Original Research Article

Phytochemical Analysis, Antioxidant and antimicrobial activity of aquatic plant *Trapa natans* L.- medicinal aquatic plant

Abstract

Trapa natans L.; (water chestnut) is an aquatic plant. The whole plant has various medicinal values but it is less known. The purpose of the study was carried out to reveal phytochemicals and their antioxidant and antimicrobial activity of the whole plant *Trapa natans.* The whole plants were shade dried and powdered. The solvents used to extract were ethanol, methanol, aqueous, chloroform and hexane. The extracts were tested for the antimicrobial activity by disc diffusion method. The bacterial strains used were *Straphylococcus aureus, Enterococcus faecalis, Lactobacillus salivarius, Bacillus subtilis, Streptococcus mutans, Klebsilla pneumonia, E.coli, Pseudomonas aeruginosa, Proteus vulgaris* and *Proteus mirabilis*. The strains used for fungal activity were *Aspergillus flavus, Aspergillus niger, Penicillium notatum, Rhizopus stolonifer* and *Candida albicans.*

Antimicrobial activities of the extract were determined by zone of inhibition by using the disc diffusion method. Maximum antibacterial activity was obtained in ethanol and methanol extract. Hexane and aqueous showed least activity in gram positive strain and did not show any activity in gram negative strain. Antifungal activity showed maximum zone in *Candida albicans* in methanol extract. Hexane and aqueous extract did not show any activity. The present investigation on the plant *Trapa natans* has revealed that the ethanol and methanol showed maximum antimicrobial activity.

Keywords: *Trapa natans,* Antibacterial, Disc diffusion assay, Antioxidant, Ascorbic acid, Gallic acid.

Introduction

Plants are great reservoir of phytochemicals which possess diverse therapeutic properties. Phytochemical from medicinal plants protect against many chronic degenerative diseases [1]. For their numerous medicinal possibilities, including their anti-mutagenic, antibacterial, antioxidant, and anticancer properties, plants have been investigated extensively over the years [2].

Depending on their inherent qualities, aquatic plants have benefits for both the economy and the environment. Some species are eaten by humans, while others offer medical benefits and still others are excellent suppliers of vitamins and minerals. Aquatic plants are utilised as food and for animal feed because of their high protein and carbohydrate content [3].

An annual aquatic floating plant called Trapa natans (Water chestnut) grows in ponds and lakes all throughout the Indian subcontinent [4]. It is grown commercially in several regions of India for its edible seasonal fruit. The plants have historically been utilised in India for a number of significant

medical uses. It has been used as a nutritive, appetiser, astringent, diuretic, aphrodisiac, cooling, tonic, and it is also beneficial for inflammation, lumbago, sore throats, bilious diseases, bronchitis, and weariness. Fruits are used to make liniments that treat sunburn and rheumatism sores [5,6,7]. The stem juice is excellent for treating eye conditions and serves as a poultice to help tumours shrink [8]. Carbohydrates, minerals, calcium, phosphate, iron, copper, manganese, magnesium, sodium, and potassium are all present in the plant. Vitamins including thiamine, riboflavin, nicotinic acid, vitamin C, vitamin A, D-amylase, and significant amounts of phosphorylases are found in the kernels. Folklore medicine has acknowledged the fruits' therapeutic benefits as a treatment for a number of ailments. They also have very high concentrations of antioxidants that are not nutrients, such as flavonoids, flavones, and total phenolic contents. The pericarp includes tannins, flavonoids, and glycosides, but the seed contains carbohydrates, saponins, phytosterols, fixed oils, and fat [9]. Little information on *Trapa natans* leaves was revealed by the review. The fruit of the *Trapa natans* plant has been the subject of several research. The primary goal of the current study is to increase the level of antibacterial and antioxidant activity in *Trapa natans* L whole plant extract.

Materials and methods

Collection of plants:

T.natans plants were collected from Iraniel at Thuckaly in Kanyakumari district. To get rid of extraneous stuff, water was used to wash the collected entire plants. For further investigation, these plant components were pulverised after being shade-dried.

Sample extraction:

Soxhlet extraction was used to create crude plant extract. By using the soxhlet extraction method, the powdered plant material (25gm) was extracted using ethanol, methanol, water, chloroform, and hexane at 40–80 °C depending on the evaporation point of the solvent. Using a solvent with increasing polarity from ethanol, water, hexane, chloroform, and methanol, respectively, the extraction was done. The extraction procedure continues until the solvent in the extractor's syphon tube becomes colourless. The extract was then placed in a beaker and heated between 30 and 40 degrees Celsius until the solvent had evaporated. For use in phytochemical analysis, the dried extract was stored in a refrigerator at 4^{o}C.

Phytochemical analysis:

Standard phytochemical assays were conducted for the examination of qualitative and quantitative testing to identify the composition of the different extracts [10, 11,12]. Carbohydrates, proteins, alkaloids, flavonoids, glycosides, terpenoids, steroids, phenol, tannin, and saponin were all examined in the phytochemical assays.

Antimicrobial activity:

Test organisms:

The test microorganisms used for antimicrobial analysis included strains of Gram positive are *Straphylococcus aureus, Enterococcus faecalis, Lactobacillus salivarius, Bacillus subtilis, Streptococcus mutans*. The strains of negative bacteria were *Klebsilla pneumonia, E.coli, Pseudomonas aeruginosa, Proteus vulgaris* and *Proteus mirabilis*. The fungal strains *Aspergillus flavus, Aspergillus niger, Penicillium notatum, Rhizopus stolonifer* and *Candida albicans* were used for the assay. The strains were obtained from Microbial Type Culture Collection and Gene Bank (**MTCC**) Chandigarh. The bacterial strains were maintained on Nutrient Agar (NA).

Preparation of culture:

Pure cultures were transferred from the plate to nutrient agar plates and subcultured for 24 hours at 37°C. Inoculum was prepared by aseptically adding the fresh culture into 2 ml of sterile 0.145 mol/L saline tube and the cell density was adjusted to 0.5 McFarland turbidity standard to yield a bacterial suspension of 1.5×108 cfu/ml. Standardized inoculums seed for antimicrobial test.

Antimicrobial assay:

The medium was made by combining 1000 ml of distilled water with 38 g of Muller Hinton Agar Medium (Hi Media). The dissolved medium was autoclaved at 15 Lbs pressure at 121^0C for 15 min (pH 7.3). The autoclaved medium was cooled, mixed well and poured petriplates (25 ml/plate) the plates were swabbed with Pathogenic Bacteria culture. Finally, The Sample or Sample loaded Disc was then placed on the surface of Mullar-Hinton medium and the plates were kept for incubation at 37°C for 24 hours. At the conclusion of incubation, inhibition zones surrounding the disc were inspected and measured using a clear ruler in millimetres. The disc included, the zone of inhibition's size was expressed in millimetres. It was believed that the absence of zone inhibition meant there was no activity (Pulido and Bravo, 2000 ; Suja et al., 2016). When the zone of inhibition is less than 7 mm, the activities are described as resistant, followed by moderate (8–10 mm) and sensitive (greater than 11 mm) (Lizcano et al., 2012).

Antifungal assay:

Antibiotic susceptibility tests were determined by agar disc diffusion method [13]. In an SDA agar plate, fungi strains were swabbed using sterilised cotton swabs. Up to 40 µl of each concentration of the extract were respectively introduced in the sterile discs using sterile pipettes. Following the disc's placement on the SDA medium, the compound was given five minutes to diffuse before the plates underwent a 48-hour incubation period at 22°C. At the conclusion of incubation, inhibition zones surrounding the disc were inspected and measured using a clear ruler in millimetres.

ANTIOXIDANT ACTIVITY

Chemicals

2,2′ - diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid , ethylenediaminetetraacetic acid (EDTA) were obtained from Hi–Media Pvt. Ltd. Mumbai, India.

Sample extraction:

Soxhlet extraction was used to create crude plant extract. Soxhlet extraction was used to extract the 25g of powdered plant material using ethanol and methanol at various temperatures depending on the solvent's evaporation point. A solvent with increasing polarity that was made from ethanol and methanol was used to carry out the extraction. The extraction procedure continues until the solvent in the extractor's syphon tube becomes colourless. The extract was then placed in a beaker and heated between 30 and 40 degrees Celsius until the solvent had evaporated. For subsequent investigation, the dried extract was stored in a refrigerator at 4^{o}C.

Determination of antioxidant activity

Nitric oxide radical scavenging assay

The Griess reaction was used to quantify the amount of nitrite ions produced when oxygen and sodium nitro prusside mix to form nitric oxide. This assay was done by the procedure described by [14]. The reaction mixture contained 3.0 ml of 10 mM sodium nitroprusside in phosphate buffered saline (pH 7.4) and various concentration of (50, 100, 150, 200 and 250 µg/ml) extracts. The resulting solution was then incubated at 25°C for 60 minutes. To the incubated sample 5.0 ml of Griess reagent (1% sulphanilamide, 0.1% NEDD in 2% H3PO4) was added and the absorbance of the chromophore formed was measured at 546 nm against a reagent blank. Percentage inhibition of the nitrite ions generated is observed. The standard ascorbic acid was used for comparison. The free radical scavenging activity was determined by evaluating % inhibition as above.

Percentage of inhibition = [(control OD−sample OD) / (control OD)]×100

Ferrous ions chelating ability

The ferrous ion chelating potential of the extracts was evaluated by method [15]. The reaction mixture contained 1.0 ml of various concentrations of the extracts (50, 100, 150, 200 and 250 µg/ml) and 0.05 ml of 2 mM FeCl3. The reaction was initiated by the addition of 0.2 ml of 5 mM ferrozine. The reaction mixture was vigorously agitated and allowed to stand at room temperature for 10 minutes before the reaction mixture's absorbance at 562 nm was measured in comparison to a reagent blank. The reaction mixture's lower absorbance suggested a greater capacity to chelate ferrous ions. The only reagent absent from the control was the sample. A reference standard for comparison was gallic acid.

Percentage of inhibition = [(Control- Test)/control]×100

DPPH radical scavenging activity

The free radical scavenging activity of the fractions was measured in vitro by 2,2′ - diphenyl-1-picrylhydrazyl (DPPH) assay according to the standard method [16]. The stock solution was prepared by dissolving 24 mg DPPH with 100 ml of ethanol stored at 20°C until required. After diluting the DPPH solution with ethanol, a 3 ml aliquot of the working solution was coupled with 1 ml of methanol

extracts at different concentrations (50, 100, 150, 200, and 250 µg/ml). The reaction mixture was well shaken and then allowed to stand at room temperature in the dark for 15 minutes. The absorbance at 517 nm was then determined. Ascorbic acid served as a benchmark for comparison in the market.

Percentage of inhibition = [(control OD−sample OD) / (control OD)]×100

Phosphomolybdenum Assay (PM)

The Total Antioxidant assay of the plant fractions was determined by phosphomolybdenum method as described [17]. Briefly, 0.3 mL of the different solvent fractions and standard drugs (50, 100, 150, 200 and 250 µg/ml) were taken in test tubes and dissolved in 3 mL of reagent solution (0.6 M sulphuric acid, 4 mM ammonium molybdate and 28 mM sodium phosphate). The test tubes were covered and incubated at 95°C in a water bath for 95 min. The mixture was allowed to cool to room temperature and the absorbance was measured at 695 nm. A mixture containing distilled water instead of the samples served as control. Ascorbic acid was used as standard drug. Higher absorbance indicates higher total antioxidant potential.

[(absorbance of control– absorbance of sample)/(absorbance of control)] ×100

Hydroxyl radical scavenging activity

The reaction mixture contained 0.8 mL of phosphate buffer solution (50 mmol L-1, pH 7.4), 0.2 mL of a sample of different concentrations (50, 100, 150, 200 and 250 µg/ml), 0.2 mL of EDTA (1.04 mmol L-1), 0.2 mL of FeCl3(1 mmol L-1), and 0.2 mL of 2-deoxyribose (60 mmol L-1). The mixtures were kept in a water bath at 37 °C and the reaction was started by adding 0.2 mL of ascorbic acid (2 mmol L-1) and 0.2 mL of H2O2 (10 mmol L-1). 2 mL of cold thiobarbituric acid (10 g L-1) and 2 mL of HCl (25%), both added to the reaction mixture, were added after 1 hour of incubation at 37 °C. The mixture was heated for 15 minutes at 100 °C before being cooled with water. Using a spectrophotometer, the solution's absorbance at 532 nm was determined. The proportion of 2-deoxyribose oxidation on hydroxyl radicals that was inhibited was used to measure the capacity of hydroxyl radical scavenging [9]. Ascorbic acid was used as standard for comparison. The scavenging percentage was calculated according to the following formula:

Scavenging effect (%) = [(control OD−sample OD)/(control OD)]×100

Statistical analysis

All the analysis were repeated thrice and the results were presented as mean ± SD. The IC50 value were calculated by using the software ED50.

Result and Discussion :

Using the disc diffusion method, the antibacterial properties of ethanol, methanol, hexane, chloroform, and aqueous extracts of entire plants were examined. The qualitative analysis of the plant sample were reported in Table.1. Results from the phytochemical investigation showed whether or not

phytochemicals were present, as well as their quantitative measurement in particular solvents. Ethanol solvent included carbohydrates, flavonoids, phenol, tannin, and saponin. It was evident that phenol, tannin, saponin, and carbohydrates were present in the methanol extract. Only steroid and glycoside were visible in hexane. The presence of protein, flavonoids, glycosides, steroids, and saponin was shown by chloroform extract. In the current experiment, aqueous extract demonstrated the presence of alkaloid, flavonoid, terpenoid, and tannin. According to earlier research, utilising solvents with different polarity in an extraction process is crucial to understanding the relationship between solvent and extraction effectiveness (Anjaria et al., 2002). Hexane mainly extracts pigments (Smith *et al.*, 1992) and chloroform is said to be the best solvent for extraction of non-polar biological active compounds. Earlier studies have showed that methanol can extract a high amount of saponins from leaves[18].

Table.1 Qualitative analysis of *Trapa natans*

Phytochemical	**Plant Extract**				
	Ethanol	**Methanol**	**Hexane**	**Chloroform**	**Aqueous**
Carbohydrate	+	+	–	–	–
Protein	–	–	–	+	–
Alkaloid	–	–	–	–	+
Flavonoid	+	–	–	+	+
Glycoside	–	–	+	+	–
Terpenoid	–	–	–	–	+
Steroid	–	–	+	+	–
Phenol		+	+	–	–
Tannin	+	+	–	–	+
Saponin	+	+	–	+	–

+: Present ; - : absent

The results of quantitative analysis were determined in three concentration (25, 50, 100) reported in Table 2. Tannin are extracted maximum in aqueous at conc. 100 (94 ± 1.41 mg/ G). Minimum in steroid content at conc.100 in hexane solvent (36.3 ± 1.02 mg/ G).

Table 2 Quantitative analysis of *Trapa natans*

Phytochemical content	**Concentration (µg/ml)**	**Sample Extract**			
		Ethanol	**Methanol**	**Hexane**	**Chloroform**
Carbohydrate	25	25.6 ± 1.08 mg/ G	20 ± 0.70 mg/ G	_	_
	50	52.3 ± 1.08 mg/ G	45 ± 0.70 mg/ G		_
	100	74 ± 1.41 mg/ G	53.6 ± 1.08 mg/ G		_
Protein	25	_	_	_	13.8 ± 0.35 mg/ G
	50	_	_	_	37.6 ± 1.08 mg/ G
	100	_	_	_	70 ± 1.41 mg/ G
Alkaloid	25	_	_	_	_
	50	_	_	_	_
	100	_	_	_	_
Flavonoid	25	20 ± 0.70 mg/ G	_	_	11.5 ± 0.35 mg/G
	50	46.3 ± 1.08 mg/ G	_	_	35.6 ± 1.04 mg/ G
	100	65 ± 1.42 mg/ G	_	_	44.6 ± 1.08 mg/ G
Glycoside	25	_	_	13 ± 0.70 mg/ G	15 ± 0.70 mg/ G
	50	_	_	25.3 ± 0.81 mg/ G	31 ± 0.70 mg/ G
	100	_	_	47.6 ± 1.08 mg/ G	55 ± 0.70 mg/ G
Terpenoid	25	_	_	_	_
	50	_	_	_	_
	100	_	_	_	_
Steroid	25	_	_	11 ± 0.70 mg/ G	14 ± 0.70 mg/ G

	50	_	_	19.8 ± 0.88 mg/ G	29.3 ± 1.08 mg/ G
	100	_	_	36.3 ± 1.02 mg/ G	38.6 ± 1.41 mg/ G
Phenol	25	15.5 ± 0.9 mg/ G	17.1 ± 0.73 mg/ G	_	_
	50	27.3 ± 0.81 mg/ G	27.3 ± 0.81 mg/ G	_	_
	100	46 ± 1.41 mg/ G	46 ± 1.41 mg/ G	_	_
Tannin	25	24 ± 0.70 mg/ G	31 ± 0.70 mg/ G	_	_
	50	48.6 ± 1.08 mg/ G	48.6 ± 1.0 mg/ G	_	_
	100	64.6 ± 1.05 mg/ G	79 ± 1.41 mg/ G	_	_
Saponin	25	21 ± 0.70 mg/ G	28 ± 0.70 mg/ G	_	7.5 ± 0.93 mg/ G
	50	36 ± 0.70 mg/ G	44 ± 0.70 mg/ G	_	25 ± 0.70 mg/ G
	100	53 ± 1.43 mg/ G	67.3 ± 1.08 mg/ G	_	47.3 ± 1.08 mg/ G

G: Gallic acid;

The antibacterial activity showed in Table 3 five gram positive and five negative strains were used for analysis. The extract ethanol showed higher inhibition zone against the organism. *Staphylococcus aureus*, an ethanol-grown gram-positive bacterial strain, displayed 18 mm, 21 mm in *Enterococcus faecalis*, and 21 mm in *Streptococcus mutans.* 18mm of *Lactobacillus salivarius* in ethanol. *Proteus mirabilis, Klebsilla pneumonia, E. coli*, *Pseudomonas aeruginosa,* and *Proteus vulgaris* had highest activity in the gram negative strain, respectively, at 10mm, 15mm, 14mm, and 11mm. The standard antibiotics used for antibacterial activity was *Streptomycin- S* 25. *Staphylococcus aureus, Enterococcus faecalis, Lactobacillus salivarius, Streptococcus mutans, and Bacillus substilis* did not exhibit any action in the hexane extract when exposed to 11 mm of hexane. Aqueous and chloroform likewise had the least action in gramme positive bacteria. Aqueous and hexane did not exhibit any action in gram-negative media.

Table 3. Antibacterial activity of *Trapa natans*

Bacterial strains	**Solvents**					**PC**
	Ethanol	**Methanol**	**Hexane**	**Aqueous**	**Chloroform**	
Staphylococcus aureus (G+) MTCC 916	18mm	17mm	11mm	12mm	13mm	21mm
Enterococcus faecalis (G+) MTCC 439	21mm	18mm	7mm	9mm	11mm	20mm

Lactobacillus salivarius (G+) MTCC 1026	18mm	15mm	9mm	10mm	13mm	18mm
Bacillus subtilis (G+) MTCC 1134	9mm	9mm	_	_	8mm	21mm
Streptococcus mutans (G+) MTCC 916	21mm	18mm	9mm	9mm	11mm	20mm
Klebsilla pneumonia (G-) MTCC 530	10mm	8mm	_	_	7mm	22mm
E.coli (G-) MTCC 1671	15mm	15mm	_	_	11mm	18mm
Pseudomonas aeruginosa (G-) MTCC 741	14mm	14mm	_	_	9mm	19mm
Proteus vulgaris (G-) MTCC 426	17mm	17mm	_	_	10mm	18mm
Proteus mirabilis (G-) MTCC 1429	11mm	11mm	_	_	7mm	24mm

PC – Positive control (Streptomycin- S25)

NC- Negative control (plain disc)

mm – Millimeter

G+ -Gram positive organism

G- - Gram Negative organism

The antifungal activity assay result of *Trapa natans* is summarized in Table 4. The highest levels of inhibition against *Candida albicans* and *Aspergillus flavus* fungi in methanol extract were 18 mm and 15 mm, respectively. Fluconazole was the go-to medication for fungus activity. The ethanol extract's moderate suppression of the *Aspergillus flavus* 13mm, *Rhizopus stolonifer* 8mm, and Candida albicans 17mm strains. In chloroform extract, the minimum inhibitory zone for *Aspergillus flavus* is 8 mm, while for Candida albicans, it is 11 mm. In the entire plant, Trapa natans L., *Aspergillus niger* and *Penicillium notatum* did not produce any fungal activity.

Table 4 Antifungal activity of *Trapa natans*

Fungal Strains	**Plant extract**					**PC**
	Ethanol	**Methanol**	**Hexane**	**Aqueous**	**Chloroform**	
*Aspergillus flavus(*F) MTCC 535	13mm	15mm	_	_	8mm	23mm

Aspergillus niger(F) MTCC 281	_	_	_	_	_	29mm
Penicillium notatum (F) MTCC 2647	_	_	_	_	_	13mm
Rhizopus stolonifer (F) MTCC 162	8mm	_	_	_	_	19mm
Candida albicans(F) MTCC 183	17mm	18mm	_	_	11mm	24mm

PC : Positive control (fluconazole)

NC : Negative control (Plain disc)

__ : no zone

Mm : millimeter

Earlier work reported that the antimicrobial activity of Methanolic extracts of *Phyllanthus emblica* on different pathogenic organisms using disc diffusion method have showed maximum zone of inhibition. Methanol extract of plant *Walsura trifoliate* showed significant antimicrobial activity [19]. Ethanol extraction has been shown to be the most effective extraction method for isolating the bioactive phytocompound [20]. Previous studies also indicate that ethanol was the best solvent for extracting antimicrobial substances from plants [15]. In the present investigation the methanol and ethanol extract shows better results in the whole plant of *Trapa natans*.

Nitric oxide scavenging activity

Since its discovery as a new signal molecule, nitric oxide (NO) has been linked to a number of physiological functions in the human body[18]. Along with playing a position in physiological processes, it also takes part in the pathogenic processes that underlie a wide range of illnesses, including as multiple sclerosis, stroke, inflammatory bowel disease, sepsis, and septic shock. The evidence for NO's role in neuronal cell death in Parkinson's disease (PD), other neurodegenerative diseases including Alzheimer's disease, and its modulation of neurotoxin-induced cell damage is growing [3.21,22]. In the present study, nitric oxide assay was carried out at different concentration (50,100,150,200 and 250 µg/ ml). The findings of the nitric oxide assay's percentage of inhibition are shown in Table 5 and Figure 1. The outcomes shown that the high dosage concentration of ethanol (67.2±0.61 at 250 µg/ ml) increases the percentage of inhibition of nitric oxide scavenging activity. Methanol extract displays 50.1±0.73 whereas ascorbic acid standard compound displayed 89.3±1.77 in 250 µg/ml. The extract's IC50 value was calculated, and the outcomes are shown in Table 5. The significant IC50 values for the ethanol extract (95.6), methanol (139.8), and the reference chemical ascorbic acid are all displayed (119.9).

Table.5. Nitric oxide scavenging activity

Concentration	% of inhibition		
	Ethanol	Methanol	Standard (Ascorbic Acid)
50 µg/ ml	18.4 ± 0.4	7.5 ± 0.35	24.8 ± 1.20
100 µg/ ml	40.4 ± 0.70	15.1 ± 0.54	36.7 ± 1.17
150 µg/ ml	48.1 ± 0.81	27.2 ± 0.78	50.0 ± 0.70
200 µg/ ml	57.2 ± 0.74	37.6 ± 0.86	74.9 ± 1.30
250 µg/ ml	67.2 ± 0.61	50.1 ± 0.73	89.3 ± 1.77
IC 50	95.6	139.8	119.9

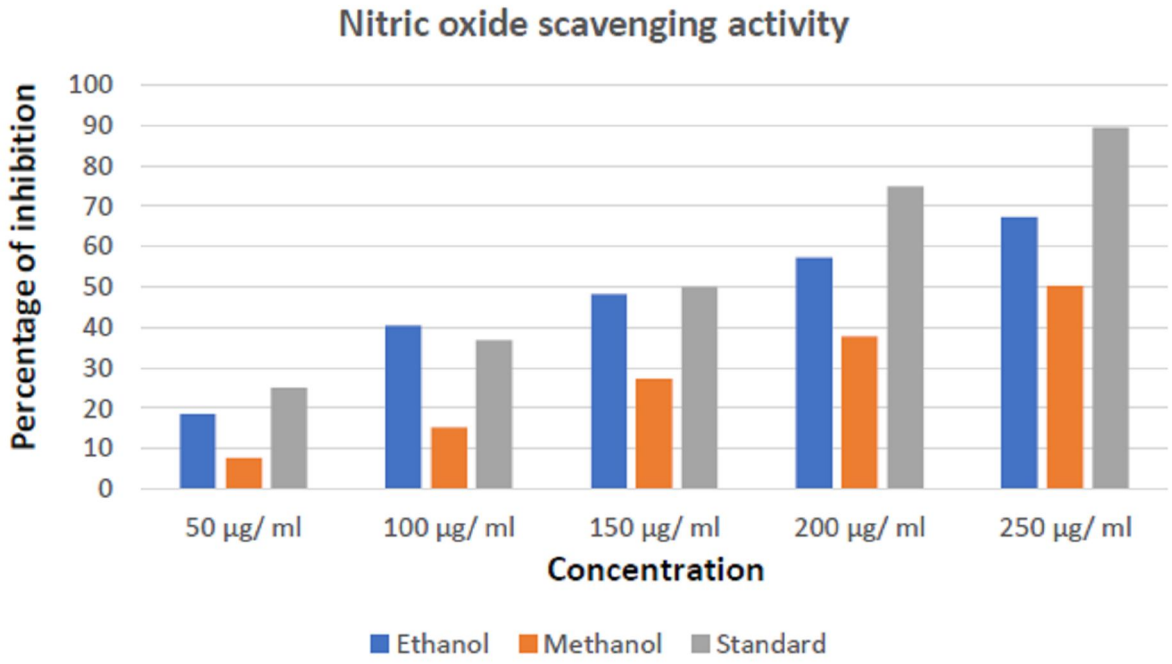

Fig.1 Nitric oxide scavenging activity of different concentration of Ethanol, Methanol and Ascorbic acid in plant sample *Trapa natans*. . Each value represents the mean ± SEM

Ferrous ion chelating activity

Because it is necessary for the transfer of oxygen, respiration, and the action of several enzymes, iron is crucial for life. However, because of its high level of reactivity, this metal catalyses oxidative changes in lipids, proteins, and other elements of the cell [23]. The roduction of the ferrous ion ferrozine complex served as a gauge of the leaf and root extracts' capacity to chelate metals. Ferrozine combines with ferrous ions forming a red coloured complex which absorbs at 562n m [24]. The amount of blue colour produced in the ferric-reducing power assay directly correlates with the extracts' antioxidant activity [19]. In the present study, the antioxidant assay ferric ion chelating activity assay was evaluated.

The percentage of inhibition was calculated, the result reported in Table.6 and fig.2. The percentage of inhibition for the extract ethanol is 69.1±0.54, methanol is 62.2±0.74, and the reference compound gallic acid is 85.1±0.73 at high dosage concentration in 250 µg/ ml. The IC50 value was calculated, the results shown in table.6. The IC50 values of the extract ethanol shows 129.9, the methanol 136.9 and the standard compound gallic acid shows 136.9.

Table 6.Ferrous ion chelating activity

Concentration	% of inhibition		
	Ethanol	**Methanol**	**Standard (GallicAcid)**
50 µg/ ml	12.2 ± 0.45	8.5 ± 0.35	21.2 ± 0.74
100 µg/ ml	25.9 ± 0.60	19.2 ± 0.48	34.2 ± 0.43
150 µg/ ml	38.0 ± 0.70	35.0 ± 0.70	48.2 ± 0.45
200 µg/ ml	58.2 ± 0.96	49.2 ± 0.48	66.9 ± 1.37
250 µg/ ml	69.1 ± 0.54	62.2 ± 0.74	85.1 ± 0.73
IC 50	129.9	136.9	124.0

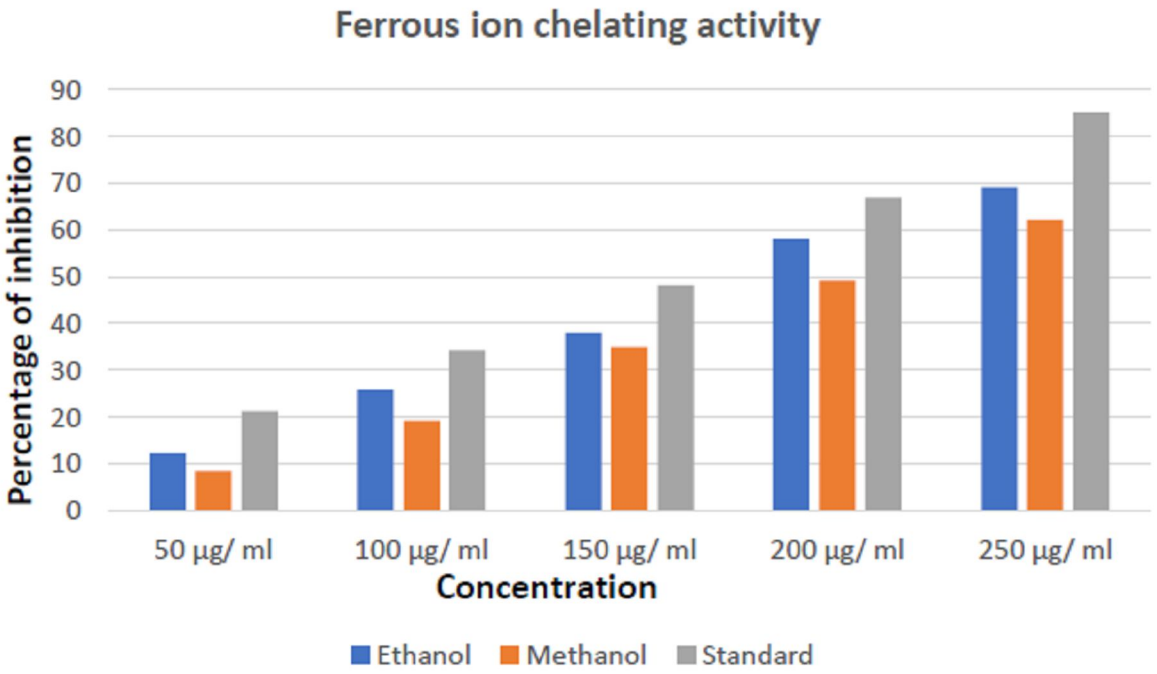

Fig.2 Ferrous ion chelating activity of different concentration of Ethanol, Methanol and Ascorbic acid in plant sample *Trapa natans.* . Each value represents the mean ± SEM

DPPH radical scavenging activity

DPPH is a free-radical generating compound and has been widely used to evaluate the free-radical scavenging ability of various antioxidant compounds [25]. The DPPH scavenging activity in the current investigation was measured by the percentage of inhibition at various concentrations (50, 100, 150, 200, and 250 µg/ ml). The results shown in table.7 and fig.3. At a concentration of 250 µg/ ml in the DPPH experiment, the percentage of inhibition in methanol was 76.2±0.81, ethanol was 76.1±0.71, and the reference component ascorbic acid exhibited 84.2±0.92 of inhibition.. The IC50 values were calculated, the results shown in table.7. IC50 value of methanol 122.8, the ethanol shows 113.4 and the standard compound shows 60.6. Earlier study showed that the prominent DPPH scavenging capacity of solvent free lipid based extract, methanolic extract and Ayurvedic Ghrita of *B. monnieri* [26].

Table 7. DPPH radical scavenging activity

Concentration	% of inhibition		
	Ethanol	**Methanol**	**Standard (Ascorbic acid)**
50 µg/ ml	21.5 ± 0.36	16.2 ± 0.53	32.4 ± 0.61
100 µg/ ml	28.2 ± 0.48	31.2 ± 0.88	57.0 ± 0.4
150 µg/ ml	55.8 ± 0.85	46.2 ± 0.61	71.1 ± 0.81
200 µg/ ml	63.6 ± 1.08	62.2 ± 0.52	75.2 ± 0.92
250 µg/ ml	76.1 ± 0.71	76.2 ± 0.81	84.2 ± 0.92
IC 50	113.4	122.8	60.6

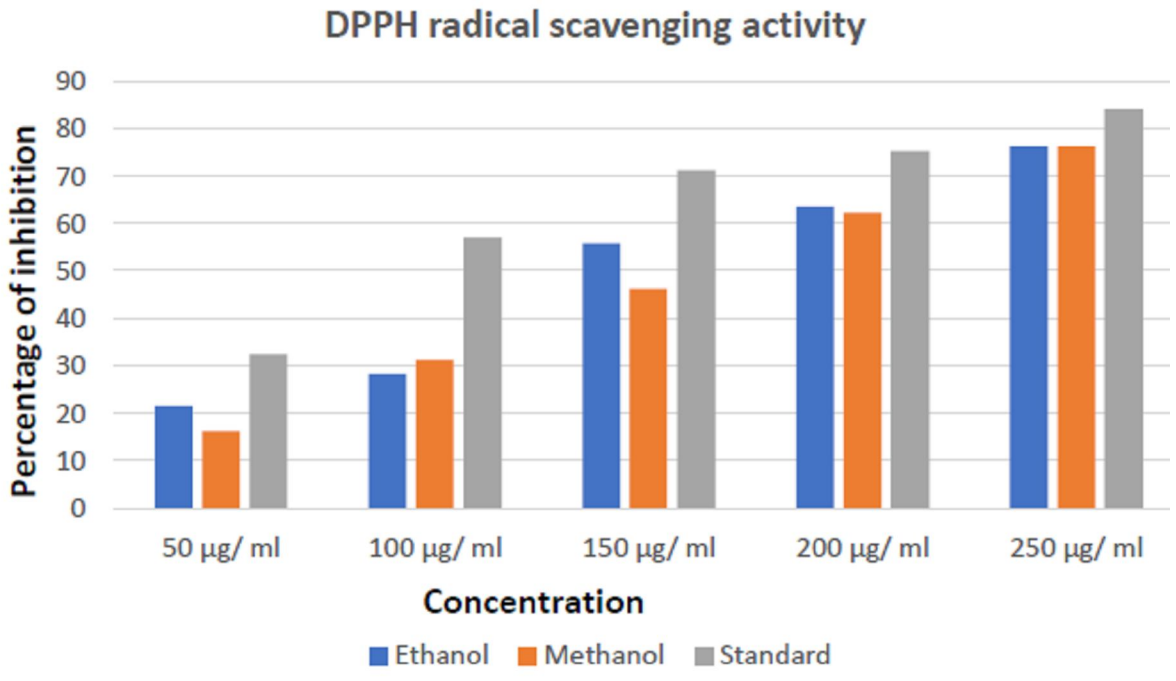

Fig.3 DPPH radical scavenging activity of different concentration of Ethanol, Methanol and Ascorbic acid in plant sample *Trapa natans*. . Each value represents the mean ± SEM

Phosphomolybdenum assay

When using the phosphomolybdenum test, a quantitative technique for assessing antioxidant ability [27]. The present study in plant *Trapa natans* , the antioxidant activity in phosphomolybdenum the percentage of inhibition the results shown in table.8 and fig.4. The percentage of inhibition shown superior activity at concentrations of 250 µg/ ml; in these, methanol exhibits activity of 59.2±0.76, ethanol exhibits activity of 66.1±0.60, and the reference molecule exhibits activity of 68.5±0.35. After calculating the IC50 values, the results are displayed in Table 8. Ethanol has an IC50 value of 127.5, methanol of 135.5, and ascorbic acid, a reference chemical, of 124.1. According to a recent research, the methanol extract of W. trifoliata root exhibited -Glucosidase inhibition and antioxidant activity, suggesting that it can be useful in preventing or reversing the progression of a number of disorders linked to oxidative stress [28].

Table 8. Phosphomolybdenum assay

Concentration	% of inhibition		
	Ethanol	**Methanol**	**Standard (Ascorbic acid)**
50 µg/ ml	16.5 ± 0.35	12.3 ± 0.48	19.5 ± 0.88
100 µg/ ml	26.0 ± 0.70	18.5 ± 0.35	26.1 ± 1.06
150 µg/ ml	38.1 ± 0.73	31.2 ± 0.48	37.6 ± 0.83
200 µg/ ml	46.3 ± 0.43	45.1 ± 0.54	52.6 ± 1.08
250 µg/ ml	66.1 ± 0.60	59.2 ± 0.76	68.5 ± 0.35
IC 50	127.5	135.5	124.1

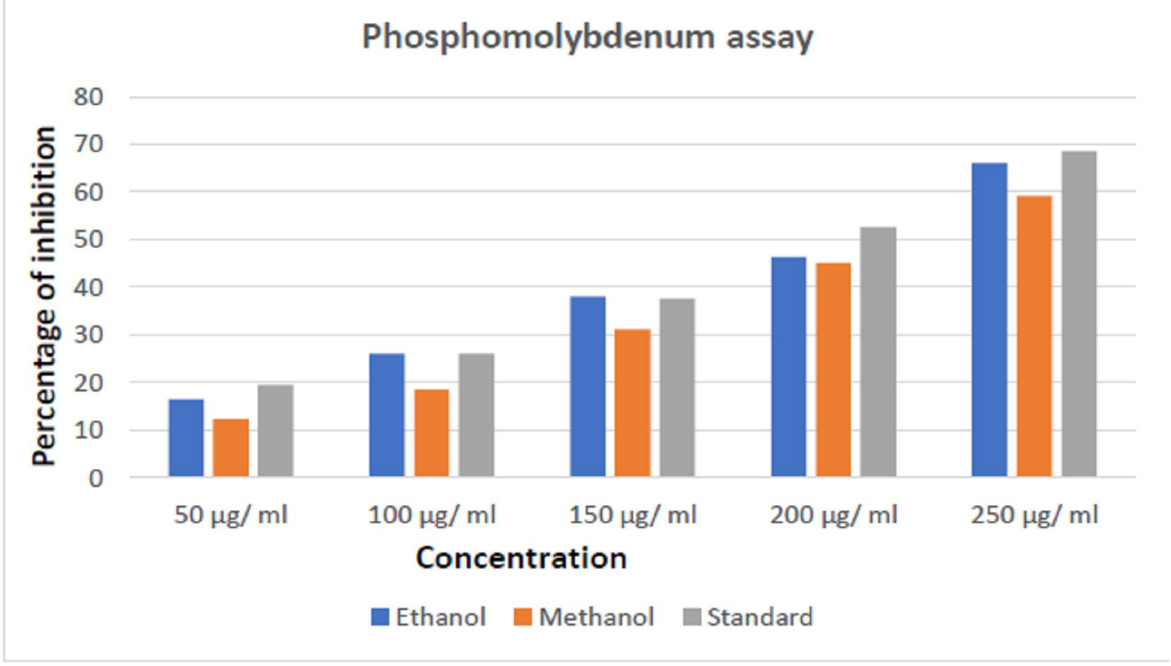

Fig.4 Phosphomolybdenum activity of different concentration of Ethanol, Methanol and Ascorbic acid in plant sample *Trapa natans*. . Each value represents the mean ± SEM

Hydroxyl radical scavenging activity

Although H_2O_2 by itself is not particularly reactive, it occasionally causes cytotoxicity by generating hydroxyl radicals in the cell. Therefore, eliminating H_2O_2 is crucial for the entire food system. Antioxidants' ability to scavenge H_2O_2 may result from the transfer of electrons to H_2O_2, which then neutralises to water [29]. In the present study, the antioxidant assay of hydroxyl radical scavenging activity the percentage of inhibition was calculated, the results shown in table.9 and fig.5. At a concentration of 250 µg/ ml, the methanol displays 58.3±0.43, the ethanol displays 63.8± 0.54, and the reference compound displays 72.2±0.45. Better activity may be seen in the extract ethanol IC50 value. 121.4, 132.0 for methanol, and 108.4 for ascorbic acid, a common chemical. An earlier study found that the plant extract *Trapa natans* L. had larger inhibition zones against organisms when the solvent ethanol was used.

Table 9. Hydroxyl radical scavenging activity

Concentration	% of inhibition		
	Ethanol	**Methanol**	**Standard (Ascorbic acid)**
50 µg/ ml	17.0 ± 0.63	12.1 ± 0.64	22.3 ± 0.43
100 µg/ ml	24.8 ± 0.73	21.1 ± 0.57	32.2 ± 0.47
150 µg/ ml	35.8 ± 1.24	29.2 ± 0.51	46.2 ± 0.50
200 µg/ ml	53.2 ± 0.77	46.2 ± 0.50	62.0 ± 0.63
250 µg/ ml	63.8 ± 0.54	58.3 ± 0.43	72.2 ± 0.45
IC 50	121.4	132.0	108.4

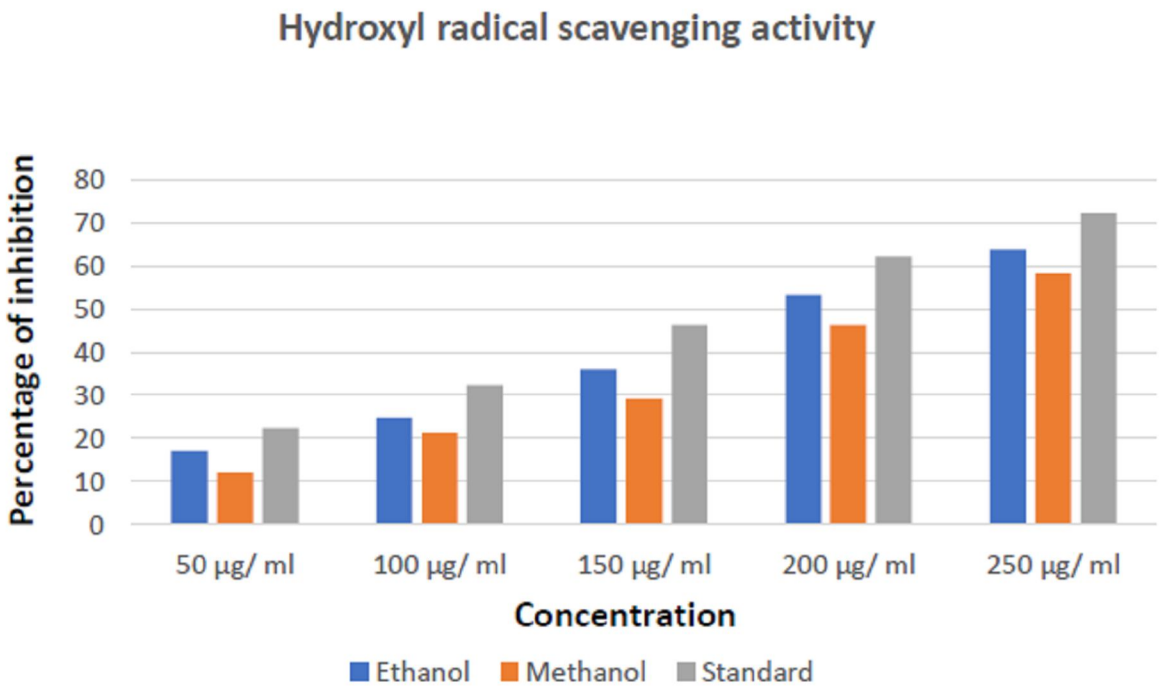

Fig.5 Hydroxyl radical scavenging activity of different concentration of Ethanol, Methanol and Ascorbic acid in plant sample *Trapa natans.* . Each value represents the mean ± SEM

Conclusion

From the above study, it concluded that the methanol and ethanol extract showed significant antimicrobial activity. It also supports traditionally that the plant *Trapa natans* has some major bioactive components which leads to further research work. It gives an awareness to keep up and save the fresh water plants. The present work it concluded that the ethanol extract of aquatic plant *Trapa natansL* posses the significant antioxidant activity which is beneficially used for the disease that related to oxidative stress. From this, antioxidant activity it gives a path for the isolation of active compounds as a future study.

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