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DEVELOPMENT, CHARACTERIZATION AND EVALUATION OF POLY HERBAL WATER PURIFYING SACHET

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ABSTRACT

To develop a formulation this aids in antimicrobial and heavy metals removal property from water with merit of formulation's portability and ecofriendly nature. Article includes use of herbs having potent antimicrobial and heavy metals removal property. A group of 4 herbs were taken and assayed against blank, ethanol and standard for antimicrobial property by disc diffusion method. Then herbs were assayed concentration wise for its most effective concentration to be utilized by disc diffusion method. For heavy metals removal activity herbal water was analyzed with limit test of water. Other than these 2 parameters water was analyzed with various BSI specifications for drinking water for ex- pH, turbidity, TDS, taste, color, and odor. Conclusion- Proposed article aims at justifying developing a formulation having mini side effects, ecofriendly nature, and portability, economic, doesn't require power consumption and is very effective in natural calamities situation and remote areas.

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INTRODUCTION

Aim of study

The aim of study is to formulate and evaluate a formulation that has herbs with application of anti-microbial activity as well as can remove harmful heavy water from drinking water [1].

Objective of study

To provide a water purification system based on natural phenomenon to purify water with minimum side effects. To provide a cost effective system to purify water to be effectively used in rural areas where there is scarcity of good quality of water. To make the system portable and user friendly [2]. To design a system in which essential minerals remain intact. To design a formulation that with advantage to purify water it also lowers risk for illness, also boost your mood at the same time.

Importance of water:

A basic need which plays very important role "water". Because of some deliberate human activities and natural calamities water is getting polluted, not only chemically but also biologically. Therefore it becomes important to remove harmful microbes and heavy metals at the same time as they affect health [3]. Therefore drinking water must be microbe free and in limit to heavy metals, which is important consideration in the prevention of water borne disease [4].

Types of impurities:

Dissolved impurities include inorganic salts (Ca, Mg, Cl, NO₃, SO₄) and Organic matter (Humic and Fulvic acids, Pesticides, Detergents etc.), Suspended impurities include Colloidal (Clay, Pests, Acids), Inorganic (Sand Particles), Organic (Industrial, Domestic), living matter like Micro-organisms (Bacteria, Algae, Virus), Macro-organisms (Fish, Shrimps, Worms, Larvae). [5]

Test for impurity:

Test for heavy metals

Table 1: List of test of impurities of heavy metals.

S. No.	Class	Test
1.	Qualitative	Limit test
2.	Quantitative	Atomic absorption Spectroscopy Flame Photometry Fluorimetry [6]

For living matter (microorganisms):

- Presumptive test for coliform group of bacteria.
- Water analysis for total bacterial population by disc diffusion method against standard, blank, ethanol, herbal extract
- Water analysis for total bacterial population by disc diffusion method against various dilutions of herbal extract.
- ISI specification for drinking water.

The provision of potable water is an enormous undertaking, especially in developing countries. [7]. Heavy metals are present in varying concentrations in all ecosystems being toxic, they have adverse effects on the environment and human health [8].

Limitations of RO water purifier:

- Removes essential minerals: While RO water purifier removes dissolved impurities it removes natural mineral such (Na, Ca, Mg, Fe) causing a mineral deficiency in the body.
- Not kills bacteria, viruses: RO water purifier does not kill water borne disease-causing bacteria and viruses.
- Water taste altered
- More time to purify
- Water wastage
- Expensive
- RO membrane breakage: Chlorine can damage RO membrane. It is advisable to replace RO membrane once in a year. [9]

Advantages of herbs as water purifier:

One of the reasons why ancients relied on Ayurveda herb infused water because it helped in healing a number of diseases. Water is a powerful ingredient in Ayurveda and when you soak herbs it increases the healing power [8]. Herbs Like Tulsi (*Ocimum Sanctum*), Neem (*Azadirachta indica*) and Cilantro (*Coriandrum sativum*), Clove (*syzonium aromaticum*) can be used for water purification and are easily available [10].

MATERIAL AND METHOD

Collection and processing of plants requirements

Plants were collected from RGPV botanical garden and local market and authenticated by Sofia College of Botany Bhopal.

Extraction of phytochemical constituents of plants

A Soxhlet is basically a laboratory apparatus invented in 1879 by Franz von Soxhlet. It is generally used for compounds having limited solubility in a solvent, and whose impurity is insoluble in that solvent. 10g Leaves were washed with distilled water and then defatted with petroleum ether followed by maceration with 70% ethanol 100ml for 12 hours and filtered through Whatmann filter paper allowed to concentrate to be used as extract.

Microbiological study

Preparation of dilutions

Plant extracts(1mg) was diluted in distilled water (10 ml) to prepare stock solution, further 1ml from the above solution was taken and diluted with distilled water(10) to prepare sub-stock. From this sub-stock solution prepare further serial dilutions i.e. 10 μ g/ml, 20 μ g/ml, 30 μ g/ml, 40 μ g/ml.

Sterilized disc were then prepared with Whatman filter paper which were cut circular in shape, disc were washed with ethanol then discs were dried in 37°C incubator for 15 minutes and then placed under UV aseptic chamber for further sterilisation.

Bacteria Culture

Bacteria to be used for the test were obtained from various contaminated water bodies as well as tap water i.e. water was taken as bacterial sample.

Disc Diffusion Method

Disc diffusion method for antimicrobial susceptibility testing was carried out according to the standard method by Bauer et al. (1966) to assess the presence of antibacterial activities of the plant extracts.

Invitro antibacterial activity of plant extract



Fig 1: culture plate showing comparative study of blank, ethanol, standard and coriender, tulsi, clove, neem extract Fig 1: culture plate showing comparative study of blank, ethanol, standard and coriender, tulsi, clove, neem extract



Fig 2: culture plate showing mix(tulsi+neem+clove+coriender)extract.

Minimum Inhibition Concentrations

MIC was determined using Inhibitory Concentrations in Diffusion (ICD) method (Guerin-Faubleee et al., 1996).

Invitro antibacterial MIC(minimum inhibitory concentration) activity of plant extract



Fig 3: culture plate showing comparative study of concentration (10 μ g/ml, 20 μ g/ml, 30 μ g/ml, 40 μ g/ml) clove, coriender, tulsi, neem extract

Physiochemical characterization and preliminary qualitative phytochemical investigation

Phytochemical screening-

Phytochemical subjective investigation incorporates appraisal of plant extricates and ethanol watery answers for nearness of phytochemical constituents.

1. Test for phenolic content

Chlorogenic acid test-Treat the test solution with aqueous ammonia and expose to air gradually.

2. Test for flavonoids

1 ml of extract in a test tube + few drops of dilute NaOH solution. An intense yellow color was appeared in the test tube. It became colorless on addition of a few drops of dilute hydrochloric acid that indicated the presence of flavonoids.

3. Limit test for heavy metals

The Indian Pharmacopoeia has adopted three methods for the limit test of heavy metals.

Method: Use for the substance which gives clear colorless solution in sodium hydroxide solution.

Table 2: Limit test for heavy metals.

TEST SAMPLE	STANDARD COMPOUND
Solution is prepared as per the monograph and 25 ml is transferred in Nessler's cylinder or weigh specific amount of substance and dissolve in 20 ml of water and add 5 ml of dilute sodium hydroxide solution. Make up the volume to 50 ml with water	Take 2 ml of standard lead solution Add 5 ml of dilute sodium hydroxide solution and make up the volume to 50 ml with water
Add 5 drops of sodium sulphide solution Mix and set aside for 5 min View downwards over a white surface	Add 5 drops of sodium sulphide solution Mix and set aside for 5 min View downwards over a white surface

Observation:

The color produce in sample solution should not be greater than standard solution. If color produces in sample solution is less than the standard solution, the sample will pass the limit test of heavy metals and vice versa.

Qualitative characterization of compound

TLC (Thin layer chromatography)

TLC technique deals with separation, identification and estimation of various components present in the sample.

- Stationary phase : Prepared silica gel G coated plates
- Mobile phase :chloroform: methanol: water (5:4:1)
- Chamber saturation time: 15 min.
- Development technique : Ascending technique
- Sample : ethanol extract
- Detection : visibly

IR (Infrared spectroscopy) Spectroscopy

IR Spectroscopy deals with infrared region of electromagnetic spectrum, i.e. light having a longer wavelength and a lower frequency than visible light. It can be analyzed in three ways: by measuring reflection, emission, and absorption .The major use of infrared spectroscopy is to determine the functional groups of molecule.

Formulation development



Fig 4: Water purifying sachet.

This formulation incorporates sachet which is little, permeable, fixed pack, regularly containing numerous herbs, which is inundated in water to soak and make water purify. These sachet is made of porous paper or cloth having an appended bit of string with a paper mark at the top that helps with expelling the sachet.

RESULT AND DISCUSSION

Percentage yield of different extract:-

Percentage yield of different extract is given in table.

Table 3: Percentage yield.

S. No.	Extract	% Yield
1.	Clove	8.9%
2.	Neem	7.2%
3.	Tulsi	6.9%
4.	Coriander	7.5%

In vitro anti-bacterial activity of plant extract

Table 4: Culture plate showing comparative study of blank, ethanol, standard and plant extract.

Herbs	Blank Zone of inhibition (mm)	Ethanol Zone of inhibition (mm)	Standard Zone of inhibition (mm)	Herbal extract Zone of inhibition (mm)
Tulsi	0	2	22	25
Neem	0	5	25	22
Clove	0	0	25	22
Coriander	0	5	23	18

Table5: Culture plate showing comparative study of concentration (10µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml) plant extract.

Plant material(crude extract)	Zone of inhibition (mm)	Zone of inhibition (mm)	Zone of inhibition (mm)	Zone of inhibition (mm)
	10µg/ml	20 µg/ml	30 µg/ml	40 µg/ml
Tulsi	0	0	20	22
Neem	0	0	17	19
Clove	2	12	17	18
Coriander	0	7	20	20

Test for phenolic content

- Tulsi-present(green brown)
- Clove-present(blackish green)
- Coriander-no distinctive color change.
- Neem-present(green blue)

Test for flavonoids

- Tulsi-present (brown)
- Clove-present (yellowish brown)
- Coriander-present (dark yellow).
- Neem-present (intense yellow).

Test for heavy metals



Fig 5: Limit test for crude extract.

Intensity of test solution when compared to test sample was low, while on comparing additional herbal water altogether with standard and herbal water sample found out to be low than standard therefore it was able to pass heavy metal limit test for water.

Characterization by TLC

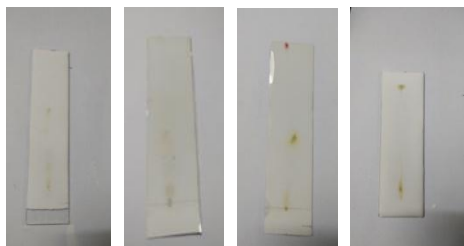


Fig 6: TLC plate for Tulsi, Clove, Coriander, Neem.

Rf value of extracted eugenol=0.62

Rf value of extracted eugenol= 0.64

Rf value of extracted azadiractin=0.57

Rf value of extracted linalool =0.31

Characterization by IR

Most convenient technique for identification of functional group and compound .Comparison of standard and extract is shown in given spectra:

Tulsi

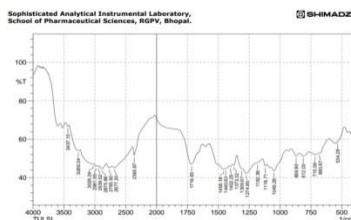


Fig 7: IR spectra Tulsi extract.

Table 6: Observation table of interpretation of IR spectra-TULSI.

S. No.	Peak	Functional group
1.	1458.18	CH ₂
2.	2875.86	OCH ₃
3.	1458.18	Benzene
4.	3437.15	OH
5.	1440.83	Aromatic ring
6.	1049.28	Primary alcohol

Clove

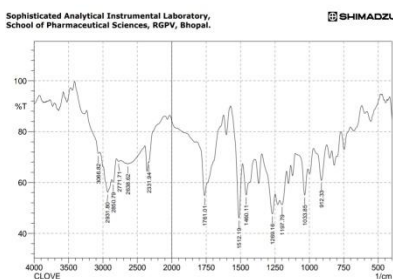


Fig 8: IR spectra Clove extract.

Table 7: Observation table of interpretation of IR spectra-CLOVE.

S. No.	Peak	Functional group
1.	NH ₃	3066.82
2.	OCH ₃	2850.79
3.	C=C	2931.8
4.	OH	1269.16
5.	Aromatic ring	1460.11
6.	CH ₃	1460.11
7.	Phenol	1197.79

Neem

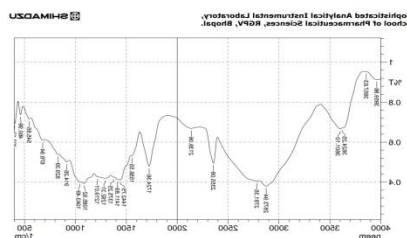


Fig 8: IR spectra Neem extract.

Table 8: Observation table of interpretation of IR spectra- NEEM.

S. No.	Peak	Functional group
1.	C=O	2873.94
2.	CH ₃	1375.25
3.	Benzene	1043.49
4.	CH ₃ COOH	1724.36
5.	Phenol	1219.01

Coriander

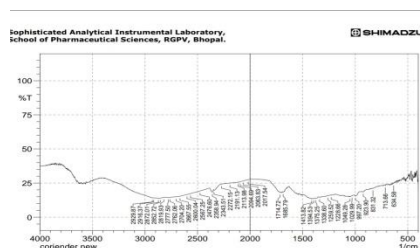


Fig :- IR spectra Coriander extract.

Table 9: Observation table of interpretation of IR spectra-CORIANDER.

S. No.	Peak	Functional group
1.	1259.52	OH
2.	1029.99	NH ₂
3.	1049.28	Primary alcohol
4.	1338.6	S
5.	2872.01	CH ₃
6.	630	C=C

Standard for Safe Drinking Water [The Bureau of Indian Standards (BIS)]

Table 10: Standard for water testing parameters.

Test parameter	Acceptable limit	Permissible limit (In the absence of alternate source of water)	Limit after treatment with herbal water
pH value	6.5-8.5 No relaxation	No relaxation	7.2
Turbidity	1	5	3
E.coli presence/absence	Shall not be detectable in any 100ml sample	Shall not be detectable in any 100ml sample	Absent
TDS (Total dissolved solids) mg/l, max	500	2000	820
Taste	Agreeable	Agreeable	Herbal taste
Colour	Transparent	Transparent	Slight yellowish
Odour	Agreeable	Agreeable	Nil
Heavy metals(limit test)	Brown	Transparent	Slight yellowish(due to herbs)

CONCLUSION

Plants with aid of negligible side effects are richest source of chemical entity. Present study includes utilization of some medicinal plants after extraction and phytochemical investigation. Ethanol extract obtained proved to be a positive result. Formulation obtained abides to antimicrobial and heavy metal removal property of water sample. Formulation is a cost effective and portable system. Formulation provides purification of water to a lot extent focusing on various contaminants in water. Formulation seems to be very effective as a future perspective as it seems eliminate impurities of drinking water.

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“God, goals, growing and glowing”

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ABBREVIATIONS

Ca	-Calcium,
Mg	-Magnesium,
Cl	-Chlorine,
NO ₃ -	Nitrite,
SO ₄ -	Sulphate,
RO -	Reverse Osmosis,
ICD-	Concentrations in Diffusion Inhibitory (ICD),
TLC	-Thin layer chromatography,
NaOH	-Sodium hydroxide,
Mm	-Micrometer,
µg/ml	-microgram per millilitre,
TLC	-Thin layer chromatography,
Rf	-Retention factor,
IR	-Infra red

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