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RESEARCH ARTICLE

Harvesting the potential of medicinal plant extracts for anti-diarrhoeal treatment and water purification

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Abstract

Methanol extracts from 23 plants were tested for antimicrobial activity against five pathogenic bacteria responsible for diarrhoeal disease: *Vibrio cholerae, Escherichia coli, Salmonella enterica, Shigella sonii* and *Staphylococcus aureus*. Among the tested plants, seven plant extract demonstrated antimicrobial activity. *Terminalia chebula* (Retz) (Hirda) and *Terminalia bellirica* (Gaertn.) Roxb. (Behada) fruit extracts demonstrated maximum antimicrobial activity. MIC of *T. chebula* (Retz) and *T. bellirica* (Gaertn) Roxb. fruit extracts were determined by using agar well diffusion method. It was found that *V. cholerae, E. coli*, and *Salmonella enterica* were sensitive to *T. chebula* (Retz) extract at 60 µg level, while *Shigella boydii* and *S. aureus* were sensitive at 240 and 1920 µg levels, respectively. In the case of *T. bellirica, V. cholerae* was sensitive to the extract at the level of 60 µg, *E. coli* and *Salmonella enterica* at 120 µg and *Shigella boydii* and *S. aureus* at the level of 960 µg. Phytochemical analyses of the plant extracts revealed the presence of diverse bioactive metabolites like terpenes, carotenoids, cardiac glycosides, saponins, phenolic glycosides, phloroglucides, anthrocenocides, flavonoids, tannins, quinones and coumarins. Methanol extract of *T. bellirica* (Gaertn.) Roxb. reduced the bacterial load of tap water at a concentration of 0.6% (w/v) pointing to its usefulness in water purification.

Keywords: *Terminalia chebula* (Retz); *Terminalia bellirica* (Gaertn) Roxb; Antimicrobial Activity; Diarrhoea; Agar well diffusion; Phytochemical Analyses.

1. Introduction

Exposure to an unhygienic environment and various pathogens in water and food causes diarrheal disease. The prevalence of the disease is higher during high temperatures and extreme rainfall events in India. The bacterial pathogens responsible for diarrheal diseases are *Salmonella, Shigella, Staphylococcus, Vibrio, Escherichia, Campylobacter* and *Helicobacter*. Many viruses and protozoa also cause diarrheal disease. The cholera toxin causes massive fluid loss, resulting in rapid dehydration and death. According to the World Health Organization (WHO) report on cholera published on 16 December 2022, since the year 2021, there has been an increase in cholera outbreaks and their geographical distribution. As per gross estimates in the year 2020, there were 675,188 registered cases and 20,256 deaths yearly in India due to cholera (WHO, 2017; Muzembo et al., 2022). The underreporting of cholera cases and the unavailability of a national cholera control plan are recognized as the main obstacles to controlling cholera in India. In the last few decades, V. cholerae has emerged as a multidrug-resistant (MDR) enteric pathogen. Antimicrobial resistance can occur through a variety of mechanisms like resistance to beta-lactam antibiotics via efflux pumps, altered penicillin-binding sites and beta-lactamases, which cleave the beta-lactam ring and inactivate the target antibiotic. Beta-lactamase is the primary means of resistance against cephalosporins and carbapenems. The development and spread of the genes responsible for antimicrobial resistance are discussed in earlier reports (Das et al., 2020; Tompkins et al., 2021; Kulkarni et al., 2015).

Considering the adverse effects of synthetic drugs, plant-based drugs are gaining more popularity for the prevention and cure of many diseases. Among the documented species, about 9,500 plants in India have ethnobotanical importance and 7,500 species are in medicinal use (Gowthami et al., 2021). A medicinal plant can be viewed as a synthetic laboratory as it produces and contains several chemical compounds that have medicinal value (Dubey et al., 2004). The beneficial medicinal effects of plants typically result from the combinations of secondary metabolites present in the plants, such as alkaloids, steroids, tannins and phenolic compounds, which are synthesized and deposited in specific parts or all parts of the plant These complex compounds may be specific to certain taxa, i.e. families, genera and species and exhibit both preventive and therapeutic activity against infectious enteric diseases. The chemistry and mechanism of action of plant secondary metabolites have been revealed and reviewed by many researchers (Sánchez et al., 2010; Omojate et al., 2014; Matsuura et al., 2015; Morsy, 2017; Thawabteh et al., 2019; Barati & Modarresi., 2024).

Phenolic compounds represent one of the largest groups of plant secondary metabolites. They include flavonoids, isoflavonoids, tannins, lignin, coumarins and phenolic acids. According to their chemical structure and properties, tannins are divided into two main groups: hydrolyzable (HT) and condensed tannins (CT). HTs (gallotannins and ellagitannins) contain a carbohydrate, generally D-glucose, as the central core. The hydrolyzable groups of these carbohydrates are esterified with phenolic groups, such as gallic acid or ellagic acid. Immature fruits of *Terminalia chebula* (Retz) and *Terminalia bellirica* (Gaertn.) Roxb., are rich sources of hydrolyzable tannins and are ingredients of the important ayurvedic formulation 'Triphala churna'. *T. chebula* Retz (Hirda/Haritaki) is the source of a variety of biologically active phytoconstituents such as chebulic acid, chebulagic acid, gallic acid, ellagic acid and other related compounds which are responsible for antimicrobial, antioxidant, antihyperglycemic, anticancer properties and protective effects on various vital organs such as nerves, heart, kidney and liver. Traditionally, this plant is used to treat a large variety of health problems (Gupta, 2012). The fruit extract of *T. bellirica* (Gaertn.) Roxb. (Behada/Bibhitak) stimulates the secretion of insulin, enhances its action and inhibits starch digestion (Kasabri et al., 2010). Phenolic acids act essentially by reducing the adherence of organisms to the cells lining the bladder and the teeth, which ultimately lowers the incidence of urinary tract infections and dental caries. Flavonoid compounds exhibit inhibitory effects against bacterial strains such as Vibrio cholerae, Streptococcus mutans, Shigella and some viruses. Quercetin, kaempferol and quercitrin are common

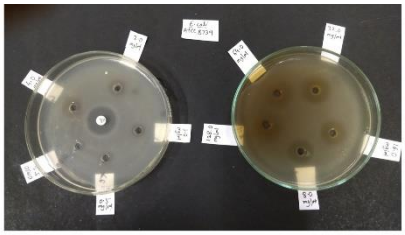

Figure 1A. Antimicrobial activity of *T. chebula* Retz against *E. coli* ATCC 8739

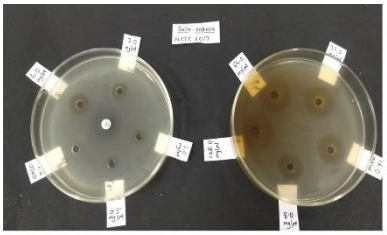

Figure 1B. Antimicrobial activity of *T. chebula* Retz against *S. enterica* NCTC 6017

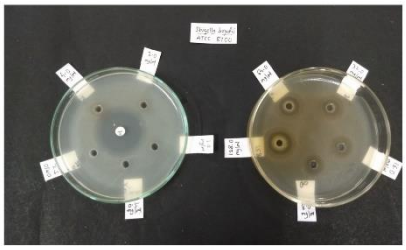

Figure 1C. Antimicrobial activity of *T. chebula* Retz against *S. boydii* ATCC 8700

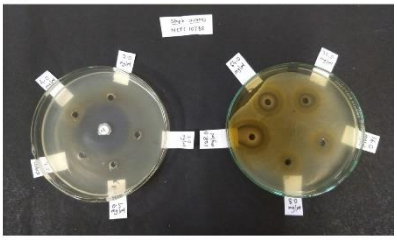

Figure 1D. Antimicrobial activity of *T. chebula* Retz against *S. Aureus* NCTC 10788

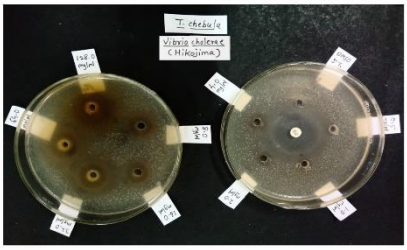

Figure 1E. Antimicrobial activity of *T. chebula* Retz against *V. cholerae* (Hikojima)

Figure 1 (A-E). Antimicrobial activity of *T. chebula* Retz fruit rind extract against tested pathogens.

flavonoids present in nearly 70% of the plants. The antimicrobial activity of flavonoids is probably due to their ability to form complexes with extracellular and soluble proteins as well as the complexation with bacterial cell walls, thereby inducing microbial cell membrane perturbations. Saponins, quinines, coumarins, anthrocenocides, terpenes and carotenoids are among the other phytochemicals present in plants (Oakenfull, 1981; Sharma et al., 2023). The objective of the present study was to evaluate the antimicrobial activity of some medicinal plants against common enteric pathogens, including Vibrio cholerae, and to identify the secondary metabolites responsible for the therapeutic property.

2. Materials and methods

2.1. Collection of plant material

A total of 23 plant species were selected based on their traditional use for stomach disorders, discussions with ayurveda practitioners and reports in the literature (Desai, 1927; Naik, 1998). The following plants were tested for antimicrobial activity in the preliminary experiment: *Aegle marmelos* (L.) Corrêa, *Adansonia digitata* L., *Lawsonia inermis* L. *Mimusops elengi* Wight, *Ocimum basilicum* L., *Limonia acidissima* L., *Tinospora cordifolia* L., *Hemidesmus indicus* (L.) R.Br., *Achyranthes aspera* L., *Impatiens balsamina* L., *Punica granatum* L., *Coriandrum sativum* L., *Foeniculum vulgare* Mill., *Trachyspermum ammi* L., *Mentha spicata* L., *Mimosa pudica* L., *Cuminum cyminum* L., *Daucus carota* L., *Ficus racemosa* L., *Terminalia bellirica* (Gaertn.) Roxb., *Terminalia chebula* (Retz)., *Datura stramonium* L. fruits, seeds, leaves, shoots and bark of the plants were collected from the local market and surrounding region of Chhatrapati Sambhajinagar. The plant parts were dried in the shade, ground and sieved. The fine powder was used for preparation of plant extracts.

2.2. Preliminary screening of plants for antimicrobial activity

Fine powder of plant parts such as fruits, seeds, leaves, shoots and bark (10 g) were added to methanol (100ml, 80%v/v) for 48 hours with intermittent shaking. After 48 hours, the mixture was filtered through ordinary filter paper and then through Whatman filter paper no.1. The filtrate was poured in a petri plate and dried at 60°C in a hot air oven. The dried extract was collected in a clean glass bottle. The preliminary screening for antimicrobial activity was carried out using agar well diffusion method with slight modification from reported earlier (Nathan et al., 1978; Perez, 1990). Muller-Hinton (MH) agar (Hi-Media) was used for the antimicrobial activity testing. For the experiment, two clinical isolates of *Vibrio cholerae* (Ogawa and Hikojima) were procured from Government Medical College and Hospital, Chhatrapati Sambhajinagar. Standard strains used for antimicrobial testing were *Escherichia coli* ATCC 8739, *Salmonella enterica* (subspp. *Enterica, Serovar– Abony*) NCTC 6017, *Shigella boydii* ATCC 8700 and *Staphylococcus aureus* NCTC 10788. The turbidity of the actively growing standard bacterial cultures was adjusted to McFarland standard 0.5 (OD of 0.08 to 0.1 at 600 nm) by adding the 24-hour culture to the sterile broth. This gives a cell concentration of approximately 1x108 CFU/ml. MH agar (1.5%) was layered in a plate and allowed to solidify. Molten MH agar (1.2%, 10 ml) was seeded with 0.1 ml bacterial culture, mixed well and layered on solidified MH agar plates. Wells were made using a cork borer (5 mm). Plant extracts (15 µl) and antibiotics, viz. ampicillin and tetracycline (positive controls) were added to the wells. The plates were kept for 20 minutes in a refrigerator and then incubated at 37°C for 24 hr. Post incubation, the zones of inhibition were recorded.

2.3. Determination of MIC for T. chebula (Retz) *and T. bellirica* (Gaertn.) Roxb.

To study the effect of the concentration of extract on the antimicrobial activity, a stock solution of dried methanol extract (256 mg) in DMSO (5%, 1 ml) was prepared by vertexing the mixture and keeping it for 24 hr at ambient conditions. After 24 hr, the mixture was centrifuged at 2000 rpm for 10 min. The stock solution consisting of the supernatant was diluted appropriately to get suitable concentrations in the range of 0.5-128 mg/ml. The antimicrobial activity was tested using the agar well diffusion method and bacterial cultures as described above. The plates were kept for 20 min in a refrigerator and then incubated at 37°C for 24 hr. The zone of inhibition was measured by using Digital Vernier Callipers (HIRA, P. No. 18621). The experiment was performed with triplicate samples and repeated twice.

2.4. Qualitative tests for phytochemical analyses

Dried and powdered plant materials were subjected to sequential extraction with lipophilic (non-polar) solvent diethyl ether (Loba Chemie Pvt. Ltd.) and then with intermediate polar solvent methanol (Fisher Scientific) followed by water, a strongly polar solvent (Tailang and Sharma, 2009; Shaikh and Patil, 2020; Morosan et al, 2021).

2.4.1. Test for ether extract

Powdered shade-dried plant material (10 g) was added to diethyl ether (100 ml) and the mixture was agitated for 12 hr on a rotary shaker. After filtering, the extract was evaporated to concentrate it to 60–70 ml. The lipophilic components were identified using the chemical reagents and methods listed below:

a) Essential oils- in an evaporating dish, ether extract (10 ml) was added and evaporated until it was dry. A pleasant fragrance indicates volatile oil.
b) Spot test for fats and lipids- a drop of ether extract was applied to a piece of filter paper and allowed to evaporate. An oily patch indicates the presence of fats and fixed oils.
c) Steroids and triterpenes (Salkowski reaction)- ether extract (10 ml) was evaporated to dryness and then dissolved in chloroform (1 ml). To this conc. sulphuric acid (1 to 3 drops) was added. The formation of red/violet colour indicates the presence of steroids.
d) Carotenoids- few drops of sulfuric acid (85%) were added to a chloroform solution of dried ether extract along the sides of a test tube. Blue colour formation at the junction of two layers indicates the presence of carotenoids.

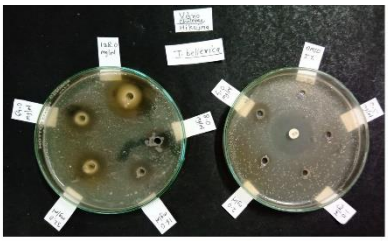

Figure 2A. Antimicrobial activity of *T. bellirica* (Gaertn) Roxb. against *V. cholerae* (Hikojima).

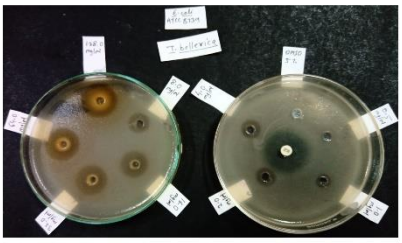

Figure 2B. Antimicrobial activity of *T. bellirica* (Gaertn) Roxb. against *E. coli* ATCC 8739.

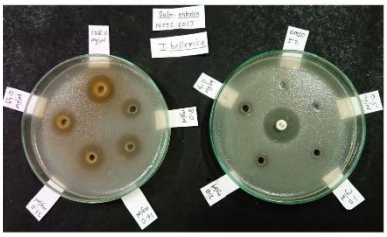

Figure 2C. Antimicrobial activity of *T. bellirica* (Gaertn) Roxb. against *S. enterica* NCTC 6017.

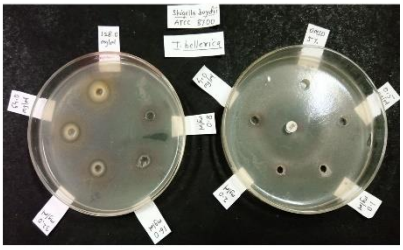

Figure 2D. Antimicrobial activity of *T. bellirica* (Gaertn) Roxb. against *S. boydii* ATCC 8700.

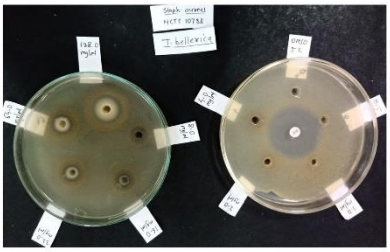

Figure 2E. Antimicrobial activity of *T. bellirica* (Gaertn) Roxb. against *S. aureus* NCTC 10788.

Figure 2 (A-E). Antimicrobial activity of *T. bellirica* (Gaertn) Roxb. fruit extract showing zones of inhibition against tested pathogens

2.4.2. Tests for alcohol extract

After extracting with diethyl ether, plant material was dried and extracted with methanol for 30 min. The filtered extract was concentrated to a minimum volume by evaporation of excess solvent. The following tests were performed for identification of phytochemicals:

a) Cardiotonic/Cardiac glycosides- (i) Keller–Killiani reaction- the residue of alcoholic extract (20 ml) was dissolved in glacial acetic acid (3 ml) containing 2 drops of $FeCl_3$ solution. Sulfuric acid (1 ml) was added along the wall of the test tube. Formation of brown ring at the junction indicates positive reaction. (ii) Trichloro acetic acid test- some quantity of alcoholic extract was dissolved in chloroform (3 ml) and mixed with trichloro acetic acid solution (90%). The appearance of a rose colour that later turned violet or bright blue indicates a positive reaction.

b) Saponins (i) Alcoholic extract was shaken with a small quantity of water. The occurrence of foam column of at least 1 cm height indicated the presence of saponins. (ii) Lead acetate test- a few drops of lead acetate solution (2%) were added to alcoholic extract (2 ml). The formation of a white precipitate indicates a positive test.

c) Phenolic glycosides- (i) Ferric sulphate test- alcoholic extract (10 ml) was evaporated to dryness. The residue was dissolved in water. To 1 ml of aqueous solution, few crystals of ferric sulphate were added. The appearance of a dark violet colour followed by precipitate formation indicates a positive test. (ii) Potassium ferrocyanide test- to an aqueous solution of alcoholic extract (2ml), 3 drops of a mixture of $FeCl_3$ (1%, 1 ml) and potassium ferrocyanide (1%, 1 ml) were added. The formation of a green-blue colour indicates a positive test.

d) Phloroglucides- conc. nitric acid (10 drops) was added to 2 ml of alcoholic extract. The formation of a dark brown colour indicates a positive test.

e) Anthrocenocides- alcoholic extract (10 ml) was evaporated to dryness. The residue was dissolved in ammonia solution (25%) or NaOH (10%) solution and stirred. The formation of cherry red or blood red colour indicates a positive test.

f) Flavonoids- (i) Lead acetate test- to alcoholic extract (1 ml), 3 to 5 drops of lead acetate solution (2%) were added. Development of orange or yellow colour indicates the presence of flavonoids. (ii) Alkaline reagent test- the alcoholic extract was mixed with NaOH (2%). Intense yellow colour formation and then loss in intensity with the addition of dilute acid indicates the presence of flavonoids.

g) Coumarins- alcoholic KOH (10%) or ammonia (1ml) was added to 5 ml of alcoholic extract. The occurrence of intense fluorescence under UV light indicates the presence of coumarin derivatives.

h) Quinones- approximately 1 ml of extract was treated with alcoholic KOH. Quinones give red to blue colouration.

i) Tannins (Ferric chloride test)- alcoholic extract (2 ml) was diluted with water (3 ml) and 3 drops of dilute solution of ferric chloride were added. The occurrence of blackish-blue colour indicates presence of gallic tannins (hydrolysable tannins) and a green-black colour indicates the presence of catechol (condensed tannins).

j) Alkaloids (Mayer's reagent test)- alcoholic extract (20ml) was evaporated and the residue was dissolved in HCl (2%, 10 ml) and 3 drops of Mayer's reagent were added. The formation of a yellow precipitate indicated the presence of alkaloids.

2.4.3. Tests for aqueous extract

After extraction with ether and methanol, the plant material was dried and extracted once again with water. The following tests for the detection of water-soluble constituents were performed:

a) Glucides- aqueous extract (3 ml) was transferred to a porcelain dish and evaporated to dryness. To this, 2 to 3 drops of conc. sulfuric acid were added and allowed to stand for 5 min. This was followed by addition of 3 to 4 drops of Molisch's reagent. A red colour indicates the presence of glucides.

b) Polyphenols- to aqueous extract (2 ml), 3 drops of a mixture of 1% $FeCl_3$ (1 ml) and 1% potassium ferrocyanide (1 ml) was added. The formation of green blue colour indicates the presence of polyphenols.

c) Polyuronides (pectin, mucilage, gums)- aqueous extract (5 ml), was added dropwise to 10 ml of alcohol or acetone. The thick precipitate was centrifuged, washed with alcohol and stained with methyl blue. The occurrence of violet or blue precipitate indicates the presence of mucilage.

d) Tannins and catechols (Styassny's reagent test)- if the alcohol extract contains both hydrolysable and condensed tannins, this test is carried out). The extract (2 ml) was boiled with a mixture of hydrochloric acid and formaldehyde (1:2 molar ratio, Styassny's reagent). Catechol condenses as a red precipitate. The precipitate was filtered and the filtrate was neutralized with sodium acetate. To this, a few drops of ferric chloride solution were added. A deep blue colour indicates a positive test for gallic tannins.

2.4.4. Treatment of tap water using plant extracts

Water extracts and methanol extracts of *T. chebula* (Retz) Retz and *T. bellirica* (Gaertn.) Roxb., were investigated for their ability to purify tap water. Stock solutions of the plant extracts were prepared in water by adding fine powder of fruit rind to hot distilled water (1 g/10 ml). Dry methanol extracts were redissolved in sterile distilled water to prepare stock solutions (0.1g/ ml). Aliquots of the stock solutions were added to tap water to test the potential of the extracts to reduce the microbial load of the tap water sample.

Table 1. Antimicrobial activity of plant extracts by agar well diffusion method

SN	Botanical names	Common name	Plant parts used	Zone of Inhibition In mm				
				E. coli	*S. enterica*	*S. sonii*	*V. cholerae* Ogawa	*S. aureus*
1	*Aegle marmelos*	Bael	Leave	10.50	10.50	11.00	10.00	10.50
2	*Adansonia digitata*	Baobab	Woody twigs	13.00	12.66	10.00	ND	12.33
3	*Achyranthus aspera*	Aghada	Herb	ND	ND	ND	ND	ND
4	*Cuminum cyminum*	Cummin seeds	Seed	ND	ND	ND	ND	ND
5	*Coriandrum sativum*	Coriander	Leave	ND	ND	ND	ND	ND
6	*Cyperus rotundus*	Nut grass	Rhizome	ND	ND	ND	ND	ND
7	*Datura stramonium*	Dhotara	Leave	14.0	11.50	14.00	ND	8.00
8	*Daucus carrota*	Carrot	Root	ND	ND	ND	ND	ND
9	*Foeniculum vulgare*	Fennel seeds	Seed	ND	ND	ND	ND	ND
10	*Ficus racemosa*	Audumbar	Woody twig	ND	ND	ND	ND	ND
11	*Hemidesmus indicus*	Anantmul	Leave	ND	ND	ND	ND	ND
12	*Impatiens balsmina*	Terada	Leave	ND	ND	ND	ND	ND
13	*Lawsonia inermis*	Mehandi	Leave	ND	13.33	14.66	17.00	13.66
			Inflorescence	ND	14.00	ND	ND	11.00
14	*Limonia acidisima*	Kavath	Leave	10.00	ND	ND	ND	10.00
15	*Mimusops elengi*	Bakul	Leave	13.33	14.00	11.00	14.00	14.33
16	*Mimosa pudica*	Lajawanti	Shoot	ND	ND	ND	ND	ND
17	*Mentha spicata*	Mint	Leave	ND	ND	ND	ND	ND
18	*Ocimum bacilicum*	Basil	Leave	ND	ND	ND	ND	ND
19	*Punica granatum*	Pomegranate	Fruit peel	ND	ND	ND	ND	ND
20	*Terminalia chebula*	Hirda	Fruit rind	24.50	25.00	31.00	29.50	22.00
21	*Terminalia bellirica*	Behada	Fruit rind	16.50	19.00	24.50	18.50	19.00
22	*Trachyspermum ammi*	Ajawain	Seed	ND	ND	ND	ND	ND
23	*Tinospora cardifolia*	Gulbel, Guduchi	Stem	10.00	ND	ND	ND	11.00
24	Ampicillin	-	-	24.00	36.00	0.00	24.00	11.00
25	Tetracycline	-	-	13.00	33.00	14.00	12.00	20.00

ND- Not detected

The mixtures were kept for 10 hours. Post-exposure, the water samples (20 µl) were inoculated in sterile nutrient broth (5ml) in test tubes and incubated at 37°C for 12hr. The growth of bacteria was determined by measuring the absorbance at 600 nm. Samples without addition of extracts served as negative controls, while sterile nutrient broth served as blank. The experiment was conducted twice with three replicates. Subsequently, the experiment was scaled up adding methanolic extract (0.8 g%) to 500 ml and 1 L water and measuring the bacterial load as described above.

3. Result and discussion

3.1. Amongst the 23 plant species that were collected for the investigations, selected plant species were authenticated at the Dept of Botany, Government Institute of Science, Chhatrapati Sambhajinagar, India. The selection was based on the preliminary screening as described earlier. The voucher samples were prepared and deposited at the same department with code- *T. bellirica* (Gaertn.) Roxb.- GISA/BOT/AP-2024-1, *T. chebula* (Retz) (Retz)-GISA/BOT/AP-2024-2.

3.2. Preliminary screening for antimicrobial activity

In the preliminary testing of antimicrobial activity agar well diffusion method tested for the crude extracts, 10 plant samples have exhibited antimicrobial activity. The highest inhibition potential was exhibited by methanolic fruit rind extracts of *T. chebula* Retz and *T. bellirica* (Gaertn.) Roxb. (Table 1). It is important to note that the *Vibrio cholerae Ogawa strain* showed resistance to tetracycline, but was found sensitive to methanolic extracts of *T. chebula* (Retz), *T. bellirica* (Gaertn.) Roxb, *Mimusops elengi* Wight and *L. inermis* L., with zones of inhibition of 29.50 mm, 18.50 mm, 14 mm and 17.0 mm, respectively. The clinical isolate *Shigella sonii* was resistant to both antibiotics, but sensitive to methanolic extracts of *T. chebula* (Retz) and *T. bellirica* (Gaertn.) Roxb., showing zones of inhibition of 31.0 mm and 24.50 mm, respectively. All the pathogens used in the preliminary testing, both Gram-positive and Gram-negative bacteria, were found to be sensitive to methanolic fruit rind extracts of *T. chebula* (Retz) and *T. bellirica* (Gaertn.) Roxb., indicating the broad-spectrum antimicrobial activity of these plant extracts. The agar well diffusion method is frequently used for testing the antimicrobial activity of plant-derived substances (Balouiri et al., 2015).

3.3. Minimum inhibitory concentration (MIC) of T. chebula (Retz) and T. bellirica (Gaertn) Roxb fruit rind extracts

To determine the MIC by agar well diffusion method, the diameter of the clear zone of inhibition was determined in mm scale and the finding was interpreted as 'sensitive', 'intermediate' or 'resistant' to the respective extract based on the standard values. Clinical and Laboratory Standards Institute (CLSI) and the European Committee for Antimicrobial Susceptibility Testing (EUCAST) provide guidelines for the interpretation of antimicrobial sensitivity testing. These guidelines were followed because there are no standard guidelines and interpretation criteria (breakpoints) for determining antimicrobial activity of plant-derived compounds (CLSI M45., 2015; CLSI M100., 2020; Bubonia-Sonie et al., 2020). The antibiogram of *V. cholerae Hikojima* indicated its resistance to amoxicillin + clavulanic acid, ceftazidime, cefepime, fosfomycin, cotrimoxazole and nitrofurantoin. Interestingly, the multiple drug-resistant strain was found sensitive to fruit extracts of *T. chebula* (Retz) and *T. bellirica* (Gaertn.) Roxb. at the level of 60 µg. The MIC values (Table 2) show that *T. chebula* (Retz) has more antimicrobial potential than *T. bellirica* (Gaertn.) Roxb.

3.4. Sequential extraction and qualitative phytochemical analyses

Qualitative phytochemical analyses were conducted for *T. chebula* (Retz) and *T. bellirica* (Gaertn.) Roxb. extracts due to the maximum antimicrobial activity. It was observed that *T. chebula* (Retz) extract, especially the fruit rind extract, had most of the phytochemicals tested (Table 3). *T. bellirica* (Gaertn.) Roxb. lacked essential oils, lipids, fats, steroids, triterpenes and carotenoids. According to reported literature, the fruits of *T. chebula* (Retz) and *T. bellirica* (Gaertn.) Roxb. contain 25-30% tannins, particularly pyrogallol-type hydrolyzable tannins, including gallic acid, ellagic acid, methyl gallate, and ethyl gallate attached to monosaccharides or oligosaccharides (He et al., 2006). Gallotannins exhibit strong antioxidant and antibacterial properties. Galloyl groups of gallotannins are hydrophobic sites that interact with aliphatic side chains of amino acids through hydrophobic association. Due to their ability of hydrophobic interactions and hydrogen bonding, gallotannins can bind with proteins and phospholipids. Hydrogen bonding also results in interaction with sugars (He et al, 2006). In earlier research, it was suggested that plant polyphenols with pyrogallol groups show higher antibacterial activity compared to those with catechol or resorcinol groups (Taguri et al., 2006).

By the virtue of diverse phytochemicals present in the selected plants, they may exert different physiological effects on the human body. The absence of possible toxic elements like cardiac glycosides makes these plants safe to use (Kim et al., 2012). Recently in-silico studies have been performed on the natural compounds of *T. chebula* (Retz) as potential therapeutic agents against the MPRO protein of SARC-CoV-2 through docking and Molecular Dynamics simulations. The results showed that daucosterol, arjunetin, maslinic acid and belliricoside may evolve as promising anti-COVID-19 drugs in the near future (Ghosh et al., 2022). The wide range of phytochemicals identified in the present studies point to the usefulness of the plant extracts for bio-medical applications.

Table 2. Effect of concentration of *Terminalia chebula* Retz and *Terminalia bellirica* (Gaertn) Roxb. extracts on the antimicrobial activity against selected pathogens.

Plant extract in well (µg)	[1]Diameter of zone of inhibition (mm)									
	Vibrio cholerae Hikojima		*E. coli* ATCC 8739		*Salmonella enterica* NCTC 6017		*Shigella boydii* ATCC8700		*S aureus* NCTC 10788	
	TC	**TB**	**TC**	**TB**	**TC**	**TB**	**TC**	**TB**	**TC**	**TB**
1920	33.70 ± 2.56	33.26 ± 3.85	30.33 ± 2.51	23.25 ± 0.38	26.07 ± 4.70	21.16 ± 2.0	20.66 ±3.05	**19.00** ± 3.12	21.91 ± 1.80	**19.48 ± 0.44**
960	29.85 ± 2.72	20.03 ±0.62	28.11 ± 4.34	21.33 ± 0.57	24.10 ±4.53	19.94 ± 1.00	18.35 ± 3.20	**16.11 ± 2.5**	**17.01 ±1.00**	**13.38 ± 1.51**
480	28.68 ± 1.77	18.83 ± 0.43	25.20 ± 2.89	19.07 ± 0.83	21.27 ±3.81	19.32 ± 0.61	16.20 ± 1.70	12.55 ± 1.2	13.94 ± 0.09	11.65 ± 1.26
240	22.69 ± 2.88	16.70 ± 0.53	22.05 ± 1.70	16.86 ± 0.23	18.66 ±2.08	17.83 ± 0.90	**15.69 ± 3.76**	9.75 ± 0.25	12.66 ± 2.30	10.22 ± 0.25
120	20.72 ± 3.70	15.46 ± 0.80	21.89 ± 1.88	**14.83** ± 0.28	16.87 ± 2.06	**15.49 ± 0.50**	12.41 ± 3.16	10.47 ± 1.02	10.70 ±3.01	9.15 ± 1.27
60	**14.77 ± 0.94**	**15.34 ± 0.59**	**17.74 ± 2.19**	12.83 ± 1.04	**16.24 ±1.82**	12.50 ± 0.86	ND	9.00 ± 0.5	ND	8.79 ± 0.61
30	10.05 ± 0.91	8.20 ± 1.5	14.27 ± 1.66	12.00 ± 1.0	11.46 ± 1.52	9.77 ± 0.25	ND	6.77 ± 0.46	ND	8.27 ± 0.75
15	7.95 ± 0.19	6.80 ±0.20	10.54 ± 1.29	7.40 ± 0.85	7.15 ± 1.00	7.03 ± 0.50	ND	ND	ND	ND
7.5	7.63 ± 2.50	6.40 ± 0.17	6.62 ± 4.16	ND	7.20 ± 0.95	ND	ND	ND	ND	ND
[2]Tet 30	28.11 ± 1.01		23.31 ± 3.48		23.07 ± 1.10		34.48 ± 0.50		31.27 ± 0.63	
[3]Amp 10	18.41 ± 0.38		20.94 ± 1.78		21.0 ± 2.0		22.81 ± 1.29		30.83 ± 3.74	

[1]Data are given as Mean ± S. D,.[2]Tetracycline, [3]Ampicillin., No zone of inhibition was observed for 5% DMSO, S- Sensitive (≥ 15 mm), I- Intermediate (12-14 mm), R- Resistant (≤ 11mm). Shaded areas indicate MIC values of the plant extracts.

3.5. Effect of the plant extracts on the microbial load of tap water.

It was observed (Table 4) that the methanol extract of *T. bellirica* (Gaertn.) Roxb. reduced the microbial load in tap water to a significant level and the reduction of microbial load was dependent on the concentration of plant extract. At 0.8 % w/v concentration of the extract, there was very low absorbance, indicating the potential of plant extract to purify the water sample. The extract of *T. chebula* (Retz) was however not found effective for purification of water sample probably on account of the low levels of antimicrobial metabolites, or the presence of some nutritive values in the extract that might work by diminishing the effectiveness of antimicrobial compounds.

4. Conclusion

The findings of present research points towards the potential of medicinal plant extracts with antimicrobial activity against *V. cholerae* and other enterobacteria responsible for diarrhoeal diseases. The methanol extract of *T. bellirica* (Gaertn.) Roxb. showed ability to reduce the microbial load in tap water pointing to the possibility of its use for cleaning drinking water, vegetables and other food commodities. The results point to the potential use of *T. chebula* (Retz) and *T. bellirica* (Gaertn.) Roxb. extracts for the control of multiple drug-resistant pathogens and the prevention of diarrhoeal diseases. It has been reported that *T. chebula* (Retz) and *T. bellirica* (Gaertn.) Roxb. trees yield approximately 40 to 50 kg of dry fruits per tree per year after attaining six years of age (Anonymous, 2008). Also, further studies can be carried out to develop methods for the production of bioactive molecules by employing biotechnological tools like plant cell culture for the production of secondary metabolites.

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Table 3. Phytochemical analysis of selected plant extracts

SN	Name of Phytochemical	*T. chebula* Retz		*T. bellirica* (Gaertn) Roxb	
		Leaf extract	Fruit rind extract	Leaf extract	Fruit rind extract
Ether extract					
1	Essential oils	-	++	-	-
2	Lipids & Fats	-	++	-	-
3	Steroids & Triterpenes	-	++	-	-
4	Carotenoids	++	++	-	-
Alcohol extract					
1	Cardiac Glycosides	-	-	-	-
2	Saponins	++	++	-	++
3	Phenolic glycosides	++	++	++	++
4	Phloroglucides	++	++	++	++
5	Anthrocenocides	++	++	++	++
6	Flavonoids	++	++	++	++
7	Coumarins	++	++	++	++
8	Quinones	++	++	++	++
9	Tannins *	++	++	++	++
10	Alkaloids	+	+	+	+
Aqueous extract					
1	Glucides	++	++	++	++
2	Polyphenols	++	++	++	++
3	Tannins *	++	++	++	++
4	Polyuronides	-	++	-	++

*Hydrolysable tannins present, - Absent, ++ Present

Table 4. Treatment of water samples using *T. chebula* Retz and *T. bellirica* (Gaertn) Roxb. extracts.

SN	Concentration of plant extract gram% (w/v)	OD_{600} of tap water sample in presence of *T. chebula* Retz extract		OD_{600} of tap water sample in presence of *T. bellirica* (Gaertn) Roxb. extract	
		Aqueous extract	Methanolic extract	Aqueous extract	Methanolic extract
1	0.0	0.258	0.258	0.258	0.258
2	0.2	1.376	0.980	0.944	0.207
3	0.4	0.976	1.049	1.091	0.168
4	0.6	1.076	1.034	0.847	0.141
5	0.8	1.126	0.979	0.575	0.048

Contribution of the authors

AAP carried out experimental work and prepared the manuscript, AVP supervised the work and carried out proof-reading of the manuscript.

Declaration of conflict of interest

The authors declare that there is no conflict of interest.

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