



Phytochemical Profiling and Antioxidant Potential of *Polygonatum neesii* (Mull. Hal.) Dozy: A Comprehensive Study

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ABSTRACT

*Bryophytes are emerging as a novel reservoir of pharmaceutical compounds with diverse therapeutic properties, widely utilized by various ethnic communities for medicinal purposes. This study investigates the phytochemical composition and antioxidant properties of *Polygonatum neesii*, a representative bryophyte species. Qualitative phytochemical analysis revealed the presence of secondary metabolites, including phenols, tannins, saponins, alkaloids, terpenoids, flavonoids, glycosides, steroids, and carbohydrates, across different solvent extracts. Quantitative analysis highlighted that ethyl acetate and methanolic extracts contained significantly higher concentrations of secondary metabolites. The antioxidant potential of these extracts was assessed using in vitro assays, including DPPH and ABTS radical scavenging assays, as well as total antioxidant capacity by the phospho-molybdenum method. The antioxidant activity of the extracts was evaluated using DPPH, ABTS and Total antioxidant assays. In the DPPH assay, the methanolic extract exhibited a stronger antioxidant activity with an IC₅₀ value of 120.3 µg/mL, while the ethyl acetate extract showed a higher IC₅₀ value of 387.61 µg/mL, indicating lower scavenging efficiency. Similarly, in the ABTS assay, the methanolic extract demonstrated an IC₅₀ value of 255.71 µg/mL and the ethyl acetate extract, which had an IC₅₀ value of 613.89 µg/mL. However, the concentration of antioxidants was higher in the ethyl acetate extract (93.72 ± 0.237 µg/mg) compared to the methanolic extract (45.22 ± 0.020 µg/mg). These findings suggest that *Polygonatum neesii* possesses significant antioxidant potential and harbors a diverse array of bioactive compounds, underscoring its potential for pharmaceutical applications. Future research could focus on isolating and characterizing specific active components to further explore its therapeutic prospects.*

Keywords: *Bryophytes, Polygonatum neesii, DPPH, ABTS, Total antioxidant assay*

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INTRODUCTION

The phytochemistry of bryophytes has been neglected for a long time because they are tiny and difficult to collect in large amounts as pure samples. Nevertheless, bryophytes have been utilized in traditional medicine to treat various conditions such as cuts, external wounds, bacterial infections, pulmonary tuberculosis, pneumonia, and neurasthenia. It is estimated that 80% of bryophytes have a sharp, acrid, and unpleasant taste, which protects them from being harmed by insects, bacteria, snails, and mammals [1]. Bryophytes are spore-producing terrestrial green plants morphologically situated between algae and pteridophytes (ferns). They are classified into three groups: Musci (mosses), Hepaticae (liverworts), and Anthocerotae (hornworts). There are approximately 24,000 species of bryophytes worldwide [2],[3]. They occur in nearly every land ecosystem [4].

Bryophytes contain various unique chemical compounds that are biologically and ecologically significant. Although most bryophytes, around 14,000 species, belong to the moss group (Bryophyta), fewer chemical compounds have been identified in mosses compared to liverworts. Mosses contain terpenoids, benzoic, cinnamic, and phthalic acid derivatives, coumarins, and some nitrogen-containing aromatic compounds, which occasionally have structural similarities to those found in vascular plants [5].

Many chemical components, such as lipophilic terpenoids, have been identified in bryophyte plants[6],[7],acetogenins[8], and bisbibenzyl compounds [9]. Bisbibenzyls exhibit a variety of biological properties, including antibacterial, antifungal, and 5-lipoxygenase inhibitory activities [1]. Many bryophyte plants have traditionally been used to treat cardiovascular diseases, bronchitis, and burns, and they also have antimicrobial, anticancer, and antifungal properties [10],[11],[12].

Phytochemicals are naturally occurring biologically active compounds in medicinal plants, fruits, vegetables, and grains. Their antioxidant properties are thought to contribute to their effectiveness in

preventing diseases [13],[14]. Reactive oxygen species (ROS) constitute a category of extremely reactive molecules produced through oxygen metabolism. Additionally, these ROS have the potential to inflict substantial harm on cells and tissues, particularly in instances of infections and various degenerative conditions such as cardiovascular disease, aging, neurodegenerative diseases like Alzheimer's disease, mutations, and cancer [15],[16]. The antioxidant activity of phenolic compounds primarily stems from their redox properties, which are crucial for adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, and decomposing peroxides [17].

The present paper investigates the phytochemical contents, and antioxidant properties of *Pogonatum neesii*.

MATERIAL AND METHODS

Plant materials and sample preparation

The *Pogonatum neesii* were collected from Kodaikanal, Tamil Nadu, and identified and authenticated by the Bryology Division of the Department of Botany at the University of Calicut. A voucher specimen, with the collection number 195032 was deposited in the Calicut University herbarium (CALI). The materials were washed, dried in a shaded environment at room temperature, and ground into powder using a mixer grinder. The ground samples were stored in a zip-lock bag for further analysis.

Preparation of Plant Extracts

The plant material underwent sequential solvent extractions with hexane, petroleum ether, chloroform, ethyl acetate, ethanol, and distilled water. The extracted components were then concentrated using a rotary evaporator and stored in sealed containers at 4°C for future use.

Qualitative Studies

Standard protocol was used for the qualitative phytochemical analysis of extracts from the mosses *P. neesii* to determine the presence of specific bioactive chemicals [18],[19].

1. Ferric Chloride test for phenolic compounds [20],[21].
2. Alkaline Reagent Test for flavonoids [22],[23].
3. Mayer's test for alkaloids [21],[24].
4. Salkowski test for terpenoids [23].
5. Biuret test for protein [25],[20].
6. Molisch's test for carbohydrates [20],[23].
7. Lead Acetate test for tannins [26].
8. Foam test for saponins [27].
9. Test for fixed oils and fats (Spot test) [27].

Quantitative Studies

Quantitative test for total flavonoid content

The total flavonoid content in the extract was evaluated by the Aluminium Chloride colorimetric method[28]. To 0.5 mL of extract, 1.5 mL of methanol, 0.1 mL of 10% AslCl3, 0.1 mL of 1 M potassium acetate (CH3COOK), and 2.8 mL of distilled water were added. The mixture was kept for incubation at room temperature for 30 minutes. The absorbance was measured at 415 nm. Quercetin was kept as the standard. TFC was calculated from the calibration graph plotted for Quercetin using the formula $T = C \times (V/M)$ where T is the total flavonoid content (mg/g) of the extracts, C is the concentration of Quercetin (mg/mL) obtained from the calibration graph, V is the volume of the extract taken (mL) and M is the weight of the extract (g). TFC is expressed as milligram Quercetin equivalent (mg QE)/g sample dry weight[28].

Quantitative test for total phenolic content

The total natural phenolic content present in the extract was determined using the Folin-Ciocalteu reagent[29]. An aliquot of 0.5 mL of the extract was treated with 2.5 mL of Folin-Ciocalteu's reagent. Mixed thoroughly and after 5 minutes 2 mL of 7.5% sodium carbonate (Na2CO3) solution was added. The reaction mixture was kept for incubation at 45°C for 15 min. The blue colour developed was read at 765 nm. Gallic acid was used as the reference standard and the TPC was estimated from the calibration graph of Gallic acid. Total Phenolic Content (TPC) in the extracts was calculated using the equation $T = C \times (V/M)$ where T is the total phenolic content (mg/g) of the extracts, C is the concentration of Gallic acid (mg/mL) obtained from the calibration graph, V is the volume of the extract taken (mL) and M is the weight of the extract (g). The results were expressed as milligrams of Gallic acid equivalents (mg GAE)/g of sample dry weight[29].

Quantitative test for total terpenoids

Dried plant extract 100mg was taken and soaked in 9mL of ethanol for 24 hours. The extract after filtration was extracted with 10mL of petroleum ether using a separating funnel. The ether extract was separated

into pre-weighed glass vials and waited for its complete drying. The ether extract was treated as total terpenoids[30].

Quantitative test for total alkaloid content

0.5mL of sample was mixed with 1ml of HCl- 0.1 N. Then 0.25ml of Dragendorffs reagent was added to the previous mixture for precipitation and the precipitate was centrifuged over 5 minutes at 3000 rpm. This precipitate was further washed with 0.25 ml of ethanol. The filtrate was discarded and the residue was then treated with 0.25ml of disodium solution (1%w/v). The brownish-black precipitate formed was then centrifuged for 5 minutes at 3000 rpm. This residue was dissolved in 0.2 ml of concentrated nitric acid and 0.1 ml was then pipetted out and mixed with 0.5 ml of thiourea solution (3% w/v). The absorbance of this solution was measured at 435 nm using a UV-VISIBLE spectrophotometer (Agilent, Cary 60) against a blank containing 0.1 ml of concentrated nitric acid and 0.25ml of thiourea solution (3% w/v), and the values obtained were interpreted using the standard graph of caffeine to get the milligram equivalents of caffeine [31].

Quantitative test for total Tannin Content

The tannins were determined by the Folin-Ciocalteu method. About 0.1 ml of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent, 1 ml of 35 % Na₂CO₃ solution and diluted to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of gallic acid (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. Absorbance for test and standard solutions was measured against the blank at 725 nm with a UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of GAE /g of extract[32].

ANTIOXIDANT ACTIVITY

DPPH (1-1-diphenyl-2-picrylhydrazine) Free Radical Scavenging Assay

Quantitative measurement of radical scavenging properties of different concentrations of the plant extract was carried out[33]. A methanolic solution of 1 mL of DPPH (0.1 mM) was added to 1 mL of different concentrations of the extract and allowed to react at room temperature for 30 min in the dark. Absorbance was measured at 517 nm. Methanol served as the blank, DPPH in methanol served as the control and ascorbic acid was taken as the reference standard. The lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The capacity to scavenge the DPPH radical was calculated using the following equation:-

$$\% \text{ radical scavenging activity} = \frac{\text{Abs. of control} - \text{Abs. of sample}}{\text{Abs. of control}} * 100$$

A graph was plotted with the percentage of inhibition against concentration. The IC₅₀ values (concentration of sample required to scavenge 50% of free radicals) were calculated from the regression equation of the graph. The smaller the IC₅₀ higher the antioxidant activity of the extracts [33].

Estimation of total antioxidant activity

The total antioxidant capacity of the extract was evaluated by the phospho-molybdenum method [34]. 1mg/mL of extract was combined with 3mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). Ascorbic acid was used as the standard and 1ml of varying concentration of standard combined with 3ml of reagent solution. The tubes containing the reaction solution were incubated at 95°C for 90 min. Then, the absorbance of the solution was measured at 695 nm using a UV-VIS spectrophotometer against blank after cooling to room temperature. The total antioxidant activity is expressed as the number of grams equivalent of ascorbic acid. The calibration curve was prepared by mixing ascorbic with methanol [34].

ABTS decolorization assay

The reaction was initiated by the addition of 200µl of diluted ABTS to 1.56-1000 µg/ml of different concentrations of sample extract and in control 50 µl of methanol was used instead of sample. Methanol is used as a blank. The absorbance was read at 734 nm and the percentage inhibition was calculated. The inhibition was calculated according to the equation:

$$\% \text{ of inhibition} = \frac{A_0 - A_1}{A_0} * 100$$

Where A₀ is the absorbance of the control, A₁ is the absorbance of test compound [35].

RESULTS AND DISCUSSION

Preliminary phytochemical analysis on various solvents such as hexane, petroleum ether, chloroform, ethyl acetate, methanol and distilled water was carried out, and the results are tabulated in Table 1. The plant revealed the presence of phenols, saponins, alkaloids, terpenoids, flavonoids, glycosides, steroids and

carbohydrates.

The analysis of *Hyophila involuta*'s phytochemical composition also indicates the existence of phenols, flavonoids, terpenoids, alkaloids, and tannins [36]. Similarly, studies on the thallus of *Bryum argenteum*, which is rich in phytochemicals such as phenolics, flavonoids, tannins, terpenoids and alkaloids. Similarly, the solvent extracts of *Dumortiera hirsuta* contain phenols, flavonoids, tannins, terpenoids, and alkaloids [37]. Significant variation was observed in the qualitative evaluation of phytochemical constituents in petroleum ether, ethyl acetate, and water extracts of *P. microstomum*[38]. The presence of glycosides, tannins, coumarins, alkaloids, saponins, flavonoids, phenols, steroids, and terpenoids is confirmed.

Table 1: Preliminary phytochemical analysis on various solvents

Tests	Hexane	Petroleum Ether	Chloroform	Ethyl Acetate	Methanol	Aqueous
Phenol	+	+	+	+	+	+
Flavonoids	-	-	+	+	+	+
Alkaloids	-	+	+	+	+	+
Terpenoids	+	+	+	+	+	+
Protein	-	-	-	+	+	+
Carbohydrate	-	-	+	+	+	+
Tannins	+	+	+	+	+	-
Saponins	-	-	+	+	+	+
Fixed oils and fats (Spot test)	+	+	-	-	-	-

Table 2: Quantitative studies of *Polygonatum neesii*

Phytochemicals	Hexane	Petroleum ether	Chloroform	Ethyl acetate	Methanol	Aqueous
Flavonoids (mgQE/g)	3.11±0.326	11.91±0.165	13.99±0.528	31.54±0.317	29.05±0.580	9.94±0.211
Phenol (mgGAE/g)	14.68±0.173	15.28±0.351	33.51±0.877	34.16±0.896	36.29±1.813	21.87±0.461
Terpenoid (mg/g)	6.13±0.233	17.26±0.433	12.08±0.582	18.03±0.484	14.07±0.190	10.10±0.321
Alkaloid (mg/g)	7.05±0.074	11.08±0.075	10.06±0.235	14.03±0.065	12.10±0.206	8.03±0.176
Tannins (mg of GAE/g)	11.45±0.352	14.83±0.321	23.53±0.362	25.09±0.404	28.08±0.705	13.40±0.375

Quantitative analysis

The result of the quantitative estimation of phenols, flavonoids, terpenoids and alkaloids and tannins were tabulated in Table 2.

Total Flavonoid content

Flavonoids are one of the major classes of polyphenolic compounds which are found in diverse groups of plants including bryophytes [39]. Flavonoids are hydroxylated phenolic substances and are known to be synthesized by plants in response to microbial infection [40]. Flavonoids have long been recognized to possess anti-allergic, anti-inflammatory, antiviral, anti-proliferative and anti-carcinogenic activities as well as to affect some aspects of mammalian metabolism [41].

The quantitative analysis of total flavonoid content in the plant extract revealed that the ethyl acetate extract exhibited the highest flavonoid concentration, measured at 31.54 mgQE/g. This was followed by the methanol extract with 29.05 mgQE/g, indicating its strong potential for flavonoid presence. Moderate levels were observed in the chloroform and petroleum ether extracts, with values of 13.99 mgQE/g and 11.91 mgQE/g, respectively. Comparatively lower flavonoid contents were detected in the distilled water (9.94 mg QE/g) and hexane extracts (3.11 mg QE/g).

The phytochemical contents of *P. neesii* were found to be higher than those of *Dumortiera hirsuta*. In examining the total flavonoid content (TFC) of *Dumortiera hirsuta* across various solvent extracts, it was observed that the acetone extract with a TFC of 21.6±0.74 mg/g, the petroleum ether extract with 15.2±1.07 mg/g, the hexane extract with 11.57±5.26 mg/g, and finally, the aqueous extract, which had the lowest TFC at 7.76±0.55 mg/g [42].

Total flavonoid content in different extracts of *P. microstomum* and found varying amounts. The ethyl acetate extract had 28.6 ± 0.37 mg of flavonoids per gram of tissue, while the petroleum ether extract had

36.3 ± 0.78 mg/gram. The water extract had the highest content of flavonoids (54.6 ± 0.14 mg per gram of tissue) [38].

The phytochemical contents of *P. neesii* were higher than species such as *Lepidozia borneensis* (TFC = 9.36 mg CE/g) and *Plagiochila beddomei* (TFC = 16.9mg QE/g) [43],[44]. Studies on the total flavonoid concentrations of 90 bryophytes from Tianmu Mountain, Zhejiang Province (China) showed that the result ranges from 1.8 to 22.3 mg/g (w/w)[45]. The ethyl acetate extracts of *Philonotis hastata* showed maximum activity of 8.51 mg/g[46]. In *H. involuta*, the maximum total flavonoid content yield (42.04 ± 0.16 mg quercetin equivalent per gram) was achieved using methanol [36]. These findings underscore the significant outcomes of the current study on *Pogonatum neesii*, which notably surpass previous research endeavors in terms of results.

Total Phenol content

The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites. The total phenolic content of the plant extracts was evaluated, with the methanol extract demonstrating the highest phenolic concentration at 36.29 mg GAE/g. This was closely followed by the ethyl acetate extract (34.16 mg GAE/g) and chloroform extract (33.51 mg GAE/g), indicating their effectiveness in phenolic compound extraction. The distilled water extract showed a moderate phenolic content of 21.87 mg GAE/g, while lower levels were observed in the petroleum ether (15.28 mg GAE/g) and hexane extracts (14.68 mg GAE/g).

The phytochemical analysis revealed that *P. neesii* exhibited higher phytochemical content compared to *Dumortiera hirsuta*. Among the various solvent extracts of *Dumortiera hirsuta*, the total phenolic content (TPC) was highest in the acetone extract, measuring 32.25 ± 0.09 mg/g. This was followed by the hexane extract with a TPC of 26.05 ± 0.21 mg/g, and the petroleum ether extract, which recorded 18.96 ± 0.10 mg/g. The aqueous extract demonstrated the lowest TPC, with a value of 15.25 ± 0.12 mg/g[42]. Total phenol content of *P. microstomum* in various extracts and observed different values. The ethyl acetate extract had 54.4 ± 0.57 mg of flavonoids per gram of tissue, while the petroleum ether extract had 84.3 ± 0.67 mg. The water extract had its highest concentration at 91.2 ± 0.81 mg per gram of tissue[38].

The methanol extraction method yields the highest phenolic content in *H. involuta*, reaching 24.06 ± 0.05 mg gallic acid equivalent per gram [36]. The phytochemical contents of *P. neesi* were higher than other liverwort species such as *Lepidozia borneensis* (TPC=12.42 mg GAE/g) and *Plagiochila beddomei* (TPC = 19.3 mg GAE/g) [43],[44]. These findings not only underscore the substantial outcomes of the current study but also represent a significant advancement over previous research efforts. These results not only highlight the noteworthy results of the present investigation, but they also signify a noteworthy progression beyond earlier research.

Total Terpenoid content

Terpenoids are the most widely described antioxidant found in secondary metabolites in bryophytes [5]. The presence of terpenoids revealed that the plants can act mainly as anti-feedants and growth disruptors and possess considerable toxicity toward insects [47]. Terpenoid also plays an important role in wound and scar healing [48]. Quantitative analysis of ethyl acetate extract was found to possess the highest amount of terpenoids (18 mg/100mg) followed by petroleum ether (17 mg/100mg), methanol (14 mg/100mg), chloroform (12mg/100mg) distilled water (10 mg/100mg) and hexane (6 mg/100mg). Hence it can be concluded that ethyl acetate extract of *Pogonatum neesii* contains the highest amount of terpenoids.

The phytochemical contents of *P. neesii* were found to be higher than those of *Dumortiera hirsuta*. In examining the total terpenoid content of *Dumortiera hirsuta* across various solvent extracts, it was observed that the total terpenoid content of the plant was determined for various solvent extracts and ranked as follows: methanol extract (4.15 ± 0.16 mg/g), hexane extract (1.09 ± 0.20 mg/g), acetone extract (0.98 ± 0.17 mg/g), petroleum ether extract (0.53 ± 0.28 mg/g), and aqueous extract (0.46 ± 0.17 mg/g)[42]. The analysis of total terpenoid content in *Thuidium tamariscellum* revealed a notable presence of terpenoids, amounting to 25.95 milligrams per gram in the moss [49].

Total Alkaloid content

The ethyl acetate extract of *Pogonatum neesii* demonstrated the highest alkaloid content at 14.03 mg/g, highlighting its superior extraction efficiency for these compounds. This was followed by the methanol extract, which contained 12.00 mg/g of alkaloids, and the petroleum ether extract with 11.08 mg/g. The chloroform extract showed a slightly lower alkaloid content at 10.06 mg/g, while the distilled water and hexane extracts recorded the lowest levels, with 8.00 mg/g and 7.05 mg/g, respectively.

The total alkaloid content of *Barbula indica*, expressed as caffeine equivalents, was notably high in the methanolic extract, reaching 32.06 ± 0.28 milligrams per gram [50]. Additionally, *Thidium gratum* exhibited a total alkaloid content of 1.60%, while *Barbula indica* showed a higher percentage at 5.60% [51]. In *Bytneria herbacea*, the total alkaloid content (TAC) was observed to vary across different plant parts, with the leaf containing 2.306% w/w, surpassing the root (0.814% w/w) and whole plant (1.319% w/w) [52]. Moreover, in *Catharanthus roseus*, the total alkaloid content was evaluated using the standard quercetin

compound, with the highest concentrations found in the benzene leaf extract ($0.61 \pm 0.03 \mu\text{g/ml}$) and stem extract ($0.60 \pm 0.02 \mu\text{g/ml}$), respectively [53]. These findings provide a comparative perspective on the alkaloid content of *Polygonatum neesii*.

Total Tannin content

The amount of tannins extracted from plant material varies significantly depending on the solvent used. Hexane extracts contain 11.45 mg of GAE/g of tannins, while petroleum ether extracts have a slightly higher content at 14.83 mg of GAE/g. Chloroform extracts show a substantial increase, yielding 23.53 mg of GAE/g. This is followed by ethyl acetate with 25.09 mg of GAE/g and methanol, which provides the highest tannin content at 28.08 mg of GAE/g. In contrast, distilled water extracts contain 13.40 mg of GAE/g of tannins. In *Bytneria herbacea*, the highest tannin content was observed in the leaf (8.148% w/w), followed by the whole plant (3.886% w/w) and the root (1.553% w/w)[52]. Conversely, in *Catharanthus roseus*, the total tannin content was assessed using standard tannic acid, with the methanol root extract exhibiting the highest concentration ($1.21 \pm 0.01 \mu\text{g/ml}$), while the benzene leaf extract displayed the lowest ($0.08 \pm 0.05 \mu\text{g/ml}$)[53]. These results provide a basis for comparing the tannin content of *Polygonatum neesii*.

Antioxidant Analysis

Antioxidant is any substance that, when present in low concentration relative to the oxidizable substrate, significantly retards or inhibition oxidation [54].

DPPH (1-1-diphenyl-2-picryl hydrazine) Free Radical Scavenging Assay

Table 3: DPPH Free Radical Scavenging Assay of *P. neesii*

Ascorbic acid		<i>P. neesii</i>		
Concentration ($\mu\text{g/ml}$)	% Inhibition	Concentration ($\mu\text{g/ml}$)	% Inhibition of Ethyl acetate extract	% Inhibition of Methanolic extract
10	30.59 ± 1.27	62.5	19.47 ± 0.01	33.795 ± 0.725
25	52.69 ± 0.32	125	33.26 ± 0.014	56.193 ± 0.509
50	70.37 ± 0.79	250	42.01 ± 0.008	64.239 ± 0.677
75	90.54 ± 0.64	500	53.17 ± 0.015	72.875 ± 0.679
IC50	$21.11 \mu\text{g/ml}$	IC50	$387.61 \mu\text{g/ml}$	$120.3 \mu\text{g/ml}$

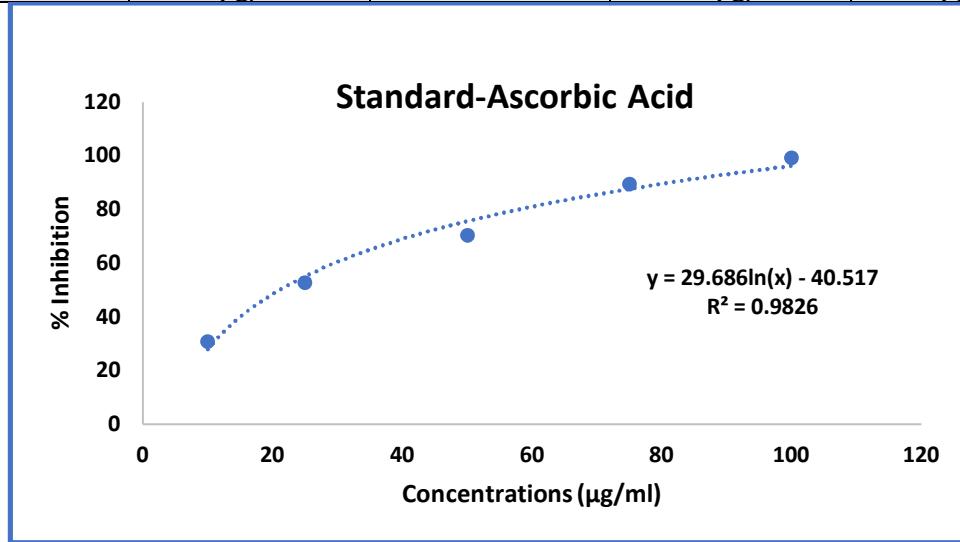


Fig. 1:Free radical scavenging activity of Ascorbic acid

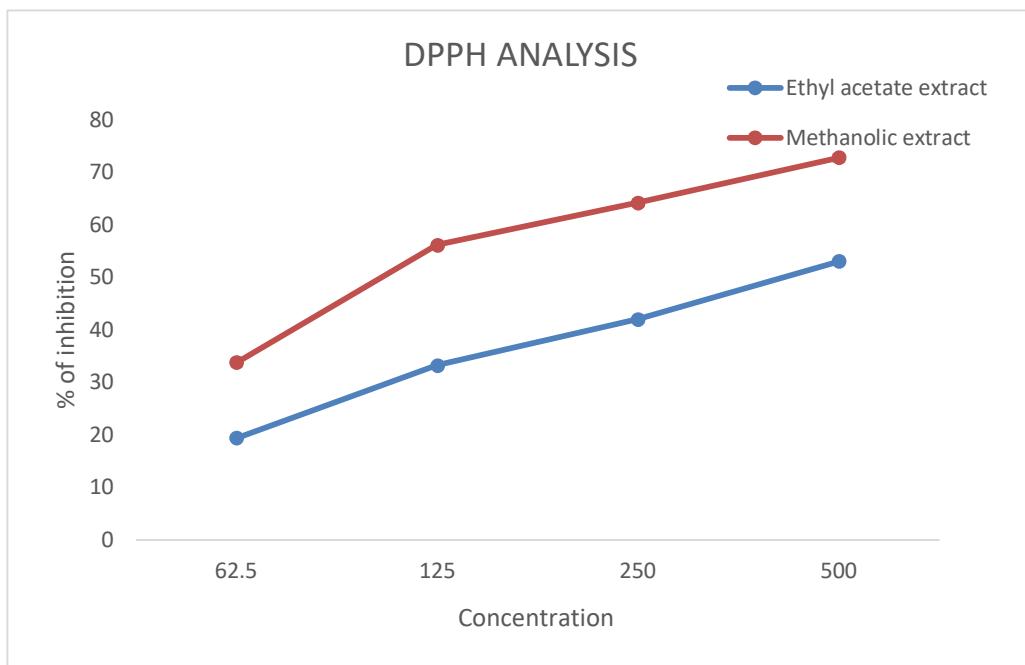


Fig. 2: DPPH Activity of Ethyl acetate and methanolic extract

DPPH is one of the stable organic nitrogen free lipophilic radical, which has been used to test the free radical scavenging ability [55]. Antioxidants, on interaction with DPPH either transfer an electron or hydrogen atom to DPPH, thus neutralizing its free radical character. The degree of discolouration indicates the radical scavenging potential of the antioxidant [56].

The antioxidant activity of *Pogonatum neesii* was assessed using the DPPH radical scavenging method, with results presented in Table 3. The DPPH radical scavenging activity, expressed as IC₅₀ values, indicates the concentration of extract required to inhibit 50% of free radicals (illustrated in Figures 1 and 2). The assay revealed that the methanol extract exhibited stronger antioxidant activity, with an IC₅₀ value of 120.3 µg/mL, compared to the ethyl acetate extract, which demonstrated an IC₅₀ value of 387.61 µg/mL.

The DPPH radical scavenging potential of various extracts of the liverwort *Dumortiera hirsuta* subsp. *hirsuta* (Sw.) Nees. The research reported distinct antioxidant capacities for the different solvent extracts. Specifically, the methanol extract exhibited a DPPH radical scavenging potential of 18.82 mg/mL, the acetone extract showed a value of 44.88 mg/mL, and the aqueous extract demonstrated a scavenging potential of 68.44 mg/mL[42].

For DPPH assay, the extract of *L. borneensis* displayed the IC₅₀ value was 216.67 ± 20.82 µg/mL[43]. The current investigation demonstrated that *Pogonatum neesii* exhibits significant antioxidant activity compared to the standard ascorbic acid.

Total Antioxidant activity

Table 4: Total Concentration of antioxidants in Ethyl acetate and Methanol extract

Sl. No:	Extract	Concentration of antioxidants in µg /mg
1	Ethyl acetate	93.72±0.237
2	Methanol	45.22±0.020

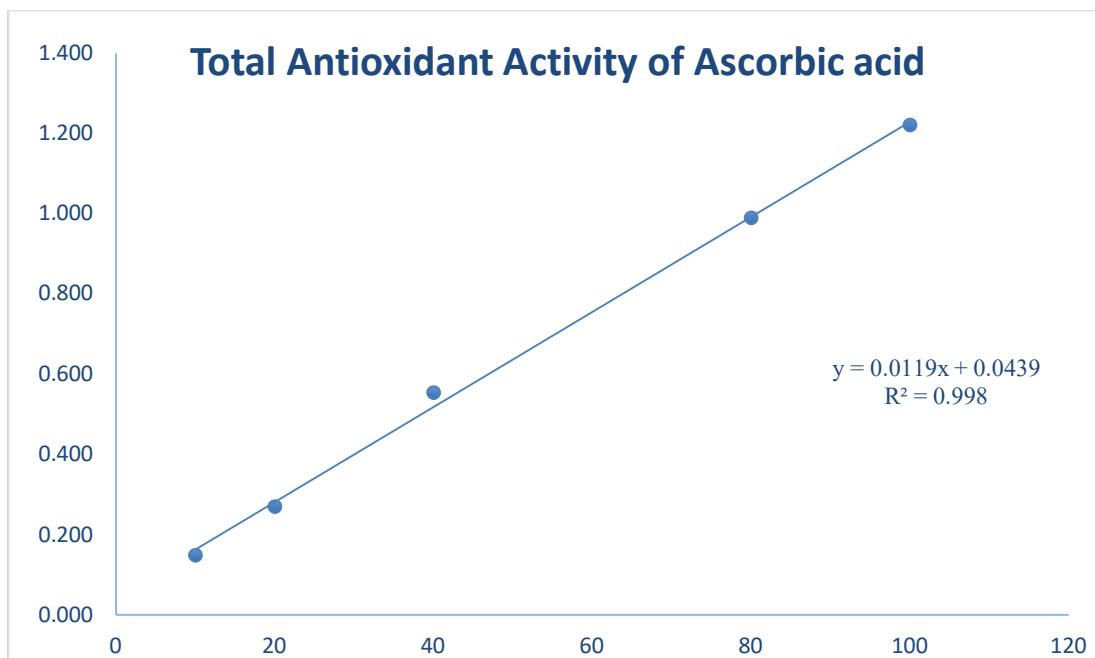


Fig. 3: Total Antioxidant Activity of Ascorbic acid

The ethyl acetate extract exhibited a notably higher concentration of antioxidants, measuring 93.71 µg per milligram of sample, compared to the methanolic extract, which displayed a concentration of 45.22 µg per milligram of sample. The total antioxidant activity of both ethyl acetate and methanolic extracts exhibits notable effectiveness.

The total antioxidant activity analyzed through the phosphomolybdenum method [57], indicates that the Methanolic extract of *Sargassum polycystum* exhibits the highest antioxidant activity with 26.84 mg of AAE/g. The ethanol extract follows with an antioxidant activity of 20.71 mg of AAE/g, while the water extract shows the lowest activity at 12.49 mg of AAE/g. These findings demonstrate the varying effectiveness of different solvent extracts in harnessing the antioxidant potential of *Sargassum polycystum*. The total antioxidant activity analyzed through the phosphomolybdenum method reveals varying concentrations among different plant species[58]. Specifically, the total antioxidant concentration in *Cuminum cyminum* is 25.28 ± 2.12 mg AAE/g, in *Trigonella foenum-graecum* is 6.92 ± 0.59 mg AAE/g, in *Coriandrum sativum* is 16.66 ± 1.2 mg AAE/g, in *Solanum lycopersicum* is 8.88 ± 0.73 mg AAE/g, and in *Papaver somniferum* is 33.16 ± 2.12 mg AAE/g. These results indicate a significant variation in antioxidant levels across the different species studied.

The total antioxidant activity analyzed through the phosphomolybdenum method reveals varying concentrations among *Enteromorpha* species [59]. The extract of *Enteromorpha* sp. demonstrated a free radical scavenging effect of $23 \pm 0.35\%$, $41 \pm 0.05\%$, $60 \pm 0.52\%$, and $81 \pm 0.5\%$ at extract concentrations of $100\mu\text{L}$, $200\mu\text{L}$, $300\mu\text{L}$, and $400\mu\text{L}$, respectively. These results indicate a dose-dependent increase in the antioxidant activity of *Enteromorpha* sp. with higher extract concentrations showing greater free radical scavenging effects.

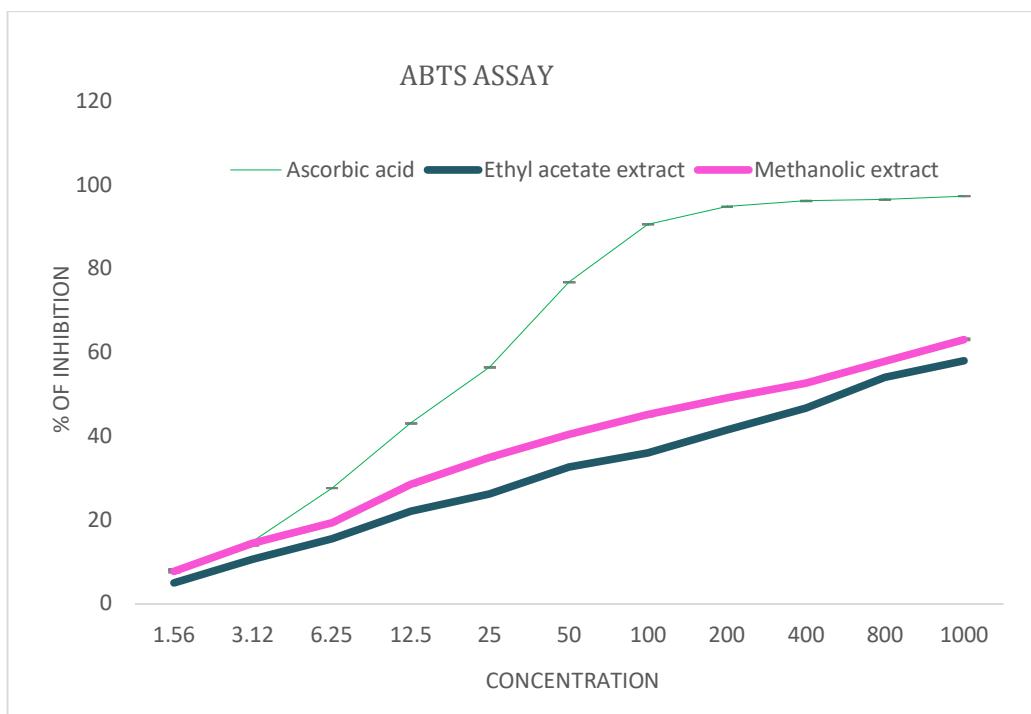
The total antioxidant activity analyzed through the phosphomolybdenum method reveals significant variation in different fractions of *Retama monosperma*. Specifically, the Hexane fraction exhibited an antioxidant concentration of 114.90 ± 1.08 mg AAE/g, the Ethyl acetate fraction showed 155.49 ± 0.96 mg AAE/g, the Ethanol fraction presented the highest concentration with 216.66 ± 0.87 mg AAE/g, and the Aqueous fraction demonstrated the lowest concentration at 42.00 ± 0.39 mg AAE/g. These results highlight the differing antioxidant potentials across various solvent extractions of *Retama monosperma*[60].

ABTS assay

ABTS assays are primarily based on the inhibition of absorbance of radical cation ABTS⁺ by antioxidants. The ABTS radical scavenging assay demonstrated a strong antioxidant activity for the standard, ascorbic acid, with an IC₅₀ value of 19.69 µg/mL. The methanolic extract of *P. neesii* demonstrated notable antioxidant activity with an IC₅₀ value of 255.71 µg/mL, indicating its effective free radical scavenging capacity. In contrast, the ethyl acetate extract displayed a significantly higher IC₅₀ value of 613.89 µg/mL, suggesting comparatively lower antioxidant potential. These results highlight the superior efficacy of the methanolic extract in neutralizing free radicals, likely due to its richer composition of antioxidant compounds. These findings emphasize the considerable variation in antioxidant activity among the extracts, reflecting differences in their bioactive compound profiles.

Table 5: ABTS Radical Scavenging Assay of *P. neesii*

Ascorbic acid		<i>P. neesii</i>	
Concentration (µg/ml)	% Inhibition	% Inhibition of Ethyl acetate extract	% Inhibition of Methanolic extract
1.56	8.20±0.007	4.98±0.209	7.65±0.292
3.12	14.79±0.016	10.57±0.219	14.35±0.451
6.25	27.57±0.011	15.51±0.260	19.30±0.115
12.5	43.03±0.021	22.01±0.276	28.45±0.266
25	56.41±0.032	26.09±0.165	34.82±0.372
50	76.72±0.043	32.62±0.278	40.28±0.165
100	90.47±0.015	35.94±0.091	45.09±0.360
200	94.72±0.021	41.35±0.098	49.05±0.317
400	96.09±0.019	46.55±0.385	52.62±0.081
800	96.39±0.015	53.99±0.222	57.82±0.053
1000	97.24±0.019	57.91±0.175	63.06±0.235
IC50	19.69 µg/ml	613.89 µg/ml	255.71 µg/ml

**Fig. 4: ABTS Radical Scavenging Assay**

Study on the Essential oil of *Syzygiella rubricaulis*, revealing its significant antioxidant properties. Their research highlighted the plant's ability to scavenge ABTS free radicals, a common method used to measure antioxidant capacity. The study found that *Syzygiella rubricaulis* exhibited an SC50 value of 343.38[61]. ABTS radical scavenging activities of *M. paleacea*, *M. linearis* and *C. conicum* were 5.97 mg/ml, 7.68 mg/ml, and 5.14 mg/ml respectively [62]. IC50 value of the methanol extract of *Marchantia polymorpha* was 0.2441 ± 0.009 mg/mL, and the ethyl acetate extract of *M. polymorpha* was 0.2126 ± 0.01 mg/mL, while Trolox exhibited an IC50 value of 0.0431 ± 0.001 mg/mL [63].

CONCLUSION

This study comprehensively demonstrates the diverse phytochemical profile and robust antioxidant capacity of *Polygonatum neesii* extracts obtained through different solvent systems. Qualitative analysis identified a wide range of secondary metabolites, including phenols, tannins, flavonoids, alkaloids, terpenoids, glycosides, saponins, steroids, and carbohydrates, emphasizing the plant's phytochemical richness. Quantitative evaluation revealed that ethyl acetate and methanol extracts were particularly abundant in these bioactive compounds, surpassing other solvents such as hexane, petroleum ether, chloroform, and distilled water. Antioxidant activity assays, including DPPH, ABTS, and total antioxidant capacity, further confirmed the potent free radical scavenging abilities of the ethyl acetate and methanol extracts.

These results highlight the potential of *Polygonatum neesii* as a valuable natural source of antioxidants, with promising applications in pharmaceuticals, nutraceuticals, and functional foods. Future research should focus on isolating and characterizing the individual bioactive compounds responsible for the observed effects, as well as investigating their mechanisms of action and potential synergistic interactions.

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