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Original Research Article

# Phytochemical composition and *in vitro* antioxidant activity of methanolic and aqueous extracts of aerial part of *Pentatropis nivalis* (Asclepiadaceae)

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**Abstract**

The objective of present study was to evaluate preliminary phytoconstitutents and *in vitro* antioxidant potential of *Pentatropis nivalis* (Asclepiadaceae). During the preliminary phytochemical analysis, methanolic and aqueous extracts of aerial part of *P. nivalis* was screened for the presence of phenolic, saponins, flavonoids, alkaloids, tannins and phytosterols. TLC of extracts were performed by using various solvent systems. Phytochemicals screening and TLC spots of MEPN and AEPN showed the presence of glycoside, steroids, terpenoids, phenolic, saponins, The qualitative tests showed that presence of secondary metabolites are more in methanolic extract than aqueous extract. The intro antioxidant potential of extracts were evaluated by DPPH and FRAP, and both the methods showed that the plant possesses good antioxidant activity.

**Keywords:** Pentatropis nivalis; Thin Layer Chromatography; Antioxidant activity; DPPH; FRAP

## Introduction

The reactive oxygen species (ROS) are the free radical with unpaired electron. During cellular metabolism (oxidation) free radical are generated some example includes superoxide anion, hydrogen peroxide, hydroxyl and nitric oxide radical. These radical are highly unstable and highly reactive in nature as they are electrically charged. They react with nucleic acid, mitochondria and enzymes which resulted cellular damage. To protect cellular damage, antioxidant defense system plays an important role. When there is imbalance between overproduction of free radical and failure of antioxidant defense system, which results in various disorder like cancer, Alzheimer's diseases, MI, Diabetes, Parkinson's diseases [1–6].

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Natural antioxidants are the chemical agents which have the ability to quench these hazardous free radicals and to neutralize their potential to attack the cells. Herbs are known to elicit antioxidant activities due to the presence of various phytoconstitutents like terpenoids, glycosides, lycopene, β-carotene, α-carotene,lutein, polyphenols, catechins, isoflavone and many other secondary metabolites [7].

The *P. nivalis* belonging to the family Asclepiadaceae. In folklore medicine it is has been documented that *P. nivalis* is used for curing inflammation, leucoderma, piles, biliousness (Ayurveda), gonorrhea, emetic, kapha [8] keeping this view the present study was carried out Phytochemical composition and *in vitro* antioxidant activity of methanolic and aqueous extracts of aerial part of *P. nivalis* (Asclepiadaceae).

## Material and Method

### *Plant Material*

Aerial part of *Pentatropis nivalis* was collected from Keshavshrushsti, Bhayandar, Mumbai. The collected plant material was authenticated by Dr. Rajendra D. Shinde, Head, Department of Botany & Director, Blatter Herbarium, St. Xavier's college, Mumbai. (Herbarium Specimen number NI-1922) The collected plant material was washed with water & then samples were air-dried at room temperature with dehumidifier. Dried samples were ground to powder using a mechanical grinder, and stored in a sealed plastic container.

### *Extraction method*

2200 g coarse powder of the sample were extracted in soxhlet extractor with methanol and water. Then the extract was evaporated by using rotatory vacuum evaporator to get semisolid mass. The concentrated extracts of aerial part of *P. nivalis* was stored in air tight close container in dark place [9].

### *Phytochemical screening of extracts*

The qualitative phytochemical screening of above extracts was performed to evaluate the types of phytoconstitutents present in the extracts [9–11].

### *Thin Layer Chromatography (TLC)*

TLC was performed with various solvent system by using Pre-coated TLC $GF_{254}$ plate was obtained from Merck Pvt. Ltd. TLC plate Size of 1.5 cm X 10 cm was taken for analysis. Plate markings were made with soft pencil. Glass capillaries were used to spot the sample for TLC applied sample volume 1-$\mu$l of sample by using capillary at distance of 1 cm .plates were observed under UV chamber and specific spray reagents were sprayed and allowed to dry. The colored spots developed on the stationary phase were marked and identified by specific reagent and their distances were measured and $R_f$ values were calculated [9, 12].

### *Detection of Spots*

Spots were detected by UV 254 nm and UV 366 nm, also dervatized by various spraying reagent [13, 14].

### *Chemicals*

Chemicals Sodium dihydrogen phosphate ($NaH_2PO_4$), Disodium Hydrogen phosphate ($Na_2HPO_4$), and Methanol were of AR grade obtained from Merck Pvt. Ltd. Pre-coated TLC $GF_{254}$ plate was obtained from Merck Pvt. Ltd. Ferric chloride was obtained from Qualigens fine chemicals. Ascorbic acid was purchased from Sisco Research Laboratories Ltd. DPPH (diphenyl-ß-picrylhydrazyl) of purity > 99.8% was purchased from Sigma Aldrich.

### *Analytical Instrument*

UV spectrophotometer (Make and model: JASCO V 550 UV spectrophotometer). The software used data acquisition and evaluation was Spectra Manager.

### *Evaluation of in-vitro antioxidant activity*

Antioxidant (*in-vitro*) activity of methanolic extract of *P.nivalis* (MEPN) and aqueous extract of *P. nivalis* (AEPN) was determined using standard ascorbic acid. All the experiments were performed in triplicate.

### *DPPH radical scavenging activity*

The DPPH assay measure the free radical scavenging capacity of the extract and has been used to evaluate the free radical scavenging ability in-vitro. Free radical scavenging capacity of MEPN & AEPN was determined using method of Chen et al. and compared with ascorbic acid. Briefly MEPN, AEPN and ascorbic acid concentration were prepared in methanol and mixed with 1ml of 0.1mM DPPH methanolic solution. The reaction mixture was incubated at 37°C for 30min and absorbance measured at 517nm [15].

$$\text{DPPH Scavenging activity (\%)} = (A_0\text{-}A_1)/A_0 \times 100$$

Where, $A_0$=absorbance of the control, A1=absorbance of the sample.

### *Ferric reducing (FRAP) assay*

The ability to reduce ferric ions was measured using the method described by Benzie and Strain the antioxidant capacity based on the ability to reduce ferric ions of extract was calculated from the linear calibration curve. The FRAP reagent was prepared fresh daily by mixing 100 ml of sodium acetate buffer (300mM, pH 3.6), 10 ml TPTZ solution (10 mM TPTZ in 40mM HCl), 10ml $FeCl_3$ (20 mM) in a ratio of 10:1:1 (v/v). FRAP reagent was warmed to 37°C on a water bath prior to use. Sample at different concentration (200, 400, 600,800 and 1000 $\mu$g) was added to 3 ml of the FRAP reagent and the mixture sample incubated for 30 min. The increase in the absorbance at 593 nm was measured. Fresh working solution of $FeSO_4$ was used for calibration [16].

## Result

### *Preliminary qualitative phytochemical analysis*

The present study revealed that the methanolic and aqueous extracts of aerial part of P. nivalis contained alkaloids,tannins,

cardiac glycosides, flavonoids, glycosides, phenols, resins, saponins, steroids and terpenoids (Table 1). However, alkaloids were detected only in methanolic extracts of aerial parts of *P. nivalis.*

**Table 1** Preliminary qualitative phytochemical analysisof methanolic and aqueous extracts of aerial part of P.nivalis.

| | | Extracts | |
|---|---|---|---|
| S. No. | Plant Constituents | MEPN | AEPN |
| 1 | Alkaloids | + | – - |
| 2 | Cardiac glycosides | ++ | + |
| 3 | Flavonoids | +++ | + |
| 4 | Glycoside | +++ | + |
| 5 | Phenols | ++ | + |
| 6 | Saponins | ++ | ++ |
| 7 | Steroids | +++ | — |
| 8 | Tannins | ++ | + |
| 9 | Terpenoids | +++ | ++ |

+++: highly present, ++: moderately present, +: Low, -: absent. MEPN: Methanolic extracts of P. nivalis AEPN: Aqueous extracts of P. nivalis.

### *Thin Layer Chromatography (TLC)*

TLC was performed on methanolic and aqueous extracts of aerial part of P. nivalis. The results are shown in Table 2.

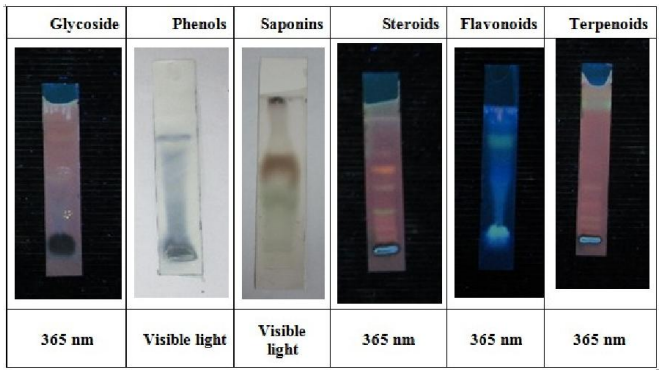


**Figure 1** TLC of MEPN

### *DPPH Assay*

The DPPH method was performed and revealed that scavenging of free radical of MEPN was found to be 25.33, 35.00, 54.00, 62.67, and 72 % at concentration of 200, 400, 600, 800 and 1000 $\mu$g/ml. respectively. The percentage of inhibition of the DPPH radical by the AEPN was 12.33, 23.67, 30.67, and 52.67 % at concentration of 200, 400, 600,800 and 1000 $\mu$g/ml. respectively. In the DPPH assay, the $IC_{50}$ of ascorbic acid was

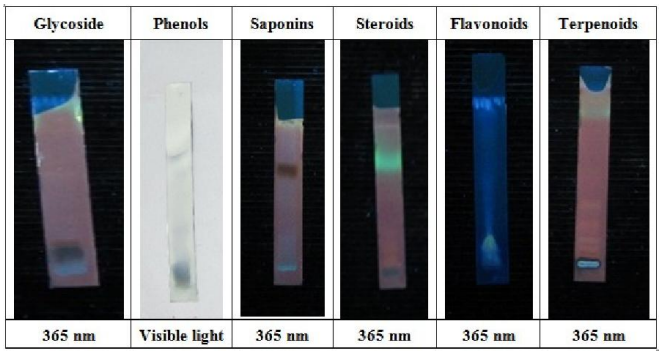


**Figure 2** TLCof AEPN

found to be 346.30$\mu$g/ml where MEPN and AEPN was 603.30 and 1049.687$\mu$g/ml respectively.

The values presented are mean ± standard deviation, n = 3. Results were analyzed using descriptive statistics.

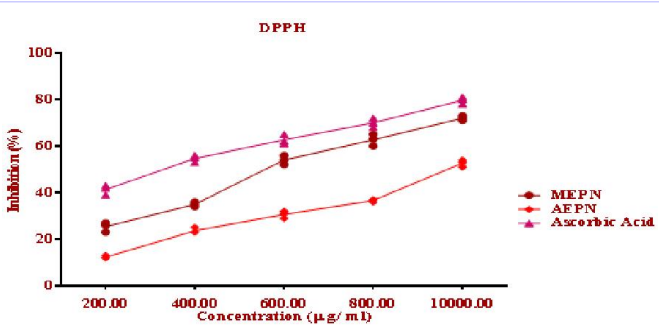


**Figure 3** DPPH radicalScavenging activity

### *FRAP Assay*

Antioxidant activity m

ethanolic and aqueous extracts of plants determined by the FRAP assay. The results are shown in Table 5.

## Discussion Conclusion

Phytochemicals screening of MEPN and AEPN was performed which showed the presence of Glycoside, steroids, saponins, flavonoids, phenols and terpenoids. Methanolic extract showed better qualitative tests for presence of secondary metabolites than aqueous extract.

TLC plate of methanolic and aqueous extracts of P. nivalis were subjected to derivatisation, various spots were observed in different solvent system. Qualitative analysis of MEPN showed presence of flavonoids, glycosides, phenols, saponins, steroids

**Table 2** TLC of methanolic and aqueous extracts of aerialpart of P. nivalis.

| S. No. | Phyto-con-stituents | Mobile phases | Spraying reagent | Spot colour | MEPN | AEPN |
|---|---|---|---|---|---|---|
| 1 | Alkaloids | Ethyl acetate: Methanol: Water (10: 1.35: 1 v/v/v) | Dragendroff's reagent followed by 10% ethanolicsulphuric acid reagent. | No Spot | – | — |
| 2 | Flavonoids | Ethyl acetate: Formic Acid: Glacial Acetic Acid: Water (10: 1.1: 1.1: 2.6 v/v/v/v) | 1% ethanolic aluminum chloride reagent | Yel-low | 0.70, 0.18 | — |
| 3 | Glyco-side | Ethyl acetate: Methanol: Water (10: 1.35: 1 v/v/v) | Anisaldehydesulphuric acid reagent | Blue | 0.83 | 0.18, 0.12 |
| 4 | Phenols | Toluene: Acetone: Formic Acid (4.5: 4.5: 1 v/v/v) | 20% sodium carbonate solution followed by Folin-ciocalteau reagent | Blue | 0.63, 0.57, 0.20, 0.12. | 0.71, 0.18. |
| 5 | Saponins | Chloroform: Glacial Acetic acid: Methanol: Water (6.4: 3.2: 1.2: 0.8 v/v/v/v) | Anisaldehyde sulphuric acid reagent | Blue | 0.59 0.35 0.14 | 0.66 |
| 6 | Steroids | Toluene: Methanol (9:1 v/v) | Anisaldehyde sulphuric acid reagent | Pur-ple | 0.86, 0.63, 0.51, 0.40, 0.24, 0.14. | — |
| 7 | Ter-penoids | n-hexane: Ethyl acetate (7.2: 2.9 v/v) | Anisaldehyde sulphuric acid reagent | Pink | 0.83, 0.43, 0.33, 0.26, 0.21 | 0.40, 0.29, 0.17. 0.10 |

**Table 3** DPPH (2, 2- diphenyl -1 –picrylhydrazyl)scavenging radical activity

| | | Percentage of Inhibition | | |
|---|---|---|---|---|
| S.No | Concentration ($\mu$g/ml) | MEPN | AEPN | Ascorbic acid |
| 1 | 200 | 25.33±1.20 | 12.33±0.33 | 41.33±1.20 |
| 2 | 400 | 35.00±0.58 | 23.67±0.66 | 54.67±0.88 |
| 3 | 600 | 54.00±1.15 | 30.67±0.88 | 62.67±1.20 |
| 4 | 800 | 62.67±1.45 | 36.67±33 | 70.00±1.15 |
| 5 | 1000 | 72.00±0.58 | 52.67±88 | 79.67±0.88 |

**Table 4** $IC_{50}$ values 2, 2- diphenyl -1 –picrylhydrazylradicalscavenging activity of MEPNand AEPN

| IC $_{50}$ values ($\mu$g/ml) | | |
|---|---|---|
| MEPN | AEPN | Ascorbic Acid |
| 603.30 | 1049.68 | 346.30 |

**Table 5** Ferricreducing antioxidant power (FRAP) assay

| S.No | Concentration ($\mu$g/ml) | Absorbance value at 593 nm. | | |
|---|---|---|---|---|
| | | MEPN | AEPN | Ascorbic acid |
| 1 | 200 | 0.11±.006 | 0.10±0.012 | 0.13±0.015 |
| 2 | 400 | 0.16±.009 | 0.13±0.006 | 0.18±0.007 |
| 3 | 600 | 0.23±.007 | 0.17±0.003 | 0.26±0.015 |
| 4 | 800 | 0.58±.009 | 0.36±0.007 | 1.39±0.081 |
| 5 | 1000 | 2.12±.012 | 1.59±0.044 | 3.97±0.120 |

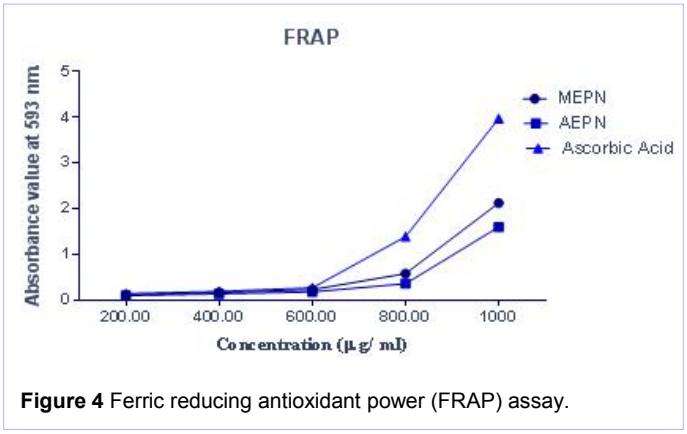


**Figure 4** Ferric reducing antioxidant power (FRAP) assay.

and terpenoids spots at Rf , 0.70, 0.83, 0.59, 0.63, 0.59, 0.83 etc. respectively and AEPN by TLC showed presence of glyco- sides, phenols, saponins, and terpenoids spots at Rf 0.18, 0.17, 0.66, 0.40 etc., Respectively. Also, The TLC studies showed that

among the two solvents, methanol extracted higher quantity of secondary metabolites of medicinal importance viz., flavonoids, glycosides,tannins,phenols, saponins, steroids and terpenoids from the aerial parts of P. nivalis.

The In-vitro antioxidant activity of both extracts of was evaluated by DPPH and FRAP, and compared with ascorbic acid as standard. From the result of both the methods it was concluded that the plant possesses good antioxidant activity, specifically

methanolic extract shore more activity in comparison to aqueous extract.

In the recent year, the use of herbal medicine and phytochemical processing of antioxidant properties have been rise due to potential effects on in the therapy of various chronic and infectious diseases. [17] The result obtained from the extract of P. nivalis have good antioxidant activity. However, extensive investigations need to be done on isolation &characterization of phytoconstitutents and to determine the in-vitro and in-vivo correlation in biological system.

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**References**

[1] Waris G, Ahsan H. Reactive oxygen species: role in the development of cancer and various chronic; 2006.
[2] Zhou C, Huang Y, Przedborski S. Oxidative stress in Parkinson's disease: a mechanism of pathogenic and therapeutic significance. Ann N. 2008;93–104.
[3] D P. Evidence of oxidative stress in Alzheimer's disease brain and antioxidant therapy: lights and shadows. Ann N. 2008;70–78.
[4] MM MBE. Oxidative stress as a mediator of cardiovascular disease. vol. 2.
[5] E WJ. Scism-Bacon JL, Glass LC. Oxidative stress in type 2 diabetes: the role of fasting and postprandial; 2006.
[6] Oxidative stress and neurodegenerative diseases: A review of upstream and downstream antioxidant therapeutic options. Curr Neuropharmacol. 2009;7–1.
[7] N T. Antioxidants- natural rejuvenators that heal, detoxify and provide nourishment.In: Sharma RK, Arora R, editors. Herbal Drugs - A Twenty First Century Perspective. New Delhi: Jaypee Brothers Medical Publishers; 2006.
[8] Kirtikar KR, Basu BD. Indian medicinal plantss, Dehradun, India; 1987.
[9] 1stChapman, Hall, editors. Harbone JB .Phytochemical Methods; 1973.
[10] CK K. Practical pharmacognosy; 1994.
[11] Khandelwal K.R.Practical Pharmacognosy Tech- nique& Experiments, NiraliPrakashan, Pune 20th; 2010.
[12] G EST. London: BailerTindal; 2002.
[13] JM B. New York: Verlogchemise Academy Press; 1966.
[14] K R. New York: Verlogchemise Academy Press; 1966.
[15] -1 -picrylhydrazyl radicalscavenging active components from Polygonummultiflorum Thunb. J Agric Food Chem. 1999;47:2226–2228.
[16] IFF SJB. The ferric reducing ability of plasma (FRAP) as a measure of Antioxidant power: The FRAP assay. Anal Biochem. 1996;239:70–76.
[17] A review on in-vitro antioxidant methods: comparisons, correlations and considerations. Int J PharmTech Res. 2010;2(2):1276–1285.