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Comparative study on *in-vitro* antioxidant and antimicrobial activity of *Trapa natans* and *Punica granatum*

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Abstract
Background: Numerous plant extracts have demonstrated antioxidant properties as well as antimicrobial effects. The hydroalcoholic (8:2) and petroleum ether extracts derived from the peels of *Trapa natans* and *Punica granatum*, obtained through the microwave-assisted extraction (MAE) method, have exhibited notable antioxidant and antimicrobial activities.
Aim: The primary aim of this study was to comparatively evaluate the in vitro antioxidant and antimicrobial activities of *Trapa natans* (water chestnut) and *Punica granatum* (pomegranate), two medicinal plants commonly used in traditional medicine for their therapeutic properties
Method: The Hydro alcoholic (8:2) and Petroleum Ether extracts of both plants were subjected to TPC, TFC, DPPH Assay, and FRAP Assay, while antimicrobial activity was assessed against selected Gram-positive and Gram-negative bacterial strains using the agar well diffusion method
Results: Results showed that both plants had significant antioxidant activity, with *Punica granatum* showing marginally superior radical scavenging efficiency in its hydro alcoholic extract when prepared via the Microwave Assisted Extraction (MAE) technique. *Punica granatum* also displayed a broader spectrum of antimicrobial activity and larger zones of inhibition
Conclusion: These findings indicate the potential of both plant extracts as natural antioxidants and antimicrobial agents, and thus reinforcing their promising applications in the development of pharmaceutical and nutraceutical products.

Keywords: *Trapa natans*, *Punica granatum*, antioxidant activity, antimicrobial activity

Introduction
Herbal medicine, a traditional practice in various cultures, uses plants as natural remedies to treat diseases and improve overall health [1,2]. Common spices and herbs are known for their antibacterial, antifungal, antioxidant, and other beneficial properties. These plant-derived remedies are safer and have a longer half-life compared to synthetic medications [3]. Traditional herbal drugs, such as Ayurveda, Traditional Chinese Medicine, and African herbal medicine, are valued for their therapeutic properties and ability to promote healing, enhance immunity, and restore balance in the body [4, 5]. *Trapa natans* (water chestnut) and *Punica granatum* (pomegranate) peel extracts exhibit significant antimicrobial properties, making them promising candidates for natural antimicrobial agents [6]. *Trapa natans* contains bioactive compounds like tannins, flavonoids, and polyphenols that inhibit the growth of various bacterial strains by disrupting microbial cell membranes and interfering with essential metabolic processes. *Punica granatum* peel extract is rich in ellagitannins, gallic acid, and other phenolic compounds that exert strong antibacterial effects by inducing oxidative stress in microbial cells and preventing biofilm formation. Both extracts have demonstrated efficacy against a wide range of pathogens, suggesting their potential use in pharmaceutical, cosmetic, and food preservation applications [6, 7]. Both *Trapa natans* and *Punica granatum* are rich in potent antioxidant compounds that contribute significantly to their health-promoting properties [5, 8]. The high levels of polyphenols, flavonoids, and tannins in *Trapa natans* scavenge free radicals and mitigate oxidative stress, while *Punica granatum* neutralizes reactive oxygen species (ROS), inhibits lipid peroxidation, and enhances the activity of endogenous antioxidant enzymes [4, 9, 10]. Collectively, these extracts present a formidable natural antioxidant potential, supporting their utilization in functional foods, nutraceuticals, and therapeutic applications aimed at combating oxidative stress-related diseases [3, 11].

Therefore, the present study has been designed to evaluate the *in vitro* antioxidant & antimicrobial activity of different peel extracts of *Trapa natans & Punica granatum* fruits [12].

Materials and methods

2.1. Collection, identification & processing of Samples: The two species, *Trapa natans & Punica granatum* were collected from Barasat, North 24 Parganas, Kolkata: 700127 in the month of December 2024 and identified and authenticated by the Scientist in charge, Botanical Survey of India, P.O. Botanical Garden, Shibpur, Howrah: 711103. Fruits were thoroughly washed, cleaned under running tap water then distilled water to remove impurities. The outer hardcover peels were removed from the fruit and air-dried. The dried samples were crushed and ground into powder using a mixer grinder and stored in airtight bottles at room temperature.

2.2. Preparation of the Extracts: The study involved extracting 50 grams of dried and powdered peel from *Trapa natans* and *Punica granatum* using a solvent-to-solid ratio of 10:1. Two solvents were used: petroleum ether and a hydroalcoholic mixture of ethanol and water (8:2). The process was carried out using microwave power between 300 and 500 watts for 10 to 15 minutes, with periodic stirring. The temperature was kept below 60°C to prevent degradation of phytochemicals. After extraction, the extracts were filtered, evaporated, and concentrated using a rotary evaporator set at 40°C under reduced pressure. The final extracts were stored at 4°C for further analysis [13].

2.3 Determination of Total Phenolic Content

2.3.1 Preparation of Standard Gallic Acid for Calibration Curve: The study used the Folin–Ciocalteu colorimetric method to determine the total phenolic contents (TPC) of extracts. A standard gallic acid solution was prepared by dissolving 10 mg in methanol. Different concentrations of gallic acid solutions were prepared, and the absorbance was measured at 765 nm using a UV-Vis spectrophotometer. The experiments were conducted in triplicates, and the average absorbance values obtained at different concentrations of gallic acid were used to plot the calibration curve.

2.3.2 Preparation of Samples for Total Phenolic Content.
Various concentrations of both hydroalcoholic and petroleum ether extracts (100, 200, 300, 400 and 500 µg/mL) were prepared. The procedure as described for standard gallic acid was followed, and absorbance for each concentration of the extracts was recorded. The samples were prepared in triplicate for each analysis, and the average value of absorbance was used to plot the calibration curve to determine the level of phenolics in the extracts. Total phenolic content of the extracts was expressed as mg gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g). The total phenolic contents in all the samples were calculated by the using the formula: C = c×V/m [where 'C' = total phenolic content mg GAE/g dry extract, 'c' = concentration of gallic acid obtained from calibrationcurve in mg/mL, 'V' = volume of extract in mL, and 'm' = mass of extract in gram] [14].

2.4: Determination of Total Flavonoid Content

2.4.1. Preparation of Standard Quercetin for Calibration Curve: Total flavonoid contents in the extracts were determined by aluminum chloride colorimetric assay. Stock solution (4 mg/mL) of quercetin was prepared by dissolving 4 mg of quercetin in 1 mL of methanol. This standard solution was diluted serially to make various concentrations of 100, 200, 300,400 and 500 µg/mL solutions. mg/mL. 1 mL quercetin of each concentration was added to the test tube containing 4 mL of distilled water. At the same time, 0.3 mL of 5% NaNO2 was added to the test tube and 0.3 mL of 10% AlCl3 after 5 min. Then, 2 mL of 1 M NaOH was added to the mixture after 6 min. The volume of the mixture was made 10 mL by immediately adding 4.4 mL of distilled water. The total flavonoids content was expressed as quercetin equivalents using the linear equation based on the calibration curve.

2.4.2. Preparation of Samples for Total Flavonoid Content
Stock solutions of 4 mg/mL concentration in methanol of the extracts were prepared, and they were diluted serially to make different concentrations (100, 200,300,400 and 500 µg/mL) solutions. Similar procedure as described for quercetin was followed for the extracts also, and the absorbance was measured by spectrophotometer at 510 nm. Readings were taken in triplicate, and the average value of absorbance was used to calculate the total flavonoid content. Thee flavonoid content was expressed as quercetin equivalent (mg QE/g) using the linear equation based on the standard calibration curve [14].

2.5 Antioxidant activity by DPPH method:

The DPPH (1, 1-Diphenyl-2-Picrylhydrazyl) Free Radical Scavenging Activity Assay was conducted using a modified method. A 0.1mM solution of DPPH was mixed with hydroalcoholic and petroleum ether extracts of *Trapa natans & Punica granatum* at different concentrations. The mixture was incubated at room temperature for 30 minutes in the dark, and absorbance was measured using a UV-Visible spectrophotometer. Ascorbic acid was used as the reference standard. Samples were prepared and measured in triplicates. The percentage of scavenging activity of each extract on DPPH radical was calculated using the equation I% = [(Ao-As)/Ao] × 100. The IC50 values of the extracts were calculated from the graph, and the IC50 represents the concentration of sample required to scavenge 50% of DPPH free radical. The IC50 of the extracts and standards were determined graphically, and the data were presented as mean values±standard deviation (n = 3) [15].

Antioxidant Activity by Ferric Reducing Antioxidant Power (FRAP) Assay: The Ferric Reducing Antioxidant Power (FRAP) assay measures a sample's antioxidant capacity by assessing its ability to reduce ferric ions to ferrous ions. This reduction forms an intense blue-colored complex with tripyridyltriazine (TPTZ), measured at 593 nm. 3 mL of hydro alcoholic and petroleum ether extracts of *Trapa natans & Punica granatum* were mixed with FRAP reagent solution. Samples were incubated at 37°C for 30 minutes, and absorbance was measured at 593 nm against a blank. Ascorbic acid was used as the standard, and the antioxidant activity was calculated as µmol Fe2+ equivalent. The % inhibition vs. concentration graph was plotted, and the IC_{50} was determined [15].

2.7 Antimicrobial Study

2.7.1 Agar Well Diffusion Assay for Antimicrobial Activity Evaluation
The Agar Well Diffusion method was used to evaluate the antimicrobial activity of various extracts. Nutrient agar plates

were prepared and inoculated with a microbial suspension. Wells (6 mm in diameter) were created in the center of the agar plate, and different concentrations of test extracts were introduced. The plates were incubated at 37°C for 24 hours for bacterial cultures and 28°C for 48 hours for fungal cultures. The zone of inhibition surrounding each well was measured in millimeters, and the antimicrobial activity was evaluated based on the size of the inhibition zone. The experiment was repeated thrice, with readings taken in three different directions [16].

Results: Percentage yield of extracts of *Trapa natans* & *Punica granatum*

The extraction efficiency of *Trapa natans* and *Punica granatum* was evaluated using two solvents: Petroleum Ether and Ethanol: Water (8:2). The results showed that the ethanol:water solvent system yielded 1.1% more for *Trapa natans* than the petroleum ether extract yield of 0.9%. In *Punica granatum*, the ethanol:water solvent system yielded 1.8% more than the petroleum ether yield of 1.2%. This indicates that solvent polarity plays a crucial role in maximizing extract yield, with ethanol:water being more effective for both plant materials. The percentage yield comparison of peel extracts of *Trapa natans* and *Punica granatum* using different solvents is provided in Table No.1 and Figure No.1.

% Yield= (Weight of Dry extract / Weight of Dry plant material) x 100%

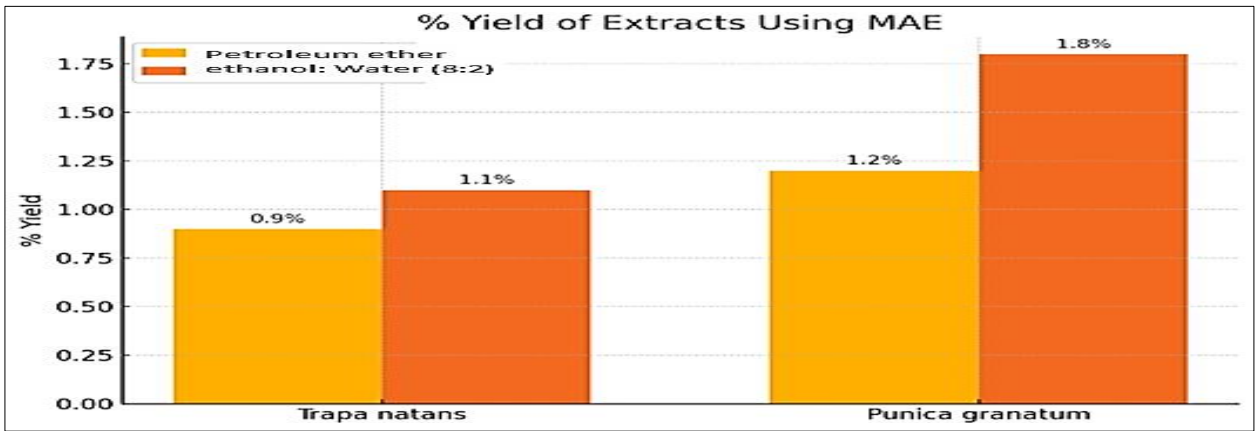

Fig 1: % yield of Pet ether & hydro alcohol extracts of *Trapa natans* and *Punica granatum*

Table 1: % yield of Pet ether & hydro alcohol extracts of *Trapa natans* and *Punica granatum*

Extraction Method (MAE)	Solvent-1 Petroleum Ether	Solvent-2 Ethanol:Water (8:1)
Trapa natans	0.9%	1.1%
Punica granatum	1.2%	1.8%

Total Phenolic Content (TPC): The study found that the Total Phenolic Content (TPC) of *Trapa natans* and *Punica granatum* extracts increased with concentration, indicating a dose-dependent polyphenol release. The hydroalcoholic extract of *P. granatum* showed the highest TPC at 500 µg/mL, followed by *T. natans*. Pet ether extracts showed lower TPC values across all concentrations. The results suggest that hydroalcoholic extraction is more efficient in recovering phenolic compounds than non-polar pet ether extraction, with observed standard deviations of approximately 6-9%. This confirms that hydroalcoholic extraction is more effective in recovering phenolic compounds. (Given in table no.2 and table no.3)

Table 2: Absorbance of Total Phenolic Content of Hydroalcoholic and Pet ether extract

Conc. (µg/mL)	Absorbance of Standard (Gallic Acid)	Pet Ether TN	Pet Ether PG	Hydroalcoholic TN	Hydroalcoholic PG
100	0.210	0.105±0.0063	0.125±0.0075	0.145±0.0087	0.155±0.0093
200	0.410	0.165±0.0102	0.195±0.0117	0.260±0.0156	0.280±0.0168
300	0.620	0.295±0.0177	0.325±0.0195	0.430±0.0258	0.450±0.0270
400	0.820	0.320±0.0192	0.410±0.0246	0.525±0.0315	0.560±0.0336
500	1.030	0.530±0.0318	0.560±0.0336	0.640±0.0384	0.660±0.0396

Table 3: Comparison of Total Phenolic Content (TPC) as Gallic Acid Equivalents (µg/mL) of two extracts at different concentration

Conc. (µg/mL)	TPC – Pet Ether TN	TPC – Pet Ether PG	TPC – Hydroalcoholic TN	TPC – Hydroalcoholic PG
100	48.78±3.25	58.54±3.80	68.29±4.10	73.17±4.40
200	78.05±4.70	92.68±5.30	124.39±7.80	134.15±8.10
300	141.46±7.60	156.10±8.10	207.32±11.80	217.07±12.40
400	153.66±8.00	197.56±9.90	253.66±14.10	270.73±14.80
500	256.10±13.00	270.73±13.50	309.76±16.80	319.51±17.20

Total Flavonoid Content (TFC)

The Total Flavonoid Content (TFC) of all extract types increases with concentration, with hydroalcoholic extracts (TN and PG) showing the highest TFC values. Hydroalcoholic PG has the highest TFC at 500 µg/mL, while Pet Ether TN has the lowest at lower concentrations. Hydroalcoholic solvents are more effective in extracting flavonoids than Pet Ether extracts, with increasing concentrations enhancing the flavonoid yield. (Given in table no.4 and table no.5)

Table 4: Absorbance of Total Flavonoid Content of Hydroalcoholic and Petroleum Ether extract

Concentration (µg/mL)	Absorbance of Standard(Quercetin)	Absorbance (Pet Ether)		Absorbance (Hydroalcoholic)	
		TN	PG	TN	PG
100	0.210	0.120±0.0072	0.130±0.0078	0.135±0.0081	0.135±0.0085
200	0.315	0.145±0.0080	0.135±0.0074	0.215±0.0129	0.230±0.0138
300	0.420	0.210±0.0121	0.215±0.0127	0.285±0.0171	0.295±0.0177
400	0.525	0.270±0.0162	0.285±0.0171	0.325±0.0195	0.345±0.0207
500	0.630	0.320±0.0072	0.330±0.0198	0.385±0.0231	0.415±0.0249

Table 5: Total Flavonoid Content (TFC) in µg/mL as Quercetin Equivalents (QE)

Conc.(µg/mL)	Pet Ether TN	Pet Ether PG	Hydroalcoholic TN	Hydroalcoholic PG
100	14.29±6.86	23.81±7.43	28.57±7.71	28.57±8.10
200	38.10±7.62	28.57±7.05	104.76±12.29	119.05±13.14
300	100.00±11.52	104.76±12.10	171.43±16.29	180.95±16.86
400	157.14±15.43	171.43±16.29	209.52±18.57	228.57±19.71
500	209.52±18.57	214.29±18.86	266.67±22.00	295.24±23.71

3.1 Correlation of TPC & TFC Data

The scatter plots show the correlation between Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) for both Pet Ether and Hydroalcoholic extracts, each for TN (test sample) and PG (plant group). The key observations include:

Hydroalcoholic Extracts (TN & PG) show a very strong positive correlation ($r \approx 0.99$ to 0.99), indicating that as the TPC increases, the TFC also increases proportionally.

Pet Ether PG displays a strong positive correlation ($r \approx 0.82$), while Pet Ether TN exhibits a moderate correlation ($r \approx 0.43$). This suggests that in Pet Ether TN, the relationship between TPC and TFC is less consistent or pronounced.

These correlations suggest that in most cases, higher phenolic content tends to coincide with higher flavonoid content, especially in hydroalcoholic extracts, where the correlation is the strongest.

The heatmap illustrates that Hydroalcoholic extracts (TN and PG) consistently show higher TPC and TFC values compared to Pet Ether extracts, indicating greater extraction efficiency. TPC values are consistently higher than TFC values across all concentrations. Both TPC and TFC increase with concentration, peaking at 500 µg/mL. The Pet Ether PG extract performs better than Pet Ether TN in extracting bioactive compounds. In summary, Hydroalcoholic solvents are more efficient in extracting phenolic and flavonoid compounds, and higher concentrations enhance the extraction of these bioactive compounds. (Given in figure no.2 and figure no.3)

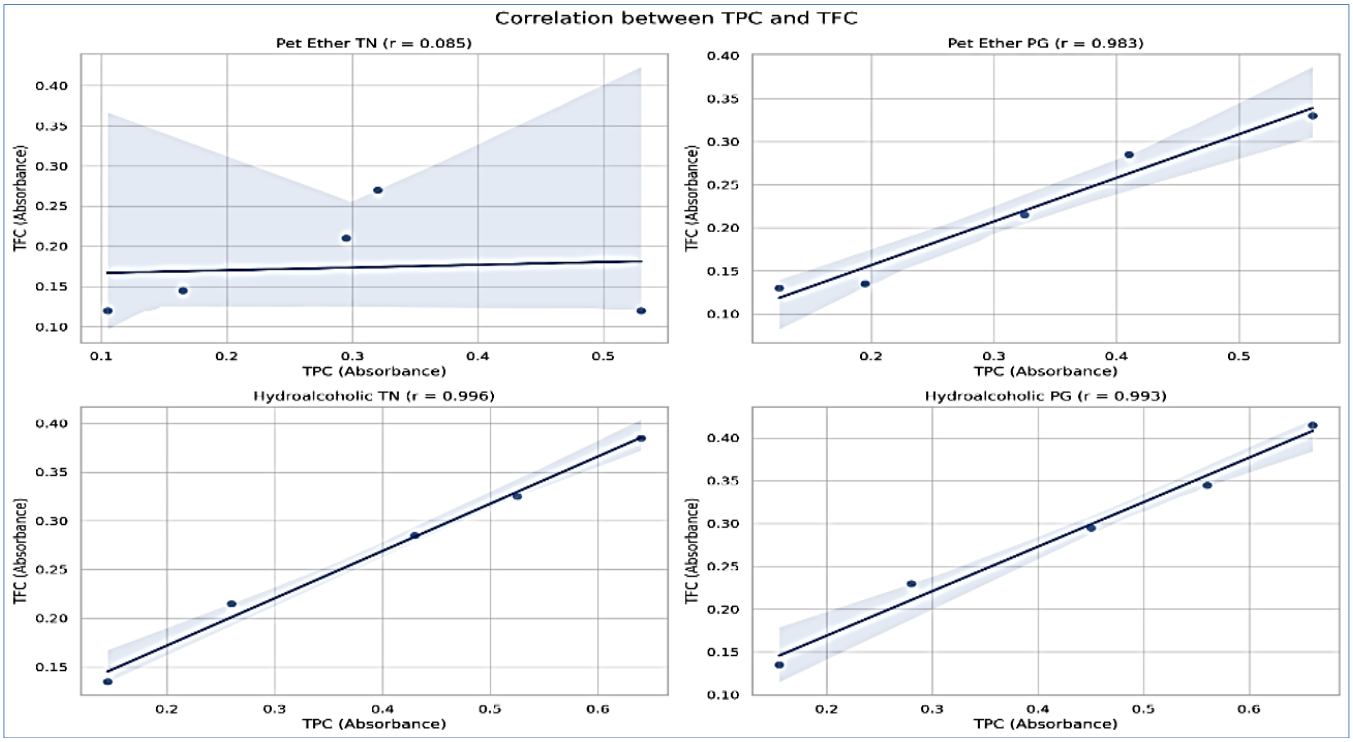

Fig 2: Correlation between Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) for both Pet Ether and Hydroalcoholic extracts

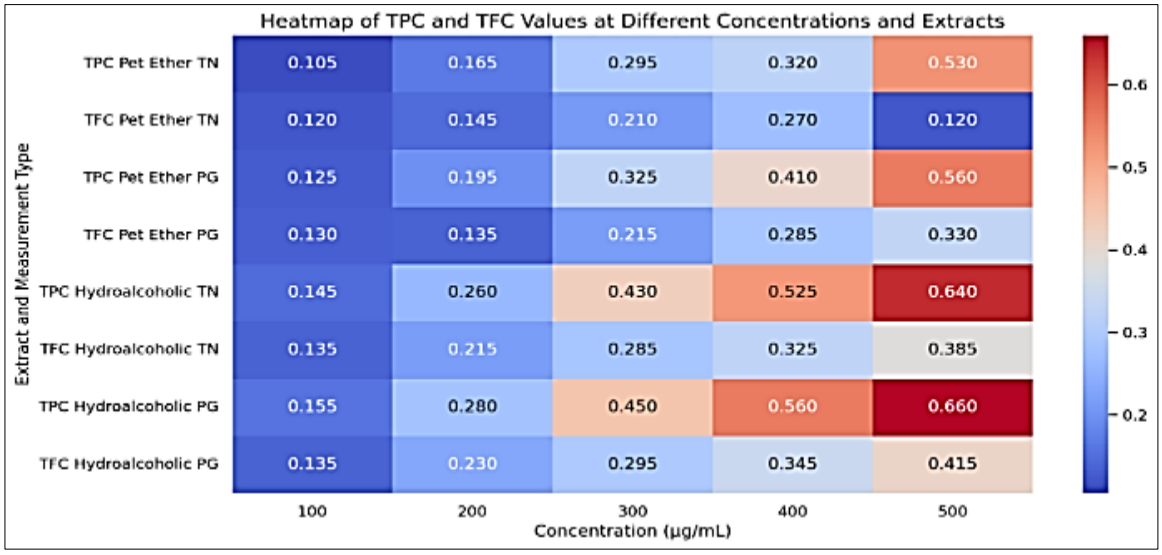

Fig 3: Heat map of TPC and TFC for the different extracts at different concentrations

3.5 DPPH Radical Scavenging Assay

The antioxidant activity of *Trapa natans* (TN) and *Punica granatum* (PG) extracts was assessed using the DPPH radical scavenging assay. Ascorbic acid showed a strong dose-dependent effect, with an IC_{50} of 175.00 µg/mL. Hydroalcoholic PG showed the highest antioxidant activity (70.83±3.06% at 400 µg/mL), indicating superior free radical scavenging capacity. Hydroalcoholic extracts showed significantly higher antioxidant potential than pet ether extracts, and PG extracts outperformed TN across both solvents. The results suggest that solvent polarity and plant species influence the efficiency of antioxidant compound extraction, with hydroalcoholic PG extract being the most potent among the tested samples. (Given in table no.6, table no.7 and figure no.4)

Table 6: Absorbance of DPPH Assay of different extracts at different concentrations

Conc. (µg/mL)	Ascorbic Acid	Control (Abs)	Pet Ether TN	Pet Ether PG	Hydroalcoholic TN	Hydroalcoholic PG
100	0.480	0.720	0.640±0.035	0.620±0.038	0.580±0.032	0.565±0.036
200	0.320	0.720	0.540±0.030	0.510±0.031	0.460±0.027	0.440±0.030
300	0.200	0.720	0.420±0.025	0.400±0.024	0.370±0.022	0.340±0.025
400	0.120	0.720	0.310±0.018	0.280±0.020	0.325±0.020	0.210±0.022

Table 7: % Inhibition (±SD) and IC_{50} Values – DPPH Assay

Concentration (µg/mL)	Ascorbic Acid	Pet Ether TN	Pet Ether PG	Hydroalcoholic TN	Hydroalcoholic PG
100	33.33%	11.11±4.86%	13.89±5.28%	19.44±4.44%	21.53±5.00%
200	55.56%	25.00±4.17%	29.17±4.31%	36.11±3.75%	38.89±4.17%
300	72.22%	41.67±3.47%	44.44±3.33%	48.61±3.06%	52.78±3.47%
400	83.33%	56.94±2.50%	61.11±2.78%	54.86±2.78%	70.83±3.06%
IC_{50} (µg/mL)	175.00	354.55	333.33	322.22	280.00

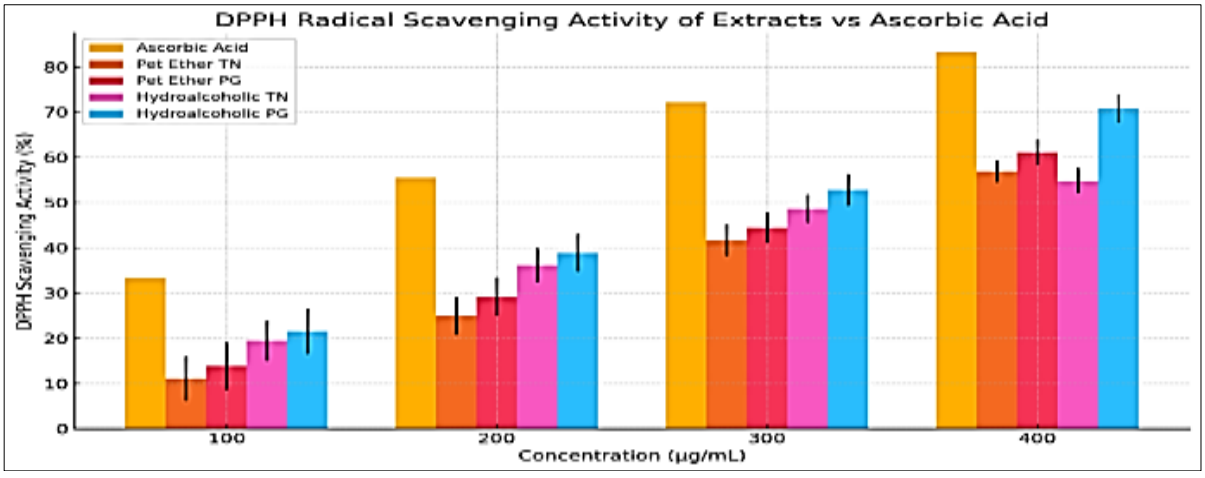

Fig 4: DPPH Radical Scavenging Activity of different extracts of TN & PG in response to standard Ascorbic acid

3.6 Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay assessed the ferric-reducing antioxidant power of *Trapa natans* and *Punica granatum* extracts, extracted using petroleum ether and hydroalcoholic solvents via Microwave-Assisted Extraction (MAE). Hydroalcoholic PG showed the highest reducing activity at 400 µg/mL, followed by Pet Ether TN and Hydroalcoholic TN. However, it had the lowest IC_{50}, indicating the strongest antioxidant potential. The results suggest that solvent type and plant source significantly influence antioxidant capacity. Hydroalcoholic PG extract demonstrated superior reducing power, indicating effective extraction of phenolic compounds. (Given in table no.8 and table no.9)

Table 8: Absorbance of different extracts of TN & PG at various concentrations with reference to stadndard Ascorbic acid by FRAP Assay

Concentration (µg/mL)	Absorbance of Standard Ascorbic Acid	Absorbance (Pet Ether)		Absorbance (Hydroalcoholic)	
		TN	PG	TN	PG
100	0.320	0.115±0.0069	0.125±0.0075	0.135±0.0081	0.160±0.0096
200	0.545	0.125±0.0075	0.155±0.0093	0.165±0.0099	0.195±0.0117
300	0.775	0.185±0.0111	0.210±0.0126	0.265±0.0159	0.280±0.0168
400	1.010	0.255±0.0153	0.295±0.0177	0.305±0.0183	0.335±0.0184

Table 9: % Inhibition (± SD) and IC_{50} Values – FRAP Assay

Concentration (µg/mL)	Pet Ether TN	Pet Ether PG	Hydroalcoholic TN	Hydroalcoholic PG
100	64.06±2.16%	50.00±3.00%	57.81±2.53%	60.94±2.34%
200	77.06±1.38%	64.22±2.15%	69.72±1.82%	71.56±1.71%
300	76.13±1.43%	63.87±2.17%	65.80±2.05%	72.90±1.63%
400	74.75±1.52%	66.83±1.99%	69.80±1.81%	70.79±1.75%
IC_{50} (µg/mL)	~275	~285	~295	~265

3.7 Comparison of antioxidant activity of different extracts measured by DPPH and FRAP assays:

- Hydroalcoholic PG consistently shows the highest antioxidant activity in both assays, with Ascorbic Acid as the benchmark.
- Pet Ether TN exhibits the lowest antioxidant activity across all concentrations in both assays, with Hydroalcoholic extracts outperforming Pet Ether extracts.
- Hydroalcoholic PG demonstrates the strongest DPPH scavenging activity (up to 70.83%) and the highest FRAP reduction (72.90%) at 400 µg/mL.
- Pet Ether TN shows the lowest DPPH (56.94%) and FRAP reduction (64.06%).

In conclusion, Hydroalcoholic PG is the most potent antioxidant extract, outperforming others in both radical scavenging and reducing power. (Given in figure no.5)

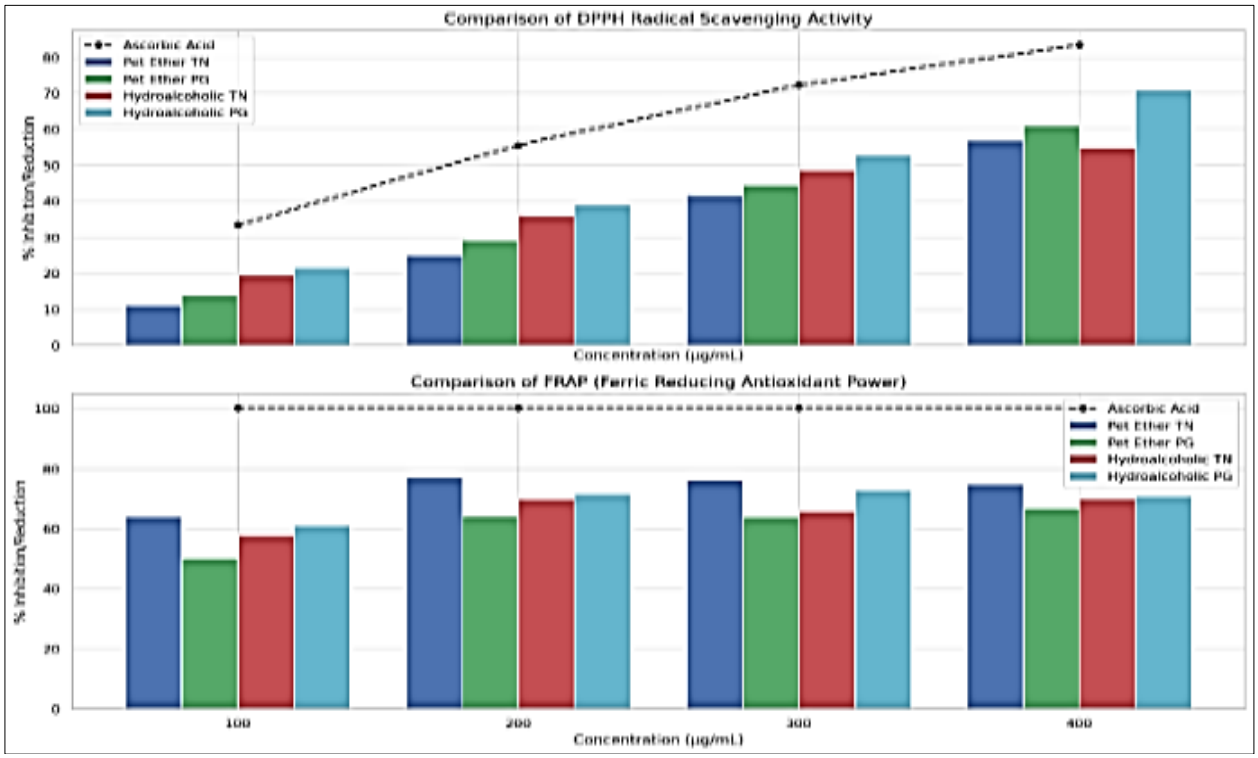

Fig 5: The bar charts highlight the antioxidant activity of different extracts measured by DPPH and FRAP assays

3.8 Antimicrobial Study: The study evaluated the antimicrobial activity of various extracts using zone of inhibition (ZDI) across concentrations of 100-400 µg/mL. The hydroalcoholic extract of *Punica granatum* (PG-MAE) showed the highest activity, with a maximum ZDI of 13.0 mm at 400 µg/mL and the lowest IC_{50} value of 175 µg/mL. *Trapa*

natans hydroalcoholic extract (TN-MAE) showed moderate activity, while pet ether extracts of both species showed weaker inhibition profiles. The standard antimicrobial agent (ZDISD) showed an IC_{50} of 125 µg/mL. (Given in table no.10 and figure no.6)

Table 10: Zone of inhibition with standard deviation (ZDISD) for TN-PE, TN-HA & PG-PE & PG-HA

Concentration (µg/mL)	Standard (ZDISD)	TN-MAE Pet Ether	TN-MAE Hydroalcoholic	PG-MAE Pet Ether	PG-MAE Hydroalcoholic
100	10.0±0.25	2.0±0.20	4.0±0.30	3.5±0.25	6.0±0.35
200	15.0±0.30	4.0±0.25	6.5±0.40	5.0±0.30	8.0±0.45
300	22.0±0.40	6.5±0.30	9.0±0.45	7.5±0.35	10.5±0.50
400	28.5±0.50	9.0±0.40	12.5±0.50	10.0±0.45	13.0±0.60
IC50	125	220	190	200	175

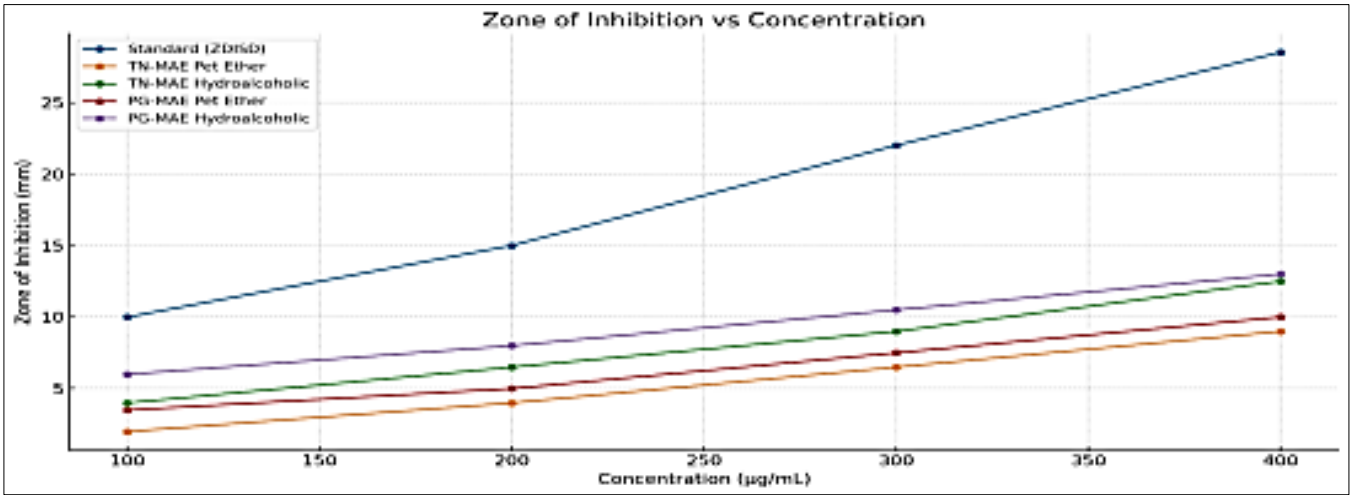

Fig 6: Antimicrobial Data of Zone of Inhibition vs Concentration for TN-PE, TN-HA & PG-PE & PG-HA

Discussion

The present study illustrates the comparative in-vitro antioxidant and antimicrobial properties of *Trapa natans* and *Punica granatum*. The investigation commenced with assessing the extraction efficiency of the two plant materials, *Trapa natans* and *Punica granatum*, employing two distinct solvent systems: Petroleum Ether and a combination of Ethanol and Water in an 8:2 ratio through Microwave assisted extraction methods. The findings indicated that the ethanol:water solvent system outperformed petroleum ether in terms of extract yield for both plants. Specifically, *Trapa natans* achieved a yield of 1.1% with the ethanol:water mixture compared to 0.9% with petroleum ether. Similarly, *Punica granatum* exhibited a yield increase, with the ethanol:water system yielding 1.8%, whereas petroleum ether provided a yield of 1.2%. These results underscore the significance of solvent polarity in optimizing extract yields, demonstrating that the ethanol:water combination is superior for extracting bioactive compounds from both *Trapa natans* and *Punica granatum*.

The study investigated the Total Phenolic Content (TPC) in extracts from *Trapa natans* and *Punica granatum*, revealing a direct correlation between concentration and polyphenol release, confirming a dose-dependent relationship. The hydroalcoholic extract of *P. granatum* achieved the highest TPC at a concentration of 500 µg/mL, surpassing that of T. natans. Conversely, pet ether extracts demonstrated significantly lower TPC values consistently across all tested concentrations. These findings highlight the superior efficiency of hydroalcoholic extraction in recovering phenolic compounds when compared to non-polar pet ether extraction, with reported standard deviations in the range of 6-9%. Thus, the study concludes that hydroalcoholic extraction is the preferred method for maximizing the recovery of phenolic compounds from these sources.

The study indicates that the Total Flavonoid Content (TFC) across all extract types exhibits an increasing trend with higher concentrations. Notably, hydroalcoholic extracts, specifically TN and PG, present the highest TFC values. The hydroalcoholic PG extract reaches its peak TFC at a concentration of 500 µg/mL. In contrast, the Pet Ether TN extract shows the lowest TFC at diminished concentrations. The findings suggest that hydroalcoholic solvents are more efficient in flavonoid extraction compared to Pet Ether extracts, signifying that as concentration rises, the yield of flavonoids also increases, underscoring the effectiveness of hydroalcoholic mediums in this context.

According to the data on the correlation between Total Phenolic Content (TPC) and Total Flavonoid Content (TFC), hydroalcoholic extracts from *Trapa natans* and *Punica granatum* exhibit an exceptionally strong positive correlation, indicated by a correlation coefficient (r) ranging from approximately 0.99 to 0.99. This suggests that as the TPC increases, there is a nearly equivalent increase in TFC. In contrast, the Pet Ether extract from PG shows a strong positive correlation ($r \approx 0.82$), while the Pet Ether extract from TN presents a moderate correlation ($r \approx 0.43$), indicating a less consistent relationship between TPC and TFC. Consequently, it can be inferred that in most instances, higher levels of phenolic compounds are associated with higher levels of flavonoids, particularly within the hydroalcoholic extracts where their correlation is maximally pronounced.

The heatmap demonstrates that Hydroalcoholic extracts, specifically from TN and PG, exhibit consistently higher values of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) compared to Pet Ether extracts, suggesting

superior extraction efficiency. It is observed that TPC values surpass TFC values across all concentration levels. Furthermore, both TPC and TFC exhibit an upward trend as concentration increases, reaching their maximum potential at a concentration of 500 µg/mL. Notably, the Pet Ether PG extract outperforms the Pet Ether TN extract in the extraction of bioactive compounds. In conclusion, Hydroalcoholic solvents are identified as more effective for extracting phenolic and flavonoid compounds, and increasing concentrations further enhance the yield of these bioactive compounds.

The study evaluated the antioxidant activity of extracts from *Trapa natans* (TN) and *Punica granatum* (PG) using the DPPH radical scavenging assay. Results revealed that ascorbic acid exhibited a strong dose-dependent effect, achieving an IC_{50} of 175.00 µg/mL. Notably, the hydroalcoholic extract of PG demonstrated the highest antioxidant activity with a scavenging percentage of 70.83±3.06% at a concentration of 400 µg/mL, indicating its superior capacity to neutralize free radicals. Comparatively, hydroalcoholic extracts were found to possess significantly greater antioxidant potential than pet ether extracts. Furthermore, PG extracts consistently outperformed those from TN across both tested solvents. The findings highlight the importance of solvent polarity and plant species in determining the extraction efficiency of antioxidant compounds, with the hydroalcoholic PG extract identified as the most potent among the samples tested.

The FRAP assay evaluated the ferric-reducing antioxidant power of extracts from *Trapa natans* and *Punica granatum*, which were obtained using petroleum ether and hydroalcoholic solvents through Microwave-Assisted Extraction (MAE). Among the extracts tested, the hydroalcoholic extract of *Punica granatum* (PG) exhibited the highest reducing activity at a concentration of 400 µg/mL. This was followed by the petroleum ether extract of *Trapa natans* (TN) and the hydroalcoholic extract of TN. Notably, the hydroalcoholic PG extract displayed the lowest IC_{50} value, signaling its strong antioxidant potential. The findings highlight that both the type of solvent and the source of the plant play a crucial role in determining the antioxidant capacity of the extracts. The superior reducing power attributed to hydroalcoholic PG suggests that it effectively extracts phenolic compounds, which are likely responsible for its antioxidant properties.

Comparison of the antioxidant activity of various extracts using DPPH and FRAP assays indicated that Hydroalcoholic PG consistently demonstrated the highest antioxidant activity in both assays, establishing Ascorbic Acid as the benchmark for comparison. In contrast, Pet Ether TN consistently exhibited the lowest antioxidant activity across all concentrations tested in both assays, with Hydroalcoholic extracts outperforming those derived from Pet Ether. Specifically, Hydroalcoholic PG exhibited the most significant DPPH scavenging activity, reaching up to 70.83%, along with a notable FRAP reduction capability of 72.90% at a concentration of 400 µg/mL. Conversely, Pet Ether TN registered the lowest figures with a DPPH scavenging capacity of 56.94% and a FRAP reduction of 64.06%. In conclusion, Hydroalcoholic PG stands out as the most potent antioxidant extract, excelling in both radical scavenging and reducing power compared to the other extracts analyzed.

The study investigated the antimicrobial properties of various extracts by measuring their zone of inhibition (ZDI) at concentrations ranging from 100 to 400 µg/mL. Among the tested extracts, the hydroalcoholic extract of *Punica granatum*, designated as PG-MAE, exhibited the most significant antimicrobial activity, achieving a maximum ZDI of 13.0 mm at a concentration of 400 µg/mL, accompanied by the lowest half maximal inhibitory concentration (IC_{50}) of 175 µg/mL. In contrast, the hydroalcoholic extract of *Trapa natans*, referred to as TN-MAE, demonstrated moderate antimicrobial activity. Furthermore, pet ether extracts from both *Punica granatum* and *Trapa natans* indicated weaker inhibition profiles when compared to the hydroalcoholic extracts. Additionally, the standard antimicrobial agent (ZDISD) yielded an IC_{50} value of 125 µg/mL, serving as a benchmark for evaluating the efficacy of the tested plant extracts.

Conclusion

In this research work Hydroalcoholic (8:2) and Petroleum Ether extracts of *Trapa natans* & *Punica granatum* were subjected to TPC, TFC, DPPH and FRAP Assay, while antimicrobial activity was assessed against selected Gram-positive and Gram-negative bacterial strains using the agar well diffusion method. Both *Trapa natans* and *Punica granatum* extracts exhibited notable antioxidant activity, with *Punica granatum* showing slightly higher radical scavenging efficiency in the hydroalcoholic extract with the MAE procedure. In terms of antimicrobial potential, *Punica granatum* demonstrated a broader spectrum of activity and higher zones of inhibition when compared to *Trapa natans*. Ultimately, the findings will contribute to the growing body of evidence supporting plant-based antioxidants and antimicrobials, paving the way for further exploration of their active compounds and therapeutic benefits. These findings highlight the potential of both plants as natural sources of therapeutic agents. *Punica granatum* may be more effective as a natural antioxidant supplement, whereas *Trapa natans* could serve as a promising antimicrobial agent. Further research, including phytochemical isolation and in vivo studies, is warranted to better understand the mechanisms of action and to develop effective formulations for pharmaceutical or nutraceutical applications.

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