^6	19	1.90×10^8
7.	10^{-7}	24	2.40×10^9	4	40×10^7	3	30×10^7	8	80×10^7
8.	10^{-8}	20	2.00×10^{10}	3	30×10^8	3	30×10^8	4	40×10^8
9.	10^{-9}	17	1.70×10^{11}	1	10×10^{10}	2	20×10^{10}	1	10×10^9

Cfu= No. of colonies counted on plate/volume of sample x dilution

NC= Not countable.

1.	10^{-1}	NC	-	NC	-	NC	-	NC	-
2.	10^{-2}	NC	-	98	9.80×10^4	NC	-	NC	-
3.	10^{-3}	NC	-	95	9.50×10^5	101	10.10×10^5	75	7.50×10^5
4.	10^{-4}	105	10.5×10^6	52	5.20×10^6	67	6.7×10^6	30	3.00×10^6
5.	10^{-5}	65	6.5×10^7	35	3.50×10^7	23	2.30×10^7	20	2.00×10^7
6.	10^{-6}	63	6.3×10^8	24	2.40×10^8	8	80×10^6	8	80×10^6
7.	10^{-7}	26	2.6×10^9	22	2.20×10^9	7	70×10^7	6	60×10^7
8.	10^{-8}	24	2.4×10^{10}	5	50×10^8	3	30×10^8	5	50×10^8
9.	10^{-9}	19	1.90×10^{11}	2	20×10^9	2	20×10^9	4	40×10^9

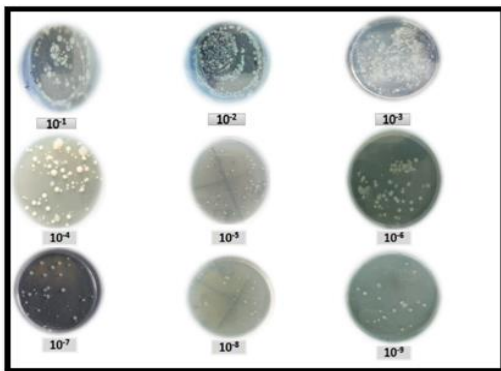


Figure: Showing bacterial load in household sewage wastewater

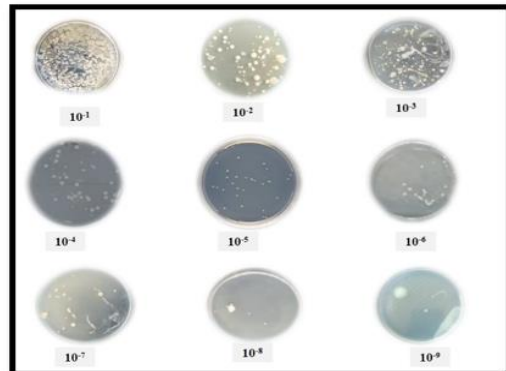


Figure: Showing bacterial load in treated household sewage waste water *Azadirachta Indica*

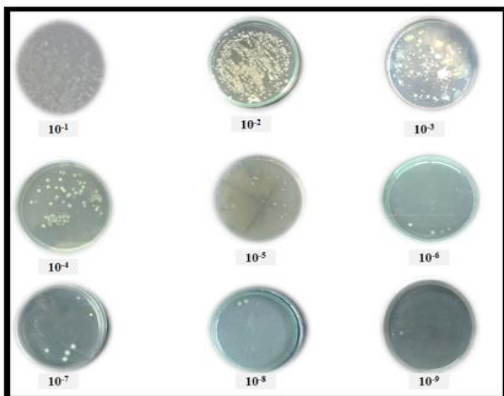


Figure: Showing bacterial load in treated household sewage waste water *Ocimum tenuiflorum*.



Figure: Showing bacterial load in treated House wastewater *Pinus roxburghii*.

Comparative bacterial load of kitchen wastewater and treated water with different medicinal plants

Through this graphical representation, we take the bacterial load on the y-axis and samples on the x-axis. The given output on the graph clearly shows that the bacterial load on the treated kitchen waste water is comparatively less as compared to contaminated kitchen wastewater. The color blue, red, green, and purple shows the serial dilutions of treated kitchen waste water and contaminated kitchen wastewater. The *Ocimum tenuiflorum* shows the best result in kitchen wastewater by giving a much lesser amount of bacterial load as compared to two other medicinal plants.

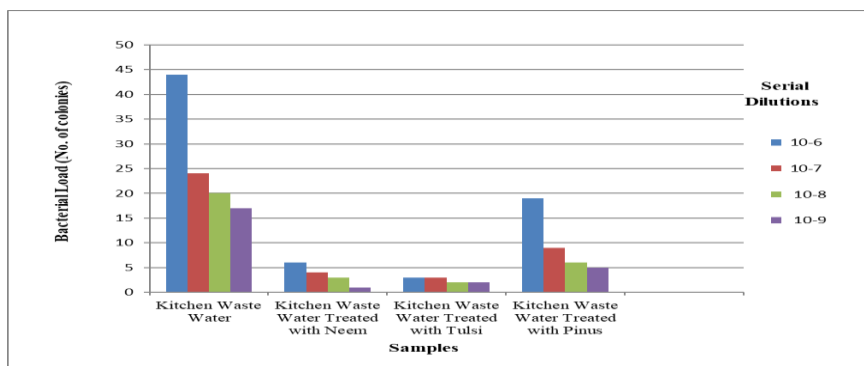


Figure: Graphical representation of comparative bacterial load of kitchen waste water and treated water with different medicinal plants

Through this graphical representation, we take the bacterial load on the y-axis and samples on the x-axis. The given output on the graph clearly shows that the bacterial load on the treated household sewage wastewater is comparatively less as compared to contaminated household sewage wastewater. The color blue, red, green, and purple shows the serial dilutions of treated household sewage waste water and contaminated household sewage wastewater. The *Pinus roxburghii* shows the best result in household sewage wastewater by giving a much lesser amount of bacterial load as compared to two other medicinal plants.

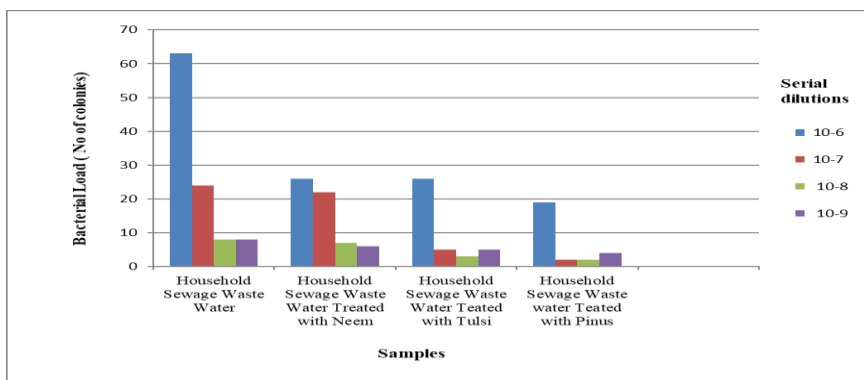


Figure: Comparative bacterial load of household sewage water and treated water with different medicinal plants

Discussion

As we observed that medicinal plants were effective against the contaminated water. We found that *Ocimum tenuiflorum* shows the best result in kitchen wastewater and *Pinus roxburghii* shows the best result in household sewage wastewater. *Pinus roxburghii* shows more effectiveness against the bacterial load in household sewage wastewater, whereas, in a previous study, the amble plant showed more effectiveness in the household sewage wastewater (Crump et.al.2004). In the Neem plant comparatively, we found that this plant showed less effectiveness against the bacterial load in kitchen wastewater and also household sewage wastewater, whereas in a previous study Neem plant showed more effectiveness against the bacterial load in kitchen wastewater (Accra et.al 1990). In contrast, Abubakar et al, (2006), the seeds of *Moringa oleifera*, and *Jatropha curcas* were used for the treatment of wastewater. Wastewater was collected from Yelwa village in Bauchi, Nigeria. They found that 90% reduction in bacterial load in *Moringa oleifera* as compared to other plants.

Conclusion

In the present investigation, we used medicinal plants (Neem, Tulsi, and *Pinus*) against contaminated water such as kitchen and sewage wastewater. For the study, we collected different water and medicinal plants from the local area of the Hamirpur district of Himachal Pradesh. The given experiment shows us that medicinal plants like *Azadirachta India*, *Ocimum tenuiflorum*, and *Pinus roxburghii* are very effective to remove the impurities of contaminated water. Comparatively, we found that the *Ocimum tenuiflorum* showed more effectiveness against bacterial contamination in the Kitchen Waste Water. *Pinus roxburghii* showed more effectiveness against bacterial contamination in the Household Sewage Waste Water. This study showed evidence of medicinal plants is quite effective to purify aminated water.

Future scope

- Medicinal plants are considered rich resources of ingredients that can be used in drug development pharmacoepial, non- pharmacoepial, or synthetic drugs.
- A part of that, these plants play a critical role in the development of human cultures around the whole world.
- Medicinal plants are also used for water purification.
- Medicinal Plants can be used in big sewage treatment plants for pure water instead of using chemical components

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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