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Evaluation of Total Phenolic, Total Flavonoid and Antimicrobial Activity of Fruit Peel and Leaves Extracts of *Trapa natans* L.

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ABSTRACT

The current study's objective was to identify the antibacterial properties and phytochemical content of *Trapa natans* L. (Family: *Trapaceae*) Fruit peel and Leaves extract. The well recognized testing protocol described in the literature was used to determine the qualitative analytical evaluation of various phytochemical components and the quantitative analytical evaluation of total phenolics and flavonoids. By using the Folins Ciocalteau reagent and the aluminium chloride method, quantitative analysis of phenolics and flavonoids were conducted, respectively. *S. mutans*, *S. bongori* and *Escherichia coli* were subjected to an *in vitro* antimicrobial capability test utilising the well diffusion assay technique and standard ofloxacin and ciprofloxacin (10–30 micro g/ml). The millimetre (mm) diameter of the zone of inhibition was used to calculate the antibacterial activity. According to the phytochemical examination, tannins, glycosides, flavonoids, alkaloids, etc. were present. The hydroalcoholic extract of following plants: *Trapa natans* L fruit peel and leaves had antibacterial activity against all pathogens in a concentration-dependent manner, but it was less potent than conventional medicine. It has been determined that the presence of these phytochemicals is what causes the plant to exhibit antibacterial action. Future research may focus on isolating and modifying the phytochemicals that are responsible for these actions.

Keywords: *Trapa natans* L; Antimicrobial action; phytochemical analysis and infectious illnesses.

INTRODUCTION

Despite the vast number of synthetic medications that have been developed, people continue to employ plant preparations as therapeutic instruments, a practice that is rooted in popular culture. The primary cause of this has been linked to the increased expenses of allopathic medications and the restricted availability of these drugs, particularly in developing nations where the majority of the population mostly relies on natural resources to treat illnesses pertaining to primary care (World Health Organization, 2013). In the process of discovering new medications, natural products have shown to be the most valuable source of chemical compounds. In their natural state and as models for medicinal chemistry, secondary metabolites derived from both marine and terrestrial organisms have been used extensively in the treatment of many illnesses (Saadia *et al.*, 2019). By providing a foundation for the creation of novel pharmaceuticals, medicinal plants are essential to the growth and progress of contemporary research. Also numerous contemporary medications were derived from medicinal plants by using plant material as a traditional medical practice or folk remedy (Ibrahim and Kebede , 2020). Plants have been utilised as an alternative or traditional medicine for many years to treat a variety of infections-related illnesses (YI TS, 2015 and Paul and Sinha, 2016). There are many different compounds found in plants used in traditional medicine that can be utilised to treat viral and chronic illnesses. Remedy for acute or chronic ailments is the primary use of medicinal herbs. A lot of physiologically active ingredients are frequently present in them. As to the World Health Organisation (WHO), almost 80% of the global population depends mostly on traditional medicine derived from plants for their primary healthcare needs. Herbal medications are highly sought after for primary healthcare in both developed and developing nations because of their broad range of biological and therapeutic capabilities, higher safety margins, and cheaper cost (Goyal, *et al.*, 2007 and Cragg, *et al,*. 1997). Herbal remedies have become more widely used to treat all diseases as a result of the common belief that natural therapy is secure, accessible, and has fewer adverse effects. Many plants are less expensive, more widely available, and less likely to have negative effects than standard treatments, especially in developing nations. These elements could account for their widespread acceptance and uses (Sofowora, 2011). Some studies have shown that certain plants offer medicinal advantages (Ukpabi and Akubugwo 2011, Idu *et al*., 2009, Banso and Adeyemo, 2007). Furthermore, a rich source of antibacterial chemicals is provided by medicinal plants (Kubmarawa *et al.*, 2007). The scientific community has been obliged to consider novel antibacterial compounds derived from other sources, such as medicinal plants, as a result (Stockwell and Thomson, 1978). Certain plant extracts and phytochemicals have been shown to possess antibacterial qualities, making them valuable tools for medical interventions. Numerous investigations demonstrating its efficacy have been carried out in various nations in the past few years (Benoit, 2006 and Senatore, 2007). A thorough assessment is necessary for clinical trials aimed at comprehending the pharmacokinetics, bioavailability, efficacy, safety, and medication interactions of recently identified bioactive substances and their formulations (extracts) (Sasidharan, 2011). As a result of the extraction and characterization of numerous phytochemicals from these green factories, several drugs with high activity profiles have been created (Mandal *et al.*, 2007). In fact, because of the increased market and public demand, many therapeutic plants are currently in danger of going extinct or losing their genetic variety (Misra, 2009). The survival of drug-resistant organisms and the increasing evolutionary adaptations of pathogenic organisms to routinely used antibiotics have reduced the efficiency of currently used antimicrobials. Additionally, antibiotics have been linked with adverse effects, forcing the search for alternative medicines from unusual sources like plants. According to estimates, over 80% population of the world's depends on vegetables and plants to meet their essential medical necessities (WHO, 2002). Plants continue to be a significant origin of medications utilized in commerce. Numerous synthetic medications have been proved for their efficiency. The utilization of natural products has increased recently, including active plant extracts are often evaluated for the creation of novel treatments (Joshi *et al.*, 2019). Phytochemicals produced in the plant's secondary metabolism are responsible for many plants' antimicrobial characteristics, which have led to their widespread use (Medina *et al.*, 2005 and Romero *et al*., 2005). Many different types of secondary metabolites, including flavonoids, glycosides, alkaloids, tannins, and terpenoids, are abundant in plants and have been shown to have antimicrobial effects *in vitro* (Dahanukar *et al*., 2000 and Cowan, 1999).

The current work intends to investigate the antimicrobial activity of petroleum ether, chloroform, ethyl acetate, and 50% ethanolic extracts from *Trapa natans* L. fruit peel and leaves against bacterial species since there are limited details regarding the antimicrobial action of *Trapa natans* L. fruit peel and leaves. Additionally, To determine which bioactive components have antibacterial qualities, initial phytochemical experiments are performed on the extracts of leaves and fruit peels. The total phenol, flavonoid, and alkaloids content were assessed by spectrophotometric technique.

2. MATERIALS AND METHODS

2.1 Plant Materials

The chosen *Trapa natans* L. (family: Trapaceae) from the neighbourhood pond were taken in December 2022 and stored at 18^0 C in Kanpur, India. The *Trapa natans* L. plant's fruit peel and leaves was identified and submitted to the Christ Church College in Kanpur Department of Botany for taxonomic authentication. To remove any last bits of dust particles and other undesirable elements, the plant's leaves and fruit skin were separated and cleansed with distilled water. Fruit peel and leaf fragments were allowed to air dry at normal room temperature. To get the powdered plant specimens, dry plant samples were chopped and processed further. For later use, the sample powders were retained. apart in a dry, sterile, and clean container.

2.2 Chemicals

Every chemical employed in this investigation were given by Hi Media Laboratories Private Limited, SD Fine-Chemical Limited., and SRL Private Limited (all in Mumbai, India). All of the materials utilized throughout this experiment were of analytical quality.

The NBRI (CSIR) in Lucknow provided the test organisms, which were *Streptococcus mutans*, *Salmonella bongori*, and *Escherichia coli*.

2.3 Maceration-Based Extraction

150 g of powdered *Trapa natans* L. fruit peel and leaves separately were extracted using the maceration process and a wide range of solvents, such as petroleum ether, chloroform, ethyl acetate, and 50% ethanol. At temperatures over their boiling points, the extracts are evaporated. The yield percentage of each dried extracts was then determined. After that, each of the four extracts was reduced in a rotary evaporator and kept for later use at 4°C in sealed containers. **(**Table 1).

2.4 Phytochemical Screening of the Extract

The raw extracts are then subjected to preliminary phytochemical screening as per the standard procedure's techniques, evaluated for the existence or non-existence of primary or secondary metabolites (Harborne, 1973).

Alkaloids, carbohydrates, glycosides, tannins, saponins, phytosterols, proteins, amino acids, and flavonoids were among the phytoconstituents that were qualitatively evaluated in the fruit peel and leaves of *Trapa natans* L (Pradhan *et al*., 2019 and Parkhe *et al*., 2018). Tests such as the Mayer's, Dragendroff's, and Wagner's tests for alkaloids, the Gelatin, Ferric chloride, and Vanillin hydrochloride tests for tannins and phenolic components, the Million, Ninhydrin, and Xanthoproteic tests for proteins and amino acids, the Salkowski test, the Sulphur Powder test for sterols and triterpenoids, the Molisch, Benedict, and Barfoed tests, the Bromine water test for carbohydrates, and the Foam test for saponins were used to determine the various components present in the leaf extracts and fruit peel of *Trapa natans* L (Shefali *et al*., 2013). (Table-2).

2.4.1 Total Phenol Determination

The method used by Parkhe *et al.* was used to calculate the total phenolic content. By using the modified Folin-Ciocalteu technique, the extracts' total polyphenol content (TPC) was determined (Fazel *et al*., 2008 and Wolfe *et al*., 2003). Before undergoing additional examination, the leaf extract were stored at 4°C in the dark. 2ml. of the extract or standard, 1ml of the Folin-Ciocalteu reagent (previously diluted with water 1:10v/v), and 1 ml of sodium carbonate (7.5 mg/ml) were used to build up a reaction mixture. After 30 minutes of incubation at 40 °C, the absorbance was measured at 765 nm using a UV-Visible spectrophotometer. The gallic acid standard graph was used to calculate the total phenolic content, and as milligrams/100 milligrams gallic acid equivalent (mg/g GAE), the total phenolic content was reported (Lipika and Alak, 2014). (Table-3, 4).

2.4.2 Total Flavonoids Determination

The total flavonoid content was evaluated by using the Parkhe *et al.* approach. To examine the total amount of flavonoids present in the extracts, we employed spectrophotometry (Fazel *et al.*, 2008, Kumazawa *et al.*, 2004 and Woisky and Salatino, 1998). After adding 1 ml of 2% $AlCl_3$ solution to 3 ml of extract or standard and allowing the mixture to remain at room temperature for 15 minutes, the absorbance at 420 nm of the reaction mixture was measured using a UV-VIS spectrophotometer. The amount of flavonoids was assessed using the quercetin standard graph, and the results were presented as quercetin equivalent (mg/100mg) (Table-5, 6).

2.5 Antimicrobial Activity of *Trapa natans* L. Fruit Peel and Leaves

The well diffusion method was utilized to measure the antibacterial activity of the *Trapa natans* L. extract. The standard formulation used IP-grade ofloxacin and ciprofloxacin. For 24 hours, *S. Mutans*, *S. bongoriand* and *Escherichia coli* were cultivated to investigate their antibacterial efficacy. For each extracted phytochemical, three concentrations—25, 50, and 100 mg/ml were used in antibiogram investigations.

2.5.1 Preparation of Culture Media

The standard protocol for microbiological media preparation was supplied by HI-MEDIA Laboratories Pvt. Ltd. in Mumbai. Mueller-Hinton Agar (MHA) and Nutrient Broth (NB) were the media utilised for the antibacterial activity. They were ready and autoclaved for 15–30 minutes at 121^0C and 15 psi.

2.5.2 Plate Preparation

Pre-sterilized petri dishes with a 95 mm diameter were filled with 30 ml of pre-autoclaved Mueller-Hinton agar (MHA). Room temperature was used to allow these petri plates to harden.

2.5.3 Well Diffusion Method

Following the plates' solidification, a freshly made microbial broth culture suspension (about 0.2 ml) was individually sterilized and used an L-shaped glass spreader to distribute the suspension over the Mueller-Hinton agar (MHA) media in an aseptic setting with laminar air flow. Then, using an 8 mm diameter borer, wells were created in each plate. Approximately 0.2 ml of the extracts of fruit peel and leaves were each put into these wells separately.

In order to completely inhibit the growth of additional microorganisms in a circular area or zone surrounding the hole containing leaf and fruit peel extract, this method relies on the diffusion of extracts from the hole through the solidified agar layer of the petri dish. In the incubator, petri plates were incubated for 24 hours at 37^0C. Following incubation, the well or holes' diameter of the clear zone of inhibition was measured in millimetres and compared to a reference medication.[29]

A crucial step is the rapid placement of antibiotic wells on the agar surfaces following inoculation with the organism under study. Never use inoculums generated from overnight broth cultures that have not been diluted. After being incubated for 24 hours at 37^0C, the plates were assessed to look for distinct zones of inhibition surrounded the wells that had been impregnated with a specific drug concentration. The diameter of each wall's zone of inhibition was measured (Table-7, 8).

3. RESULTS AND DISCUSSION

In order to determine the actual extraction yield, the raw extracts that were generated during each subsequent maceration extraction phase were concentrated on a water bath by completely evaporating the solvents. The extraction yield from fruit peel and leaves of *Trapa natans* prepared with solvents such as petroleum ether, chloroform, ethyl acetate, and 50% ethanol is shown in Table 1.

Table 1. Extractive values of *Trapa natans* L. fruit peel and Leaves

Sr. No	Extracts	% Yield (W/W)		Colour of extractive	
		fruit peel	**Leaves**	**fruit peel**	**Leaves**
1	Petroleum ether	0.89	0.92	Light Yellow	Dark green
2	Chloroform	3.15	3.9	Brown	Brown
3	Ethyl acetate	4.68	4.17	Brown	Yellow
4	50% Ethanol	15.32	15. 03	Brown	Brown

3.1 Phytochemical Screening of the Extract

The outcomes of a qualitative phytochemical analysis of the raw powder leaves and fruit peel of *Trapa natans* L. are presented in Table 2. In a hydroalcoholic extract of the plant, flavonoids, alkaloids, saponins, phenolics, carbohydrates, and tannin were found.

Table 2. Outcome of *Trapa natans* L. fruit peel and leaves phytochemical screening

S. No.	Constituents	Chloroform extract		Ethyl acetate extract		50% Ethanolic extract		Petroleum ether	
		fruit peel	leaves	fruit peel	leaves	fruit peel	leaves	fruit peel	leaves
1.	**Alkaloids**								
	Hager's Test:	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve
	Wagner's Test:	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve
2	**Glycosides**								
	Legal's Test:	-ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve
3	**Flavonoids**								
	Lead acetate Test:	-ve	+ve	+ve	+ve	+ve	-ve	+ve	-ve
	Alkaline test:	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve
4	**Diterpenes** Copper acetate Test:	-ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve
5	**Phenol** Ferric Chloride Test:	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve
6	**Proteins** Xanthoproteic Test:	-ve	+ve	-ve	-ve	+ve	-ve	-ve	+ve
7	**Carbohydrate** Fehling's Test:	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve
8	**Saponins** Froth Test:	-ve	+ve	-ve	+ve	+ve	-ve	+ve	-ve
9	**Tannins** Gelatin test:	-ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve
10	**Triterpenoid**	-ve	-ve	-ve	+ve	+ve	-ve	-ve	+ve

+ve = Positive, -ve= Negative

3.2 Total Phenol Determination

Calibration curve of gallic acid:

Gallic acid's standard curve was generated and plotted in distilled water to provide information on absorption. With the use of this data, the range of Beer's law and the regression coefficient are calculated (Table 3, 4 and Figure 1).

Table 3. Absorbance of gallic acid in different concentration

At λ_{max} 765nm	
Concentration	**Absorbance**
200 µg/ml	0.036 ± 0.003
400 µg/ml	0.095 ± 0.001
600 µg/ml	0.154 ± 0.004
800 µg/ml	0.223 ± 0.002
1000 µg/ml	0.282 ± 0.001

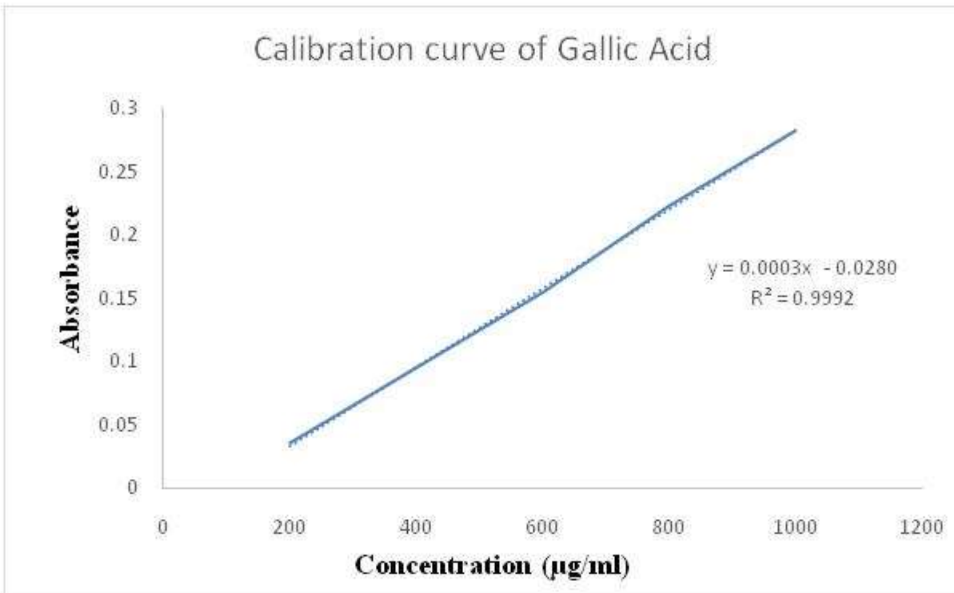

Figure 1. Gallic acid calibration curve

Calculation for TP/g of extract:
We may calculate the total phenol content using the gallic acid standard equation.

$$y = 0.0003x - 0.028$$

Where y is an absorbance and X is a concentration.

Table 4: Following data shows the presence of total phenol in different solvent extract

Plant Parts	Extracts	Absorbance	Concentration (µg/ml)	Concentration (mg/100mg)
Trapa natans L. leaves	Petroleum ether	0.782	2616± 0.017	2.616± 0.001
	Chloroform	0.987	3299.33± 0.011	3.299± 0.001
	Ethyl acetate	1.488	4969.33± 0.023	4.969± 0.007
	50% Ethanolic	2.563	8552.67± 0.016	8.553± 0.003
Trapa natans L. fruit peel	Petroleum ether	0.913	3052.67± 0.018	3.053± 0.009
	Chloroform	0.992	3316± 0.014	3.316± 0.003
	Ethyl acetate	1.733	5786± 0.019	5.786± 0.005
	50% Ethanolic	2.81	9376± 0.01	9.376± 0.002

In terms of gallic acid equivalent, the total phenolic contents of the 50% ethanolic *Trapa natans* leaves and fruit peel extract were measured by utilizing the Folin-Ciocalteu's reagent (the standard curve equation: $y = 0.0003x - 0.0285$, $R^2 = 0.9998$).

3.3 Total flavonoids determination

Calibration curve of quercetin:

Quercetin standard curve was generated and plotted in distilled water to provide information on absorption. With the use of this data, the range of Beer's law and the regression coefficient are calculated (Table 5, 6 and Figure 2).

Table 5. Absorbance of quercetin in different concentration

At λ_{max} 420nm	
Concentration	**Absorbance**
10µg/ml	0.074 ± 0.002
20µg/ml	0.139 ± 0.001
30µg/ml	0.211 ± 0.009
40µg/ml	0.272 ±0.003
50µg/ml	0.349 ± 0.001

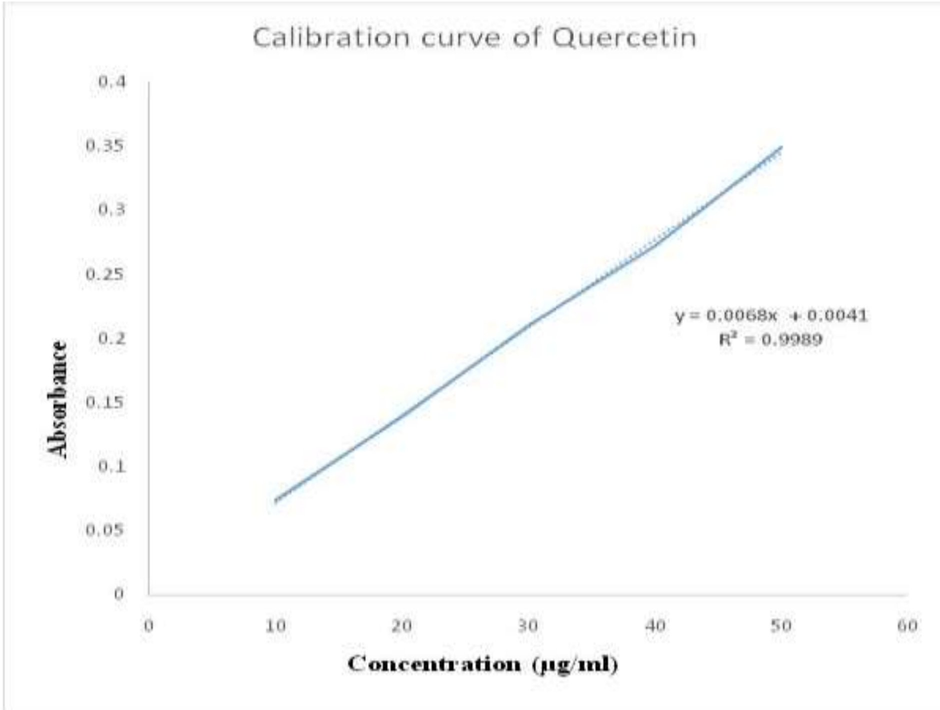

Figure 2. Quercetin calibration curve

Calculation for TF/g of extract:

Therefore, we may calculate the total Flavonoid content by utilizing the quercetin standard equation.

$$y = 0.0068x + 0.0041$$

Where y is a absorbance and X is a concentration.

Table 6. Following data shows the presence of total Flavonoid in different solvent extract

Plant Parts	Extracts	Absorbance	concentration (µg/ml)	concentration (mg/100mg)
Trapa natans L. leaves	Petroleum ether	0.859	125.77± 0.021	0.126± 0.001
	Chloroform	1.085	158.90± 0.019	0.159± 0.002
	Ethyl acetate	1.635	239.86± 0.015	0.240± 0.001
	50% Ethanolic	2.816	413.59± 0.022	0.414± 0.005
Trapa natans L. fruit peel	Petroleum ether	1.003	146.94± 0.019	0.147± 0.003
	Chloroform	1.090	159.71± 0.013	0.160± 0.002
	Ethyl acetate	1.904	279.46± 0.015	0.279± 0.001
	50% Ethanolic	3.088	453.50± 0.017	0.454± 0.002

In terms of gallic acid equivalent, the total Flavonoid contents of the *Trapa natans* leaves and fruit peel extract were measured using the 2% $AlCl_3$ solution (the standard curve equation: $y = 0.0068x + 0.0041$, $R^2 = 0.9989$).

In both industrialized and developing nations, infectious diseases caused by bacteria and microbes are the primary cause of morbidity and mortality among people. Since the development of synthetic antibiotics and their application, there has been a belief that infectious illnesses will eventually disappear. But over use of them has resulted in the emergence of drug-resistant strains, which has reduced their effectiveness. In our investigation, we found that extracts from the fruit peel and leaves of *Trapa natans* L. could inhibit the mechanism of bacterial resistance, thereby aiding in the management, cure, and elimination of sickness.

Table7. Standard medication's antimicrobial efficacy against specific selected microor ganisms

Name of microorganism	**Zone of inhibition (mm)**					
	Ofloxacin			**Ciprofloxacin**		
	10 µg/ml	**20 µg/ml**	**30 µg/ml**	**10 µg/ml**	**20 µg/ml**	**30 µg/ml**
Streptococcus mutans	13.5 ± 0.03	16 ± 0.09	18 ± 0.05	15.7 ± 0.5	19 ± 0.9	22 ± 0.4
Salmonella bongori	12 ± 0.9	13 ± 0.7	15 ± 0.5	16 ± 0.04	20 ± 0.01	23 ± 0.05
Escherichia coli	18 ± 0.1	22 ± 0.9	26 ± 0.4	17 ± 0.4	19 ± 0.5	21 ± 0.6

*** The values are in Mean ± SD, the average of three determinations**

Table 8. Antimicrobial activity of extracts of *Trapa natans* L. leaves and fruit peel against selected microbes

Plant parts	Extracts	Zone of inhibition (mm)								
		Streptococcus mutans			***Salmonella bongori***			***Escherichia coli***		
		25 mg/ ml	**50 mg/ml**	**100 mg/ml**	**25 mg/ ml**	**50 mg/ml**	**100 mg/ml**	**25 mg/ ml**	**50 mg/ml**	**100 mg/ml**
Trapa natans L. leaves	Petroleum ether	21±0.46	21±0.33	25±0.87	14±044	14±0.43	16±0.36	26±0.71	28±0.45	36±0.37
	Chloroform	12±0.37	13±0.23	14±0.25	22±0.25	22±0.45	24±0.47	22±0.63	26±0.93	26±0.91
	Ethyl acetate	25±0.34	28±0.27	28±0.23	19±0.73	20±0.63	22±0.65	28±0.66	30±0.58	36±0.84
	50% Ethanolic	22±0.48	22±0.43	24±0.97	14±0.45	14±0.45	16±0.46	53±0.53	57±0.63	62±0.32
Trapa natans L. fruit peel	Petroleum ether	23±0.62	24±0.45	23±0.54	16±0.48	18±0.50	19±0.53	13±0.33	16±0.64	23±0.91
	Chloroform	13±0.57	14±0.73	15±0.31	22±0.38	27±0.32	22±0.41	16±0.59	20±0.68	22±0.73
	Ethyl acetate	12±0.32	14±0.63	16±0.81	32±0.87	36±0.91	42±0.93	29±0.48	31±0.52	35±0.35
	50% Ethanolic	24±0.49	28±0.42	24±0.93	12±0.38	17±0.48	16±0.48	44±0.37	36±0.91	43±0.95

*** The values are in Mean ± SD, the average of three determinations**

Plants contain secondary metabolites that are vital to human nutrition and wellbeing. Based on the growth of the microbiological species examined, Tables 7 and 8 demonstrate the bioactivity of the petroleum ether, chloroform, ethyl acetate and 50% ethanolic extracts of *Trapa natans* L. fruit peel and leaves against bacteria. The zone of inhibition of the extracts was the same as the standard drug. Depending on the concentration, it works well against *S. mutans*, *S. bongori* and *Escherichia coli*.

4. CONCLUSION

In this study, *Trapa natans* L. fruit peel and leaves extracts shown broad antibacterial efficacy against a range of microbiological species. The use of the plant extract as a folkloric medicine health remedy is supported by its antibacterial efficacy, which may be connected to the identified phytoconstituents. Consequently, this plant's bioactive components can be utilised to make antimicrobial medications that are effective against a range of bacterial infections.

Future studies should concentrate on identifying these phytoconstituents, evaluating their antibacterial potential, and conducting toxicological analyses in order to create novel chemotherapeutic medications.

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AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

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CONFLICT OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

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