

Phytochemical Screening And Evaluation Of Antioxidant Activity Of Different Solvent Extracts Of Parmotrema Perlatum

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ABSTRACT

The present study aimed to evaluate the phytochemical constituents and antioxidant potential of different solvent extracts of Parmotrema perlatum. The powdered lichen material was successively extracted using hexane, chloroform, ethyl acetate, ethanol, and water, and the percentage yield was determined. Preliminary phytochemical screening revealed the presence of flavonoids in all extracts, while phenolic compounds were predominantly detected in ethanolic and aqueous extracts. Thin Layer Chromatography (TLC) analysis confirmed the presence of flavonoids and phenolic constituents by comparing R_f values with standard quercetin and gallic acid. Quantitative estimation demonstrated that the ethanolic extract possessed the highest total phenolic and flavonoid content. The antioxidant activity was evaluated using DPPH, nitric oxide, and hydrogen peroxide scavenging assays, with ascorbic acid used as the standard. The ethanolic extract exhibited concentration-dependent radical scavenging activity in all assays. Although its DPPH and nitric oxide scavenging activities were moderate compared to ascorbic acid, it showed comparatively strong hydrogen peroxide scavenging activity. The findings suggest that the antioxidant potential of Parmotrema perlatum is attributed to its rich phenolic and flavonoid content, highlighting its potential as a natural source of antioxidant compounds for pharmaceutical applications.

Keywords: Parmotrema perlatum, Phytochemical screening, Thin Layer Chromatography (TLC), Total phenolic content, Total flavonoid content, DPPH assay, Nitric oxide scavenging, Hydrogen peroxide scavenging, Antioxidant activity

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INTRODUCTION

Natural products have long served as an important source of therapeutic agents, particularly due to their rich diversity of bioactive secondary metabolites (Twajj and Hasan; 2022).

In recent years, there has been increasing interest in plant- and lichen-derived compounds because of their potential antioxidant, anti-inflammatory, antimicrobial, and anticancer properties (Tripathi et al., 2022). Oxidative stress, caused by an imbalance between free radical generation and antioxidant defense mechanisms, plays a significant role in the pathogenesis of various chronic diseases, including cancer, cardiovascular disorders, diabetes, and neurodegenerative conditions (Sharma et al., 2012). Therefore, the search for natural antioxidants has become an important area of pharmaceutical and biomedical research.

Lichens are symbiotic associations between fungi and algae or cyanobacteria and are known to produce unique secondary metabolites with diverse biological activities. *Parmotrema perlatum*, commonly known as black stone flower, is a widely distributed lichen species traditionally used in food and folk medicine (Sheikh and Dutta; 2025).

It is reported to contain various bioactive compounds such as flavonoids, phenolic compounds, tannins, and terpenoids, which are known for their antioxidant potential. However, systematic scientific evaluation of its

phytochemical profile and antioxidant activity using different solvent extracts remains essential to validate its therapeutic value (Saha et al., 2021).

Extraction using solvents of varying polarity plays an essential role in isolating different classes of phytoconstituents. Furthermore, qualitative and quantitative phytochemical screening, along with chromatographic techniques such as Thin Layer Chromatography (TLC), helps in the identification and characterization of bioactive compounds. In vitro antioxidant assays such as DPPH, nitric oxide, and hydrogen peroxide scavenging methods are widely employed to assess free radical scavenging capacity.

Therefore, the present study was designed to investigate the phytochemical constituents and evaluate the in vitro antioxidant activity of different solvent extracts of *Parmotrema perlatum*, thereby exploring its potential as a natural source of antioxidant agents for pharmaceutical applications.

MATERIAL AND METHODS

Material

The present investigation utilized dried and powdered thalli of *Parmotrema perlatum* for extraction. Analytical grade solvents such as hexane, chloroform, ethyl acetate, ethanol, and distilled water were used for successive extraction. Chemicals including DPPH, sodium nitroprusside, hydrogen peroxide, sulphanylamine,

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naphthylethylenediamine dihydrochloride, ferric chloride, and other reagents were employed for phytochemical and antioxidant studies. Standard compounds such as ascorbic acid, quercetin, and gallic acid were used as references. Silica gel TLC plates and a UV-Visible spectrophotometer were used for phytochemical identification and absorbance measurements. All reagents were of analytical grade.

Methods

Extraction using maceration method

Defatting of plant materials

50 gram of flowers of *Parmotrema perlatum* shade dried plant material were coarsely powdered and subjected to extraction with Hexane by maceration method. The extraction was continued till the defatting of the material had taken place.

Extraction by maceration process

Defatted flower of *Parmotrema perlatum* were extracted with chloroform, ethyl acetate, ethanol and water solvents by maceration method (Mukherjee, 2007). Each extraction process was carried out for 24 hours. The filtrate was separated from the residue using Whatmann filter paper. The filtrate from each solvent was collected and evaporated using a water bath at 50°C until a thick extract was obtained. The percentage yields were calculated of the dried extracts.

Determination of percentage yield

The extraction yield is evaluate of the solvent's efficiency to extracts bioactive components from the selected natural plant samples and it was defined as quantity of plant extracts recovered in mass after solvent extraction compared with the initial quantity of plant samples. After extraction, yield of the plant extracts obtained were calculated in grams and then converted it into percentage (Khandelwal; 2005, Kokate; 1994). Percentage yield measures the effectiveness of the entire extraction process. % yield is calculated using the formula below:

$$\text{Percentage Yield} = \frac{\text{Weight of Extract}}{\text{Weight of Powder drug taken}} \times 100$$

Qualitative phytochemical screening

Qualitative phytochemical screening is carried out to investigate the various classes of natural compounds present in the extract. This is accomplished using standard methods (Tiwari *et al.*, 2011). The classes of compounds identified in the extract included phenolics, flavonoids, tannins, saponins, alkaloids and protein.

Separation and Identification of phytoconstituents by TLC

The chromatographic development was carried out in a twin-trough chamber using different solvent systems. For the detection of flavonoids, a mobile phase consisting of toluene: ethyl acetate: formic acid (5:4:1) was used, while for phenols, the solvent system toluene: ethyl acetate: formic acid (7:5:1) was employed (Patel *et al.*, 2017). The TLC chamber was pre-saturated with the mobile phase for 20 minutes prior to development. After development, the retention factor (R_f) values of the separated compounds were calculated. The developed chromatographic plates were observed under visible light, short-wave UV light (254

nm), and long-wave UV light (365 nm) using a TLC visualization cabinet. Once the chromatogram was developed the R_f Value of the spot was calculated using the formula:

$$R_f = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

Quantitative estimation of Phytochemical in plant extracts

10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10-50µg/ml was prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this solution was used for the estimation of phenol. 2 ml of each extract or standard was mixed with 1 ml of folin-ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15 min for colour development. The absorbance was measured at 765 nm using a spectrophotometer (Gaur Mishra *et al.*, 2017).

Estimation of total flavonoids content

Preparation of standard solution 10mg quercetin was weighed and made up to 10ml with Methanol in a 10ml volumetric flask. From the above solution (1mg/ml), 1ml was pipetted out and made up to 10ml with methanol to get 100µg/ml. Quercetin standard solution (stock solution). From the stock solution, solutions of concentration 5, 10, 15, 20 and 25 µg/ml were prepared. 3 ml of each standard and test was mixed with 1 ml of 2% Aluminium chloride solution. The solutions were mixed well and the absorbance was measured against the blank at 420nm using UV-Visible spectrophotometer. A standard graph was plotted using various concentrations of Quercetin and their corresponding absorbance (Gaur Mishra *et al.*, 2017).

In-vitro antioxidant activity using different methods

DPPH method

The free radical scavenging activity of the extract of *Parmotrema perlatum* was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay method as described by Parkhe and Jain (2018). A stock solution of DPPH was prepared by dissolving 6 mg of DPPH in 100 mL of methanol to obtain a deep violet solution, which was stored in a dark container to prevent photodegradation. Ascorbic acid was used as the reference standard, and a stock solution (1 mg/mL) was prepared in methanol, followed by serial dilutions to obtain concentrations of 10–100 µg/mL. Similarly, the dried extract of *Parmotrema perlatum* was dissolved in methanol to prepare a 1 mg/mL stock solution, and further dilutions (10–100 µg/mL) were made. For the assay, 1.5 mL of DPPH solution was mixed with 1.5 mL of either standard or sample solution and incubated in the dark at room temperature for 30 minutes. The absorbance was measured at 517 nm using a UV-Visible spectrophotometer, with methanol as blank. A control was prepared by mixing DPPH solution with methanol, and its absorbance was used as reference. The percentage of DPPH radical scavenging activity was calculated using the formula:

$$\% \text{ Inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Where:

A_{Control} = Absorbance of control

A_{Sample} = Absorbance of test or standard

The IC_{50} value (concentration required to inhibit 50% of DPPH radicals) was calculated from the graph plotted between % inhibition and concentration.

Nitric oxide method

The nitric oxide scavenging activity of the extract was evaluated according to the method described by Marcocci et al. (1994), based on the principle that nitric oxide scavengers compete with oxygen, thereby reducing nitric oxide generation. Sodium nitroprusside (10 mM), prepared in phosphate buffer saline (PBS, pH 7.4), was used as a nitric oxide donor, which spontaneously generates nitric oxide under physiological conditions. Various concentrations of the extract and standard (ascorbic acid) ranging from 20, 40, 60, 80, and 100 $\mu\text{g/mL}$ were mixed with sodium nitroprusside solution in PBS and incubated at 25°C for 150 minutes. After incubation, freshly prepared Griess reagent, consisting of 1% sulphanilamide in 2% phosphoric acid (H_3PO_4) and 0.1% naphthylethylenediamine dihydrochloride, was added to the reaction mixture. The nitric oxide generated during incubation reacts with oxygen to form nitrite ions, which undergo diazotization with sulphanilamide followed by coupling with naphthylethylenediamine to produce a pink-colored chromophore. The absorbance of the resulting solution was measured at 546 nm using a UV-Visible spectrophotometer. A control was prepared without extract, and ascorbic acid treated similarly served as the positive control. The percentage inhibition of nitric oxide radicals was calculated using the formula:

$$\% \text{ Inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Where A_{control} is the absorption (without extract) of the control and where A_{test} is the absorption in the presence of the extract / standard.

Hydrogen peroxide method

The nitric oxide scavenging activity of the extract was evaluated according to the method described by Marcocci et al. (1994), based on the principle that nitric oxide scavengers compete with oxygen, thereby reducing nitric oxide generation. Sodium nitroprusside (10 mM), prepared in phosphate buffer saline (PBS, pH 7.4), was used as a nitric oxide donor, which spontaneously generates nitric oxide under physiological conditions. Various concentrations of the extract and standard (ascorbic acid) ranging from 20, 40, 60, 80, and 100 $\mu\text{g/mL}$ were mixed with sodium nitroprusside solution in PBS and incubated at 25°C for 150 minutes.

After incubation, freshly prepared Griess reagent, consisting of 1% sulphanilamide in 2% phosphoric acid (H_3PO_4) and 0.1% naphthylethylenediamine dihydrochloride, was added to the reaction mixture. The

nitric oxide generated during incubation reacts with oxygen to form nitrite ions, which undergo diazotization with sulphanilamide followed by coupling with naphthylethylenediamine to produce a pink-colored chromophore. The absorbance of the resulting solution was measured at 546 nm using a UV-Visible spectrophotometer. A control was prepared without extract, and ascorbic acid treated similarly served as the positive control. The percentage inhibition of nitric oxide radicals was calculated using the formula:

$$\% \text{ Inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

RESULTS AND DISCUSSION

The extraction yield results (Table 1) indicate that the aqueous extract (6.78%) exhibited the highest percentage yield, followed by the ethanolic extract (3.52%), suggesting that polar solvents are more efficient in extracting phytoconstituents from *Parmotrema perlatum*. In contrast, hexane (0.25%) and chloroform (0.66%) extracts showed lower yields, indicating a comparatively smaller proportion of non-polar constituents.

Preliminary phytochemical screening results (Table 2) revealed the presence of flavonoids in all extracts, while phenolic compounds were mainly detected in the ethanolic and aqueous extracts. Diterpenes were observed in chloroform, ethyl acetate, ethanolic, and aqueous extracts, whereas saponins were found only in the aqueous extract. Alkaloids, proteins, and sterols were absent in all extracts. These findings suggest that polar extracts are richer in secondary metabolites responsible for antioxidant activity. Thin Layer Chromatography (TLC) profiling of flavonoids (Table 3, Figure 1) showed multiple spots in the ethanolic and chloroform extracts under UV light, indicating the presence of diverse phytoconstituents. The R_f values of certain spots were comparable to the standard quercetin ($R_f \approx 0.6$), suggesting the presence of similar flavonoid compounds. Likewise, TLC analysis for phenolic compounds (Table 4, Figure 2) demonstrated that the ethanolic extract showed spots with R_f values close to standard gallic acid ($R_f \approx 0.30$), confirming the presence of phenolic constituents.

Quantitative estimation of total phenolic and flavonoid content (Table 5) showed that the ethanolic extract contained the highest phenolic content (1.20 mg/100 mg) and flavonoid content (1.57 mg/100 mg), followed by the aqueous extract. These findings correlate well with the qualitative phytochemical screening and TLC results.

In antioxidant studies, the ethanolic extract exhibited concentration-dependent radical scavenging activity. In the DPPH assay (Table 6), the ethanolic extract showed moderate antioxidant activity with an IC_{50} value of 108.19 $\mu\text{g/mL}$ compared to ascorbic acid (22.33 $\mu\text{g/mL}$). Similarly, in the nitric oxide scavenging assay (Table 7), the extract demonstrated an IC_{50} value of 93.20 $\mu\text{g/mL}$, which was lower in potency than ascorbic acid (21.87 $\mu\text{g/mL}$). However, in the hydrogen peroxide scavenging assay (Table 8), the ethanolic extract exhibited comparatively

Phytochemical Screening And Evaluation Of Antioxidant Activity Of Different Solvent Extracts Of *Parmotrema perlatum*

strong activity with an IC₅₀ value of 13.47 µg/mL, approaching that of the standard ascorbic acid (19.02 µg/mL)

Table 1: % Yield of Flower extract of *Parmotrema perlatum*

Sr. No	Extracts	% Yield (w/w)
1.	Hexane	0.25%
2.	Chloroform	0.66%
3.	Ethyl acetate	1.20%
4.	Ethanollic	3.52%
5.	Aqueous	6.78%

Table 2: Result of phytochemical screening of hexane extract of *Parmotrema perlatum*

S. No.	Constituents	Hexane extract	Chloroform extract	Ethyl acetate extract	Ethanollic extract	Aqueous extract
1.	Alkaloids Wagner's Test: Hager's Test:	-ve -ve	-ve -ve	-ve -ve	-ve -ve	-ve -ve
2.	Glycosides Conc. H ₂ SO ₄ Test:	-ve	-ve	-ve	-ve	-ve
3.	Flavonoids Lead acetate Test: Alkaline test:	+ve +ve	+ve +ve	+ve +ve	+ve +ve	+ve +ve
4.	Diterpenes Copper acetate Test:	-ve	+ve	+ve	+ve	+ve
5.	Phenol Ferric Chloride Test: Folin Ciocalteu Test:	-ve -ve	-ve -ve	-ve -ve	-ve +ve	-ve +ve
6.	Proteins Xanthoproteic Test:	-ve	-ve	-ve	-ve	-ve
7.	Carbohydrate Fehling's Test: Benedict's Test	-ve -ve	+ve -ve	-ve -ve	-ve -ve	-ve -ve
8.	Saponins Froth Test:	-ve	-ve	-ve	-ve	+ve
9.	Tannins Gelatin test:	-ve	-ve	+ve	-ve	-ve
10.	Sterols Salkowski Test:	-ve	-ve	-ve	-ve	-ve

+Ve = Positive, -Ve= Negative

Table 3: Identification of phytoconstituents by TLC of *Parmotrema perlatum* (Flavonoids)

S. No.	Standard/ Extract	Solute distance	R _f value
1.	(Quercetin) Dis. travel by mobile phase= 5cm No. of spot at normal light= 1 No. of spot at short UV = 1 No. of spot at long UV= 1	=3 =3 =3	=0.6 =0.6 =0.6

Phytochemical Screening And Evaluation Of Antioxidant Activity Of Different Solvent Extracts Of Parmotrema Perlatum

2.	(Hexane extract) No. of spot at normal light= 2 No. of spot at short UV = 4 No. of spot at long UV= 5	=4.5,4.6 =3.8, 4, 4.5, 4.9 =3.2, 3.4, 4.1, 4.5, 4.7	=0.9,0.92 =0.76,0.8,0.9,0.98 =0.64,0.68,0.82,0.9,0.94
3.	(Chloroform extract) No. of spot at normal light= 2 No. of spot at short UV = 8 No. of spot at long UV= 6	=3.1,4.5 =2.5,2.9,3.3,3.5,4, 4.5,4.8 =3.1,3.3,4.1,4.5,4.7	=0.62,0.9 =0.5,0.58,0.66,0.7,0.8,0.9,0.96 =0.62,0.66,0.8,0.82,0.9,0.94
4.	(Ethyl acetate extract) No. of spot at normal light= 0 No. of spot at short UV = 5 No. of spot at long UV= 4	=0 =2.7,3.4,3.6,4,4.5 =3.3,4.2,4.5,4.8	=0 =0.54,0.68,0.2,0.8,0.9 =0.66,0.84,0.9,0.96
5.	(Ethanol extract) No. of spot at normal light= 1 No. of spot at short UV = 7 No. of spot at long UV= 6	=3.2 =2.7,3,3.2,3.4,3.8,4.1,4.6 =2.9,3.2,4.2,4.5,4.7,4.8	=0.64 =0.54,0.6,0.64,0.68,0.76,0.82,0.92 =0.58,0.64,0.84,0.9,0.94,0.96
6.	(Aqueous extract) No. of spot at normal light= 0 No. of spot at short UV = 0 No. of spot at long UV= 0	=0 =0 =0	=0 =0 =0
Spot Sequence			
Quercetin			1 st
Hexane extract			2 nd
Chloroform extract			3 rd
Ethyl acetate extract			4 th
Ethanolic extract			5 th
Aqueous extract			6 th

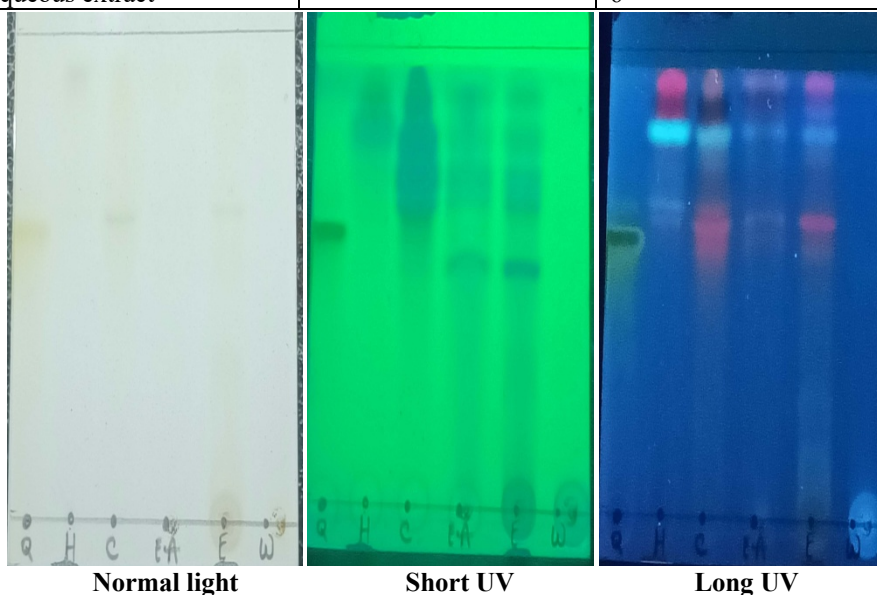


Figure 1: TLC of *Parmotrema perlatum* extracts

Table 4: Identification of phytoconstituents by TLC of *Parmotrema perlatum* (Phenol)

S. No.	Standard/ Extract	Solute distance	R _f value
1.	(Gallic acid) Dis. travel by mobile phase= 5cm No. of spot at normal light= 0 No. of spot at short UV = 1 No. of spot at long UV= 1	=1.5 =1.5 =1.5	=0.30 =0.30 =0.30
2.	(Ethanol extract) No. of spot at normal light= 1 No. of spot at short UV = 5 No. of spot at long UV= 3	=2.6 =1.8, 2.8,3.2, 3.9, 4.5 =3.9, 4.1, 4.5	=0.52 =0.36,0.56, 0.64, 0.78, 0.9 =0.78, 0.82, 0.90
3.	(Aqueous extract) No. of spot at normal light= 0 No. of spot at short UV = 1 No. of spot at long UV= 1	=0 =1.8 =1.8	=0 =0.36 =0.36
	Spot Sequence		
	Gallic acid		1 st
	Ethanol extract		2 nd
	Aqueous extract		3 rd

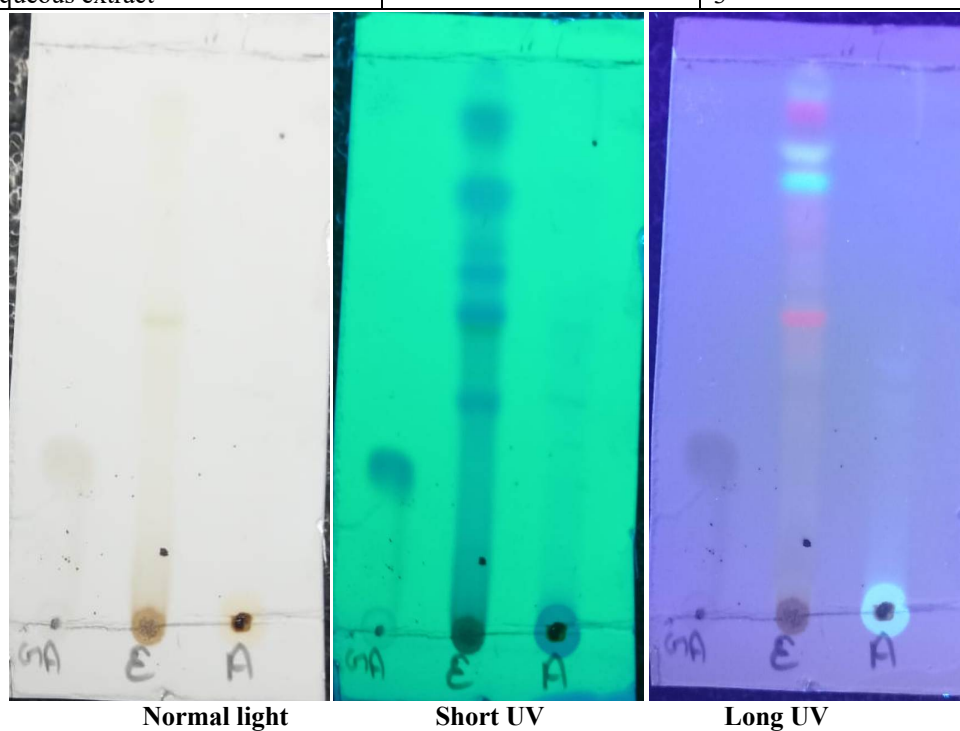


Figure 2: TLC of *Parmotrema perlatum* extracts

Table 5: Estimation of total phenol and flavonoids content of *Parmotrema perlatum*

S. No.	Extracts	Total phenol content (mg/ 100 mg of dried extract)	Total flavonoids content
1	Hexane	-	0.17
2	Chloroform	-	0.21
3	Ethyl acetate	-	0.35
4	Ethanolic	1.20	1.57
5	Aqueous	1.15	0.58

Table 6: % Inhibition of ascorbic acid and extract of *Parmotrema perlatum* using DPPH method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Ethanollic extract
1	10	41.53	31.23
2	20	47.34	32.45
3	40	62.35	35.96
4	60	72.52	40.07
5	80	80.39	43.46
6	100	88.14	49.64
IC₅₀ value		22.33	108.19

Table 7: % Inhibition of ascorbic acid and extract of *Parmotrema perlatum* using NO method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Ethanollic extract
1	20	47.72	7.82
2	40	58.84	17.29
3	60	61.54	34.87
4	80	69.37	44.05
5	100	75.45	51.49
IC₅₀ value		21.87	93.20

Table 8: % Inhibition of ascorbic acid and extract of *Parmotrema perlatum* using hydrogen peroxide method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Ethanollic extract
1	50	57.03	31.53
2	100	60.42	42.96
3	150	66.98	54.92
4	200	72.80	62.53
5	250	86.66	70.26
IC₅₀ value		19.02	13.47

CONCLUSION

The present study concludes that *Parmotrema perlatum* possesses significant phytochemical constituents, particularly flavonoids and phenolic compounds, which contribute to its antioxidant potential. Among the various extracts, the ethanolic extract showed the highest phenolic and flavonoid content and exhibited notable free radical scavenging activity in DPPH, nitric oxide, and hydrogen peroxide assays. These findings suggest that *Parmotrema perlatum* can be considered a promising natural source of antioxidant agents for potential pharmaceutical applications.

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