Research article

Assessing the effect of solvent extraction on the antioxidant activity of *Polystichum acrostichoides* and *Adiantum lunulatum* aerial parts: A comparative study

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ABSTRACT

Introduction and Aim: Free radicals have been established detrimental effects on human health, mostly because of oxidative stress. Antioxidants are essential in reducing these effects and protecting cells from oxidative damage. As a result, there is rising interest in finding natural antioxidants. In this work, *Polystichum acrostichoides* and *Adiantum lunulatum* aerial parts, both widely utilized in medical systems for their therapeutic effects and as sources of free radicals, were examined to see how solvent extraction affected their antioxidant activity.

Materials and Methods: In both plants' methanolic and hexane extracts, the amounts of phenolic and flavonoid compounds were measured. Utilizing the ABTS, DPPH, and hydroxyl radical scavenging tests, the antioxidant activity of the extracts was evaluated.

Results: In both plant species, the methanolic extracts had higher phenolic and flavonoid contents than the hexane extracts. Notably, the ABTS, DPPH, and hydroxyl radical scavenging assays revealed that the methanol extract had the best antioxidant potency. On the other hand, there was no apparent antioxidant action in the hexane extracts.

Conclusion: The results suggest that the aerial parts of *Polystichum acrostichoides* and *Adiantum lunulatum* could be effective sources of antioxidants. In the ABTS, DPPH, and hydroxyl radical scavenging experiments, the methanolic extracts had reduced IC50 values, which indicated that they had considerable antioxidant activity. These results demonstrate the potential of these ferns in the creation of innovative treatments for a range of oxidative stress-related diseases.

Keywords: Hansraj; free radicals; antioxidant; Christmas fern; DPPH; ABTS.

INTRODUCTION

Ferns, belonging to the class of vascular non-flowering plants, that have long been revered for their aesthetic appeal and ecological significance. However, beyond their aesthetic appeal, ferns have been reported to demonstrate remarkable array of therapeutic characteristics, with potential applications in the treatment of numerous maladies (1). Significant investigations have shed light on the intricate biochemistry of ferns, demonstrating the presence of several secondary metabolites with substantial pharmacological properties (2). These compounds have demonstrated diverse biological actions, such as antioxidant, anti-inflammatory, antibacterial, and anticancer characteristics. Furthermore, research has shown that fern extracts are effective in treating several illnesses, including diabetes, cancer, cardiovascular problems, and neurological disorders (3,4).

Adiantum lunulatum and *Polystichum acrostichoides* are two common fern species found in the Indian sub-continent, and they have been traditionally valued for their therapeutic properties. The beautiful ornamental plant *A. lunulatum*, also known as "Maidenhair fern or Hansraj," has long been used in Ayurveda and Unani medicine to alleviate a multitude of medical conditions, including bronchitis, asthma, and hepatic disorders (5,6). The plant has flavonoids, alkaloids, tannins, and other bioactive substances with significant anti-inflammatory, antioxidant, and hepatoprotective properties (7). *Polystichum acrostichoides*, often known as "Christmas fern," is another prominent fern with therapeutic significance. Traditionally, this herb has been used to manage rheumatism, skin infections, and gastrointestinal issues. It has many bioactive compounds which have powerful anti-inflammatory, antimicrobial, and antioxidant effects (8). The potential of these ferns as a source of novel medicinal drugs has been the subject of intensive research in recent years, and their pharmacological qualities are of great interest to the scientific community (9).

Contrary to several conventional medications, ferns have a relatively low toxicological profile. Numerous compounds that have been extracted from ferns have been confirmed to be safe and well-tolerated by humans, with few negative effects ever being documented. This makes fern-based therapies a desirable substitute for synthetic drugs, which can entail high toxicity and negative consequences (10, 11). With the rising demand for natural products and the obligation to deliver sustainable drug development approaches there is growing interest in

the pharmacognosy of ferns and the identification of their bioactive chemicals (12).

The objective of this research is to evaluate the antioxidant activities of the *P. acrostichoides* and *A. lunulatum* ferns which are well-known for their therapeutic value. Specifically, this study examines how solvent extraction affects their antioxidant activity, which may have implications for their utilization in medicines, nutraceuticals, and functional foods.

MATERIALS AND METHODS

Collection and identification of plant samples

The collection and authentication of the aerial parts of *Adiantum lunulatum Burm. f.* and *Polystichum acrostichoides (Michx) Schott*. were carried out as follows: The aerial parts of *A. lunulatum Burm. f*. was collected from Seshachalam hills, Tirupati and were authenticated by Dr. V. Rama Rao of the Central Ayurveda Research Institute, Uttarhalli, Bengaluru, with the reference number RRCBI-4546. On the other hand, the aerial parts of *P.acrostichoides (Michx) Schott*. were collected from Talakona forest, Andhra Pradesh and were authenticated by Dr. K. Madhava Chetty, a Plant Taxonomist from SV University, Tirupati, Andhra Pradesh, India with voucher number-0579.

Preparation of fern extracts

The dried plant material was mashed using a grinder and put through a sieve number 120 to obtain a coarse powder. This powder was carefully stored in labeled and tightly sealed containers for further analysis. Rotary flash extraction was employed to extract the dried powdered aerial parts of *A.lunulatum* and *P. acrostichoides* using a 1:5 ratio (drug: solvent) for each solvent used, namely hexane, chloroform, and methanol. This extraction was conducted twice, and the supernatant was collected for each trial, evaporated under reduced pressure in a rotary evaporator, and weighed to calculate the yield. The dried extracts were then stored in labelled and tightly sealed containers for subsequent investigations. The percentage of yield was calculated with the following formula:

$$\text{Percentage yield} = \frac{\text{weight of the extract} \times 100}{\text{weight of the sample taken}}$$

Quantitative phytocompound screening of the extracts

The hexane, methanol, and chloroform extracts of *A.lunulatum* and *P. acrostichoides* was subjected to quantification of the phytocompounds.

Determination of total flavonoid content

For the determination of flavonoid using the aluminium chloride method, a stock solution of Quercetin was prepared by dissolving 10mg of it in methanol using sonication for 20 minutes and making up the volume to 100ml. Sample preparation involved weighing 0.50g of the sample and adding 50ml of methanol, sonication for 20 minutes, cooling, and making up the volume. The solution was then filtered. To develop the color, 1ml of the standard and sample solution was pipetted into separate test tubes, followed by the addition of 0.3 ml of 5% $NaNO_2$ solution, 0.5ml of 2% $AlCl_3$ solution after 5 minutes, and 0.5ml of 1N NaOH solution after 6 minutes. The solutions were mixed and allowed for 10 minutes at ambient temperature. For the blank, 1ml of methanol was pipetted into a test tube and treated similarly (13,14). The spectrophotometric reading was taken at 510nm, and the content was calculated using the given formula or by preparing a calibration curve using different concentrations and extrapolating the results.

Absorbance of sample × Weight of std. in mg × Purity of standard divided by
Absorbance of standard × Weight of the sample

Determination of total phenolic content

To determine the phenolic acid, tannic acid was used as a standard. A stock solution was prepared by dissolving 100mg of tannic acid in extraction solvent by slightly warming on a water bath followed by sonication for 5 minutes. The solution was cooled to room temperature and made up to 100ml with extraction solvent. To prepare the sample, 1.0g of the sample was weighed accurately into a 100ml round-bottomed flask. 25ml of extraction solvent was added, and the mixture was sonicated for 5 minutes and refluxed on a water bath for 30 minutes. The resulting solution was cooled and decanted to a designated 100ml volumetric flask. The extraction process was repeated two more times, and the content was filtered to the same designated volumetric flask. The volume was then made up to 100ml with diluent. For the colour development procedure, standard and sample solutions were prepared. Standard solutions of 2, 5, and 10ml were pipetted out to different 100ml volumetric flasks. Folin's reagent, saturated solution of $NaCO_3$ solution, and water were added at intervals of 2 to 3 minutes. The mixture was allowed to rest for 30 minutes, after which it was made up to 100ml with water and allowed to rest for 20 more minutes (15,16). The same procedure was followed for the sample solutions. The spectrophotometric reading was then taken using water as blank at 750nm. The phenolic content was calculated using the given formula, or calibration curves were prepared and extrapolated for the results.

Absorbance of sample × Weight of std. in mg × Sample dilution × Purity of standard × 100 divided by
Absorbance of standard ×Standard dilution × Weight of the sample in mg × 100

ABTS radical scavenging assay

The ABTS (2, 2'-azinobis-ethyl-benzothiozoline-6-sulphonic acid) radical scavenging assay is a well-established method for evaluating the antioxidant activity of plant extracts and natural compounds. The assay entails combining ABTS with ammonium persulfate (APS) and allowing the mixture to react for 16 hours to generate an ABTS radical cation solution. The resulting solution is further diluted with Phosphate Buffered Saline (PBS) to achieve an absorbance of 1.000. To 225 µl of the ABTS working solution, various concentrations of the test sample and the reference standard (the highest volume collected was 50 µl) are added, resulting in a final volume of 250 µl, which is made up by adding PBS (17). As a reference standard, 1 mg of quercetin was diluted into 1 ml of PBS. At 734 nm, the absorbance was immediately recorded and the IC50 (Inhibitory concentration) values were obtained using Graph Pad Prism.

DPPH radical scavenging activity

The assay is based on the ability of the tested compound to scavenge the stable DPPH (2,2-diphenyl-1-picrylhydrazyl) radical, which is a deep violet chromophore that is reduced to a colourless compound upon reaction with an antioxidant. The DPPH solution was mixed with different concentrations of the test solution and reference standard in HPLC (High performance liquid chromatography) grade methanol, resulting in a total volume of 240 µl with 80 µl of DPPH solution. The reference standard was tested at concentrations of 0.3125, 0.625, 1.25, 2.5, 5, and 10 µg/mL, while the test samples were tested at a concentration of 1000 µg/mL and two-fold dilutions down to 15.63 µg/mL. The reaction mixture was then incubated at 25°C for 15 minutes, and the absorbance was measured at 510 nm using i3x plate readers. A control reaction was also performed without the test sample. The IC_{50} value was calculated using Graph Prism software by nonlinear regression analysis of the percentage inhibition recorded for different concentrations of test substances/standard. The relative activity of the sample was determined by comparing the IC_{50} value of the sample with the standard. For compounds showing less than 50% inhibition, the IC_{50} value was not calculated (18,19). The growth of inhibition was calculated with the following formula:

$$\% \text{ Inhibition} = \frac{(\text{A0 of Control} - \text{A1 of Sample})}{\text{A1 of Control}} \times 100$$

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging assay is a method used to evaluate the ability of antioxidants to scavenge hydroxyl radicals, which are highly reactive and cause oxidative damage to biological molecules. The procedure was conducted as described by Halliwell *et al*. The deoxyribose degradation assay is a method used to assess the ability of antioxidants to inhibit the oxidative degradation of deoxyribose, a sugar found in DNA. In this assay, a reaction mixture is prepared by combining 5.6 mM deoxyribose, 2.8 mM H_2O_2, 40 µM $FeCl_3$, 100 µM EDTA, and varying concentrations of the test sample in 2.5 mM phosphate buffer (pH 7.4) in a total volume of 1.0 mL. The reaction is initiated by the addition of 0.1 mM ascorbic acid and incubated at 37°C for 90 minutes. After incubation, 1 mL of 0.7% TBA (thiobarbituric acid) in 0.05 N KOH and 1 mL of 2.5% TCA (trichloroacetic acid) are added to the mixture. The mixture is heated at 100°C for 8 minutes, cooled, and the pink color formed is measured at 532 nm using a spectrophotometer. Controls are run without the test samples, and catechin is used as the reference standard. The percentage inhibition of deoxyribose degradation is calculated using the formula % inhibition = ((A control - A sample)/A control) × 100, where A control is the absorbance of the control reaction (without the test sample), and A sample is the absorbance of the reaction mixture containing the test sample (20). The concentration of the test sample required to inhibit 50% of the deoxyribose degradation (IC_{50} value) can be determined by nonlinear regression analysis using GraphPad Prism software. The percentage inhibition of hydroxyl radicals is calculated with the following formula:

$$\% \text{ inhibition} = \frac{\text{Absorbance (control)} - \text{Absorbance (test sample)}}{\text{Absorbance (control)}} \times 100$$

RESULTS

Preparation of extracts

The aerial parts of the ferns *Adiantum lunulatum* and *Polystichum acrostichoides* were collected for the assessment (Fig. 1) and were subjected to solvent extraction in hexane, chloroform, and methanol respectively. It was noticed that the percentage of yield was relatively higher in the methanolic extracts for both *A.lunulatum* and *P. acrostichoides* (Table 1) which is attributed to its polarity as it is a polar solvent that can dissolve a wide range of polar and nonpolar compounds, including polyphenols, flavonoids, and alkaloids, which are known to have antioxidant and other bioactive properties.

(a) *Adiantum lunulatum* (b) *Polystichum acrostichoides*

Fig. 1: Aerial parts of the ferns *Adiantum lunulatum* and *Polystichum acrostichoides*

Table 1: Percentage yield of *Adiantum lunulatum* and *Polystichum acrostichoides*

	Extract	Colour	Consistency	Weight (g)	% Yield
	Hexane	Dark green	Solid	13	2.6
	Chloroform	Dark green	Semi solid	6	1.2
	Methanol	Dark green	Semi solid	35	7
Polystichum acrostichoides	Hexane	Dark green	Solid	6	1.2
	Chloroform	Dark green	Semi solid	5	1
	Methanol	Dark green	Semi solid	15	3

Quantitative phytocompound screening

Determination of total flavonoid content

The hexane, chloroform and methanolic extracts of *A. lunulatum* and *P. acrostichoides* were subjected to quantification of flavonoids with quercetin as reference standard (Fig.2). The study presents that the methanolic extracts (Table 3) demonstrated higher flavonoid content in contrast to chloroform (Table 4) and hexane (Table 2; Fig. 2). The measurement of total flavonoids content was the highest value for the methanol extract of *A. lunulatum* (0.26 ± 0.002) at 500 µg/ml, and the lowest value for hexane extract (0.17 ± 0.003) at 500 µg/ml. The measurement of total flavonoids content was the highest value for the methanol extract of *P. acrostichoides* (0.25 ± 0.006) at 500 µg/ml, and the lowest value for hexane extract (0.17 ± 0.004) at 500 µg/ml. The hexane extracts had the least flavonoid content (Table 2; Fig. 2).

Table 2: Determination of flavonoid contents in the hexane extracts

Conc. µg/ml	Hexane Extract of *Adiantum lunulatum*				Hexane Extract of *Polystichum acrostichoides*			
	Trial 1	Trial 2	Trial 3	Mean with SD	Trial 1	Trial 2	Trial 3	Avg with SD
100	0.137	0.135	0.132	0.13 ± 0.003	0.127	0.122	0.123	0.12 ± 0.003
200	0.144	0.142	0.142	0.14 ± 0.001	0.132	0.129	0.130	0.13 ± 0.002
300	0.156	0.154	0.151	0.15 ± 0.003	0.147	0.139	0.144	0.14 ± 0.004
400	0.16	0.162	0.163	0.15 ± 0.002	0.151	0.150	0.154	0.15 ± 0.002
500	0.177	0.172	0.175	0.17 ± 0.003	0.169	0.166	0.161	0.17 ± 0.004

Table 3: Determination of flavonoid contents in the methanol extracts

Conc µg/ml	Methanol Extract of *Adiantum lunulatum*				Methanol Extract of *Polystichum acrostichoides*			
	Trial 1	Trial 2	Trial 3	Mean with SD	Trial 1	Trial 2	Trial 3	Avg with SD
100	0.095	0.094	0.084	0.09 ± 0.006	0.088	0.087	0.079	0.08 ± 0.005
200	0.133	0.132	0.133	0.13 ± 0.001	0.142	0.14	0.145	0.14 ± 0.003
300	0.177	0.176	0.178	0.18 ± 0.001	0.18	0.175	0.171	0.18 ± 0.005
400	0.206	0.205	0.209	0.21 ± 0.002	0.201	0.202	0.201	0.20 ± 0.001
500	0.264	0.265	0.262	0.26 ± 0.002	0.255	0.245	0.244	0.25 ± 0.006

Table 4: Determination of flavonoid contents in the chloroform extracts

Conc. µg/ml	Chloroform extract of *Adiantum lunulatum*				Chloroform Extract of *Polystichum acrostichoides*			
	Trial 1	Trial 2	Trial 3	Mean with SD	Trial 1	Trial 2	Trial 3	Avg with SD
100	0.086	0.085	0.088	0.09 ± 0.002	0.088	0.087	0.079	0.08 ± 0.005
200	0.115	0.104	0.108	0.11 ± 0.006	0.142	0.14	0.145	0.14 ± 0.003
300	0.154	0.153	0.155	0.15 ± 0.001	0.18	0.175	0.171	0.18 ± 0.005
400	0.171	0.169	0.17	0.17 ± 0.001	0.201	0.202	0.201	0.20 ± 0.001
500	0.222	0.221	0.222	0.22 ± 0.001	0.255	0.245	0.244	0.25 ± 0.006

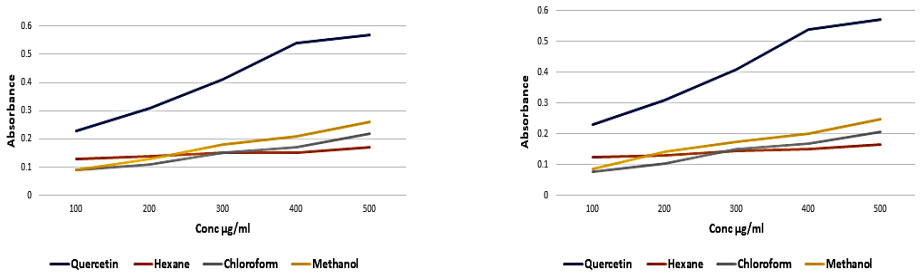


Fig. 2: Determination of total flavonoid content of the fern extracts in different solvents
(a) *Adiantum lunulatum* (b) *Polystichum acrostichoides*

Determination of total phenolic content

The hexane, chloroform and methanolic extracts of *A. lunulatum* and *P. acrostichoides* were subjected to quantification of phenolics with tannic acid as reference standard. The measurement of total phenolic content was the highest value for the methanol extract of *A. lunulatum* (0.45 ± 0.006) at 500 µg/ml, and the lowest value for hexane extract (0.29 ± 0.010) at 500 µg/ml. The measurement of total phenolic content was the highest value for the methanol extract of *P. acrostichoides* (0.39 ± 0.006) at 500 µg/ml, and the lowest value for hexane extract (0.28 ± 0.012) at 500 µg/ml (Table 6). The methanolic extracts displayed highest phenolic content (Table 6) as compared to chloroform (Table 7) and hexane (Table 5; Fig. 3).

Table 5: Determination of phenolic contents in the hexane extracts

Conc µg/ml	Hexane Extract of *Adiantum lunulatum*				Hexane Extract of *Polystichum acrostichoides*			
	Trial 1	Trial 2	Trial 3	Avg with SD	Trial 1	Trial 2	Trial 3	Avg with SD
100	0.08	0.07	0.07	0.07 ± 0.006	0.06	0.05	0.07	0.06 ± 0.010
200	0.09	0.08	0.07	0.08 ± 0.010	0.07	0.07	0.08	0.07 ± 0.006
300	0.12	0.12	0.11	0.12 ± 0.006	0.099	0.089	0.1	0.10 ± 0.006
400	0.22	0.21	0.23	0.22 ± 0.010	0.19	0.18	0.18	0.18 ± 0.006
500	0.3	0.29	0.28	0.29 ± 0.010	0.29	0.27	0.27	0.28 ± 0.012

Table 6: Determination of phenolic contents in the methanol extracts

Conc µg/ml	Methanol Extract of *Adiantum lunulatum*				Methanol Extract of *Polystichum acrostichoides*			
	Trial 1	Trial 2	Trial 3	Avg with SD	Trial 1	Trial 2	Trial 3	Avg with SD
100	0.22	0.22	0.2	0.21 ± 0.012	0.19	0.18	0.2	0.19 ± 0.010
200	0.29	0.27	0.26	0.27 ± 0.015	0.22	0.21	0.22	0.22 ± 0.006
300	0.31	0.29	0.32	0.31 ± 0.015	0.3	0.29	0.21	0.27 ± 0.049
400	0.39	0.38	0.4	0.39 ± 0.010	0.21	0.22	0.22	0.22 ± 0.006
500	0.45	0.44	0.45	0.45 ± 0.006	0.39	0.38	0.39	0.39 ± 0.006

Table 7: Determination of phenolic contents in the chloroform extracts

Conc µg/ml	Chloroform extract of *Adiantum lunulatum*				Chloroform extract of *Polystichum acrostichoides*			
	Trial 1	Trial 2	Trial 3	Avg with SD	Trial 1	Trial 2	Trial 3	Avg with SD
100	0.11	0.1	0.16	0.12 ± 0.032	0.09	0.08	0.15	0.11 ± 0.038
200	0.12	0.13	0.12	0.12 ± 0.006	0.098	0.097	0.13	0.11 ± 0.019
300	0.19	0.21	0.2	0.20 ± 0.010	0.16	0.17	0.17	0.17 ± 0.006
400	0.32	0.2	0.31	0.28 ± 0.067	0.28	0.27	0.28	0.28 ± 0.006
500	0.38	0.36	0.37	0.37 ± 0.010	0.3	0.29	0.31	0.30 ± 0.010

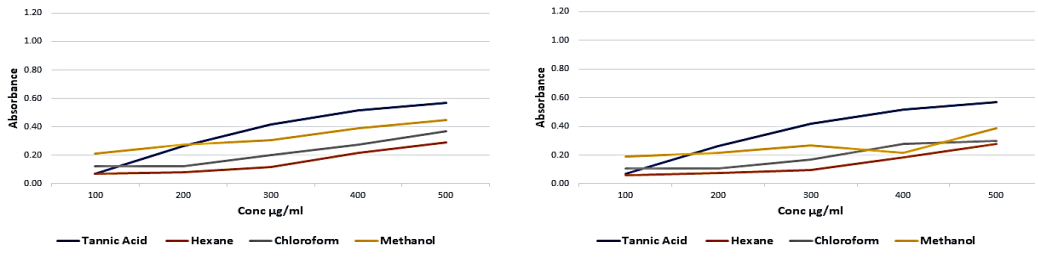


Fig. 3: Determination of total phenolic content of the fern extracts in different solvents
(a) *Adiantum lunulatum* (b) *Polystichum acrostichoides*

ABTS radical scavenging assay

The ability of plant extracts to function as antioxidants was evaluated using ABTS radical scavenging activity. ABTS is a blue-green chromophore that is reduced to a colorless form, and this test assesses an antioxidant compound's capacity to scavenge ABTS radicals and thereby prevents the development of the colorless form of ABTS. The degree of color reduction is proportional to the antioxidant activity of the tested extract. The methanolic extracts of *P. acrostichoides* and *A. lunulatum* aerial parts displayed the highest ABTS radical scavenging activity (Figs. 5 and 6). The standard Quercetin has showed IC50 value of 3.1795 µg/ml. The methanolic and chloroform extracts of *A.lunulatum* had shown activity having an IC50 value of 62.60, 163.09 µg/ml respectively (Fig. 4). The methanolic extract and chloroform extracts of *P. acrostichoides* have shown activity having an IC50 value of 77.75, 155.54 µg/ml respectively. The hexane extract has not shown significant activity in both the ferns. This suggests that these extracts have a stronger ability to scavenge free radicals and buffer against oxidative stress.

DPPH radical scavenging activity

DPPH is a stable free radical that changes color when reduced by an antioxidant. This approach is useful in determining a compound's ability to scavenge free radicals and prevent oxidative damage. Free radicals have the potential to harm cells and facilitates the progression of numerous chronic illnesses, including cancer, cardiovascular disease, and neurological disorders. Identification of natural antioxidant sources can therefore aid in the formulation of novel therapies for these disorders. The methanolic and chloroform extracts of *A.lunulatum* have shown activity having an IC50 of 61.63 and 134.161 µg/ml respectively (Fig. 6). The methanolic and chloroform extracts of *P.acrostichoides* have shown activity having an IC50 value of 64.71 and 128.54 µg/ml respectively (Fig. 7). The hexane extract has not shown significant activity in both the ferns. The findings show that the methanolic extracts of the ferns have the highest antioxidant potential, which can be attributed to their high phenolic and flavonoid content.

Hydroxyl radical scavenging activity

Due to their great reactivity and capacity to damage essentially any biological molecule, hydroxyl radicals are regarded as one of the most destructive reactive oxygen species. In the present study, the standard Catechin has showed IC50 value of 4.218 µg/ml. The methanolic and chloroform extracts of *A.lunulatum* have shown activity having an IC50 value of 54.19 and 75.76 µg/ml respectively (Fig. 8). The methanolic and chloroform extracts of *P. acrostichoides* have shown activity having an IC50 value of 53.71 and 76.58 µg/ml respectively (Fig. 9). The hexane extract has not shown significant activity in both the fern extract

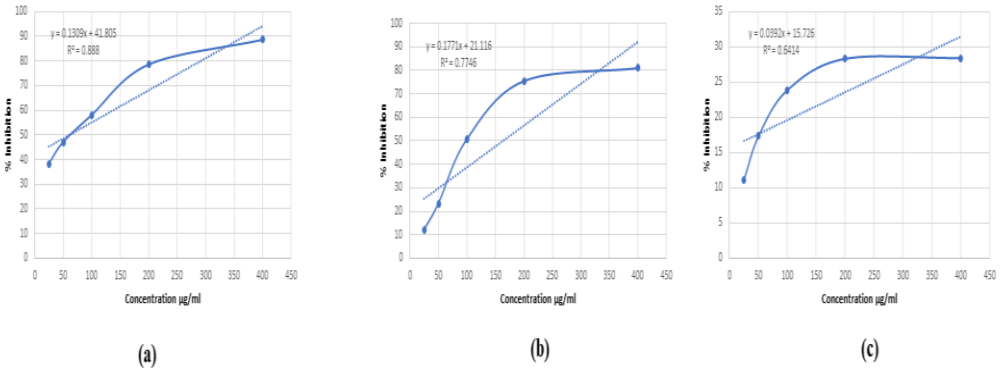


Fig. 4: ABTS radical scavenging activity of *Adiantum lunulatum* extracts
(a) Methanol extract (b) Chloroform extract (c) Hexane extract

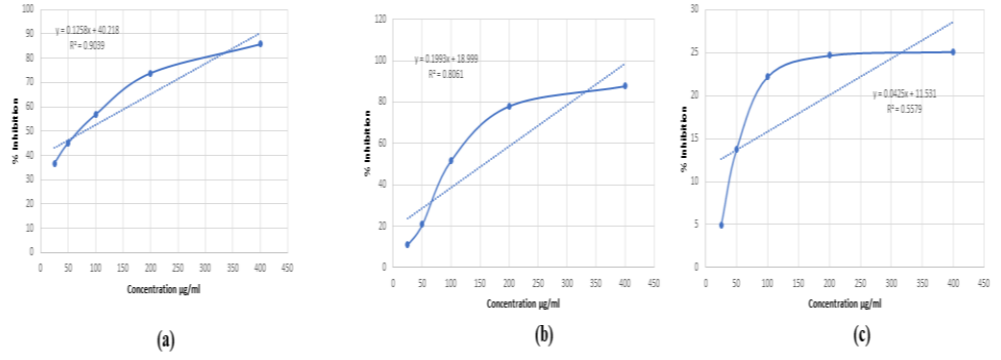
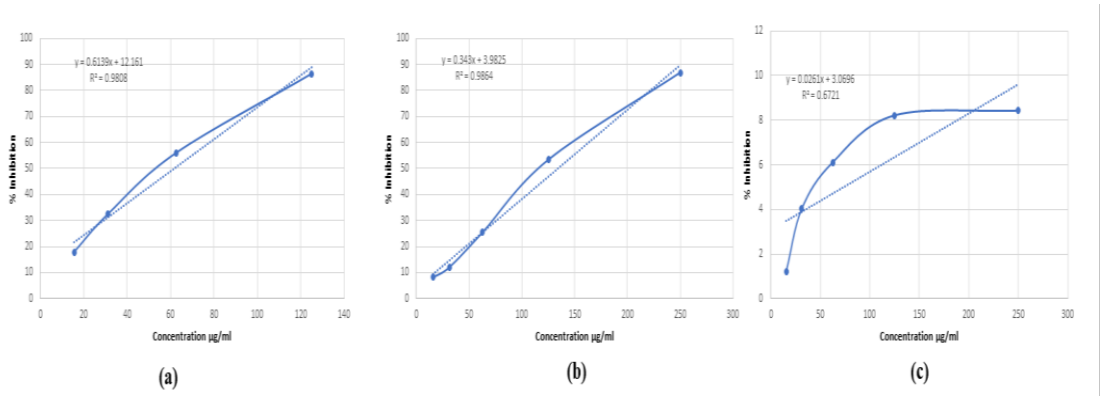
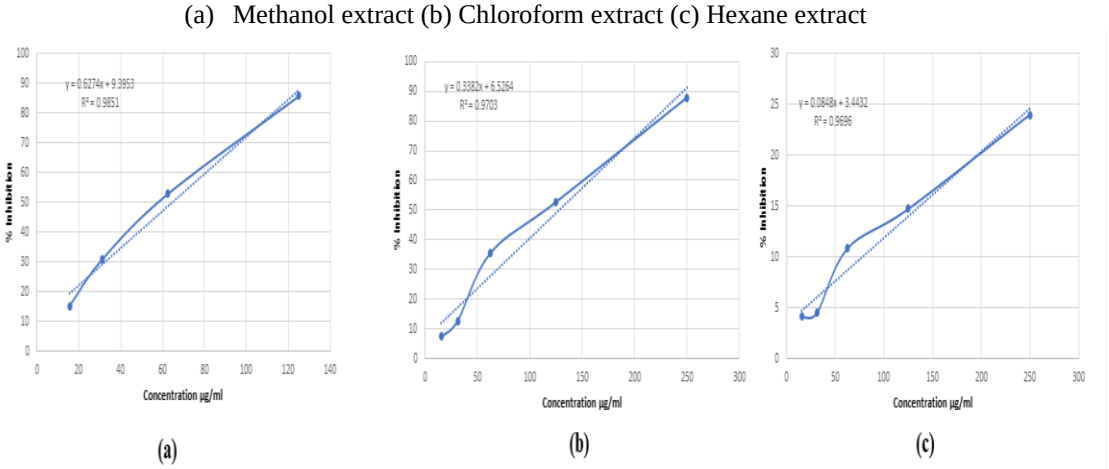


Fig. 5: ABTS radical scavenging activity of *Polystichum acrostichoides* extracts
(a) Methanol extract (b) Chloroform extract (c) Hexane extract



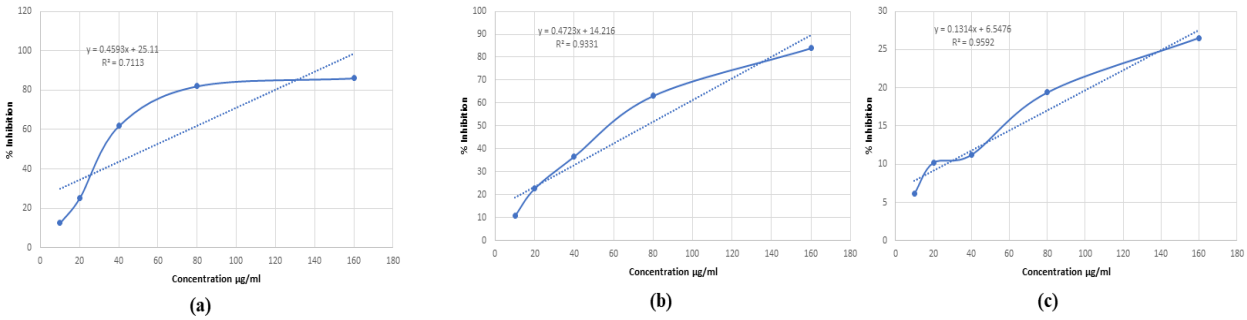
(a) (b) (c)

Fig. 6: DPPH radical scavenging activity of *Adiantum lunulatum* extracts
(a) Methanol extract (b) Chloroform extract (c) Hexane extract



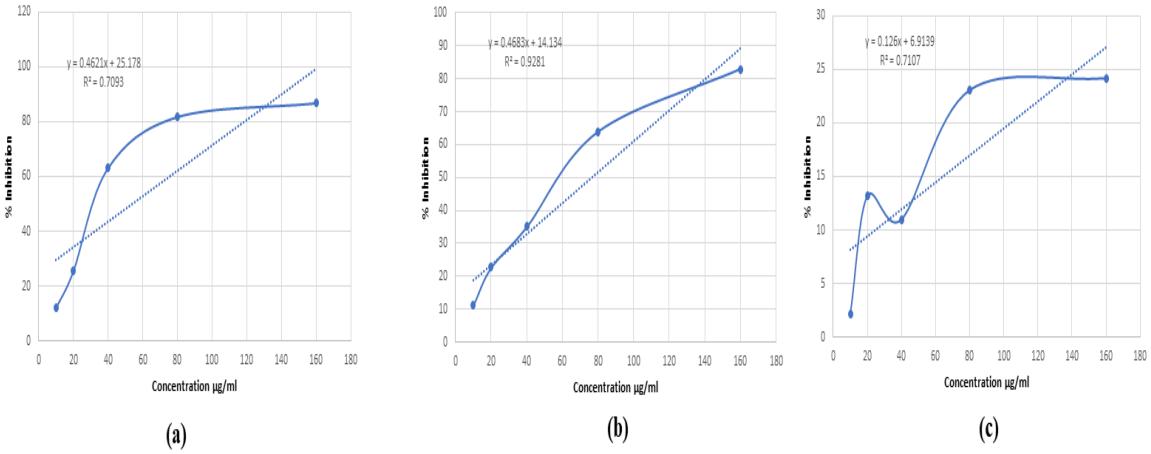
(a) (b) (c)

Fig. 7: DPPH radical scavenging activity of *Polystichum acrostichoides* extracts
(a) Methanol extract (b) Chloroform extract (c) Hexane extract



(a) (b) (c)

Fig. 8: Hydroxyl radical scavenging activity of *Adiantum lunulatum* extracts
(a) Methanol extract (b) Chloroform extract (c) Hexane extract
(b)



(a) (b) (c)

Fig. 9: Hydroxyl radical scavenging activity of *Adiantum lunulatum* extracts
Methanol extract (b) Chloroform extract (c) Hexane extract

DISCUSSION

Free radicals are extremely reactive substances with an unpaired electron in their outer orbit. They are generated either because of being exposed to external variables including pollutants, irradiation, and cigarette smoke or as a resultant of normal physiological activities like respiration, metabolism, and immunological response (20). The unpaired electron in free radicals renders them unstable and highly reactive, and they tend to react with other molecules to restore their stability. By oxidizing numerous biological components such as DNA, lipids, proteins, and carbohydrates, free radicals can cause cellular dysfunction, inflammation, and cell death. Oxidative stress is one of the key processes through which free radicals inflict cellular damage. This process has been linked to the development of many ailments, including cancer, cardiovascular disease, neurodegenerative diseases, diabetes, and inflammatory disorders (21).

Free radicals can also induce cellular damage by activating inflammatory pathways. The production of pro-inflammatory cytokines can be stimulated by free radicals, which can result in an inflammatory response that can exacerbate tissue damage. Several pathologies, including rheumatoid arthritis, asthma, and atherosclerosis, have been attributed to chronic inflammation. Free radicals can also harm cellular membranes, altering membrane permeability and permitting the release of intracellular substances (22). This process may result in cellular instability and accelerate the onset of neurodegenerative pathologies such as Parkinson's and Alzheimer's. Free radicals can also harm cellular organelles, such as mitochondria, which are essential for cellular energy generation and survival (23). Many illnesses, such as cardiovascular diseases and neurological disorders, have been linked to mitochondrial abnormalities (21).

To prevent free radical damage and lower the chance of contracting numerous diseases, antioxidants are crucial. Identification of naturally occurring chemicals with antioxidant characteristics, such as those found in plant-based sources like ferns, may provide substantial opportunities for the development of novel and effective treatment modalities against oxidative stress-related pathologies (12,24). Ferns have hoistorically valued for their therapeutic benefits, therefore in the present study the antioxidant potency of *A. lunulatum* and *P.acrostichoides* were investigated. *A. lunulatum* (Hansraj), has been utilized in Ayurveda medicine to treat respiratory illnesses due to its high flavonoids and alkaloids content (25, 26). Native Americans, on the other hand, have utilized *P. acrostichoides* to treat a variety of conditions, including fever, rheumatism, and skin diseases. The plant includes several pharmacologically potent substances, including tannins, flavonoids, and saponins, which have been demonstrated to have potent antibacterial, antipyretic, and analgesic activities (27).

The current study exemplifies that methanol was the most suitable extract with highest yield (Table 1) to ascertain the antioxidant potency of the fern. In the present study we have investigated the ABTH, DPPH and radical scavenging activity of the fern extracts. It was observed that the ABTS scavenging activity was predominantly better in the methanolic extracts of *A.lunulatum* (62.50µg/ml) and *P.acrostichoides* (77.75 µg/ml). Similar results were noticed in the DPPH and hydroxy radical scavenging assay wherein the methanolic extracts exhibited 61.33 and 64.71 µg/ml in DPPH assay and 54.19 and 53.71 µg/ml in hydroxyl radical scavenging assay. The present study highlights the potential of ferns as a natural source of antioxidants and suggests the possibilities of developing novel and effective treatment against oxidative-stress related pathologies. These findings provide valuable insights for the development of alternative therapies to combat the detrimental effects of free radicals in various disorders.

CONCLUSION

Free radicals are highly reactive and unstable chemicals that have the potential to induce cellular dysfunction, inflammation, and cell death. Antioxidants are essential for preventing free radical deterioration and lowering the chance of getting oxidative stress-related disorders. The current work employed various in vitro assays to assess the antioxidant activity of the ferns, *Adiantum lunulatum* and *Polystichum acrostichoides*. The study's findings indicate that these ferns have high antioxidant capacity, as seen by their ABTS, DPPH, and hydroxyl radical scavenging capabilities. Our findings imply that these ferns may serve as useful sources of free radical scavengers for treating oxidative stress-related illnesses. Future studies should concentrate on finding the active substances responsible for the antioxidant activity of these ferns and investigate their mechanisms of action and efficacy in in-vivo setting. This might result in the creation of fresh, efficient remedies for conditions driven by oxidative stress. Essentially, the current study lays the groundwork for future research into the therapeutic potential of ferns as a natural antioxidant source.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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