Phytochemical Constituents and in-vitro antioxidant Activity of *Aleuritopteris bicolor* Leaves, *Crinum amoenum* Bulbs, and *Drynaria coronans* Rhizomes of Nepal

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ABSTRACT

Background: Due to plant derived chemicals's potential as antioxidant agents, there has been a growing interest in using them to cure or prevent diseases. Similarly plant species like *Aleuritopteris bicolor(*AB) leaves,*Crinum amoenum*(CA) bulbs and *Drynaria coronans (*DC) rhizomes are used as traditional herbal medicine in Nepal.

Aims: This study aims to assess the qualitative and quantitative phytochemical constituents along with the antioxidant potency of extracts from the leaves of AB, bulbs of CA, and rhizomes of DC.

Methods and Materials: The qualitative phytochemical profile was assessed using thin-layer chromatography (TLC) and standard phytochemical tests. TLC was performed on silica gel 60 F254 plates (20×20 cm, layer thickness: 0.2 mm) using a solvent system of chloroform: methanol: water at (6:4:1) ratio. Plates were visualized under UV light (254 nm and 365 nm) and further developed using 1% $FeCl_3$, 10% H_2SO_4, and DPPH for antioxidant activity. Quantitative analysis of total flavonoid and phenol content was conducted using aluminum chloride and Folin-Ciocalteu reagents, respectively. The antioxidant activity was measured through the DPPH free radical scavenging assay.

Results: The selected species were found to contain flavonoid, phenol, saponin and tannin. Higher flavonoid and phenol content was found in the leaves of AB (398.861 ± 6.94 mg quercetin/ g dry extract) and rhizomes of CA (172.97 ± 1.777 gallic acid/ g dry extract) respectively whereas leaves of AB had the most potent antioxidant activity (IC_{50}= 3.233 µg/ml).

Conclusions: All the selected plant species were found to have significant constituents. Among them, the leaves of AB extract had the highest flavonoid concentration and the higher antioxidant activity, highlighting its potential for further medicinal use.

Keywords: Phytochemical screening, antioxidant, *Aleuritopteris bicolor* leaves, *Crinum amoenum* bulbs, *Drynaria coronans* rhizomes

Key Messages: This is a novel study which shows the importance of TLC evaluation of selected species from Nepal. This study could act as a basis for biological studies depending upon the different phytochemical estimation and antioxidant activity.

INTRODUCTION

Phytochemicals are the metabolites of plant species that have the potential to fight against diseases. These phytochemical constituents show antioxidant effects through which various diseases are inhibited (1,2). Since the beginning of human history, herbal plants have been a component of alternative treatment plans for various ailments (3). It is estimated that 70–80% of Asia's rural population uses medicinal herbs to treat medical conditions (4). Despite established ethno-medicinal uses of plants, a large array of herbal species around the world

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still have not been scientifically investigated (5).

Nepal, which ranks ninth among Asian nations for its floral richness, hosts 1792 to 2331 species of beneficial medicinal and aromatic plants. Among them some of this includes *Aleuritopteris bicolor* (AB) leaves, *Crinum amoenum* (CA) bulbs, and *Drynaria coronans* (DC) rhizomes (Table 1). However, only a small fraction of the plant of Nepal has been scientifically studied for their medicinal properties (3). Given the rich ethnomedicinal tradition and the potential health benefits of these plants, scientific research should prioritize the study of herbal plants. Thus, scientific studies should be concentrated on herbal plants. Following this, the current study was conducted to evaluate the phytochemical makeup of species and their response toward oxidative stress in order to determine its scientific validity. The evaluation involves thin-layer chromatography (TLC) for qualitative analysis and specific assays to determine flavonoid and phenol content, as well as antioxidant activity of the Nepalese species, to establish the scientific validity of these traditional herbal medicines from Nepal.

Figure A*: Aleuritopteris bicolor*

Figure B*: Crinum amoenum*

Figure C*: Drynaria coronans*

Table 1. Selected Nepalese plant species

SN	Species Name	Family	Local Name	Traditional Uses
1	*Aleuritopteris bicolor* (Roxb.) Fraser.Jnek	Pteridaceae	Dankernu(6)	Diarrhea,Dysentery, Gastritis (6), Cuts, fever, sinusitis(7)
2	*Crinum amoenum*	Amaryllidaceae	Hade Lasun (8)	Cholera(8)
3	*Drynariacoronans*(Wall.ex Mett.) J.Sm.exT.Moore	Pteridaceae	Kamaru(9)	Diarrhea, Constipation(9)

METHODS AND MATERIAL:

Plant Materials Collection

Under the guidance of a traditional healer, plants were gathered from Lekhnath region, Kaski district, Nepal. Species AB and DC were identified and authenticated by the taxonomist Mr. Dhan Raj Kandel (Reference No. 74) of the National Herbarium and Plant Laboratories, Godavari, Lalitpur, Nepal while the species CA was identified and authenticated by Mr. Dharmaraj Koirala (Reference No. 11) of Department of Plant Resources, Raniban, Pokhara, Nepal. The species were then registered and preserved at the Crude drug museum of Pharmacognosy laboratory, School of Health and Allied Sciences, Pokhara University under voucher numbers PUH-2022-39 (for AB), PUH-2022-41 (for CA), and PUH-2022-40 (for DC). Fresh and disease-free plants were chosen for the experiments. The leaves of

AB, bulbs of CA, and rhizomes of DC were used for the estimation of phytochemical constituents and evaluation of in-vitro antioxidant activity.

Preparation of Extract of Plant Species

Into labeled conical flasks, 80 grams of powdered dried samples from each species were poured. The samples were then cold-macerated in 70% ethanol in a 1: 10 w:v ratio. Maceration was carried out for three days with intermittent shaking. Filtration was carried out using Whatman No. 1 filter paper. The filtrates were concentrated at reduced pressure at 40°C using a rotary evaporator to obtain extracts which were further dried in a vacuum desiccators (10).

Thin Layer Chromatography (TLC) Profiling

One grams of the each dried extract from the leaves of **Aleuritopteris bicolor** (AB), bulbs of **Crinum amoenum** (CA), and rhizomes of **Drynaria coronans** (DC) was dissolved in 2 mL of methanol as sample solutions. The TLC profiling was conducted on Merck Silica Gel 60 F254 plates (analytical grade, silica gel with fluorescent indicator, aluminum backing, 20×20 cm size, 0.2 mm layer thickness, 10-12 µm particle size). The sample solution was applied to the TLC plats as spots where solvent mixture used for development was chloroform, methanol, and water in a ratio of 6:4:1. The plates were then developed for approximately 30 minutes and observed under UV light at 254 nm and 365 nm. After visualization, the plates were sprayed separately with 1% aqueous $FeCl_3$, 10% H_2SO_4 (followed by heating), and slightly submerged in a 60 mM methanolic solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). The TLC profile revealed spots corresponding to different phytochemicals: polar compounds appeared in the upper layer of the plate, observed under UV light; flavonoids (yellow spots) and saccharides (black spots) were visible after treatment with 10% H_2SO_4 and heating; and phenols were detected as brown spots after spraying with 10% $FeCl_3$ (11).

Phytochemical Analysis

Alkaloids, carbohydrates, flavonoids, glycosides, phenols, saponins and tannins were evaluated qualitatively by wagner, fehling, Alkaline reagent, salkowski's, sodium hydroxide, lead acetate, and ferric chloride test respectively. Wagner's reagent (Loba Chemie Pvt. Ltd., Mumbai, India), Fehling's solution (Central Drug House (P) Ltd., New Delhi, India), alkaline reagent (Nice Chemicals Pvt. Ltd., Cochin, India), Salkowski's reagent (Himedia Laboratories Pvt. Ltd., Mumbai, India), Sodium hydroxide (Qualigens Fine Chemicals ,Thermo Fisher Scientific), Mumbai, India), Lead acetate (Thomas Baker (Chemicals) Pvt. Ltd., Mumbai, India) and ferric chloride (Nice Chemicals Pvt. Ltd., Cochin, India)(12).

Determination of Total Flavonoid Contents

With few modifications, the aluminum chloride colorimetric approach was employed to determine the presence of flavonoids. In brief, 4 ml of distilled water was added to 1 ml of extract (1000 µg/mL in ethanol) and then 0.3 ml of sodium nitrite (5% w/v in distilled water) was added. After 5 minutes, 0.3 ml of aluminum chloride (20% w/v in distilled water) was added, and the mixture was let to stand for 6 minutes. 2 ml of sodium hydroxide (1M in distilled water) was then added. The mixture was vortexed and the absorbance was recorded at 510 nm against a blank using single beam UV-visible spectrophotometer (Agilent Cary-60, Malaysia). A calibration curve was drawn using quercetin (25µg /ml-500 µg /ml in ethanol) as standard, the total flavonoid concentration was reported as mg quercetin equivalent per gram dry extract (13).

Determination of Total Phenol content

According to the method,(14) with some modifications, the Folin Ciocalteu method was employed to determine total phenols. In brief, 5 ml of distilled water, 1 ml of Folin reagent (2N in distilled water), and 1 ml of test solution (1000 µg/mL) were combined. After 5 minutes of standing, 1 ml of 10% sodium carbonate was added and mixed. The mixture was incubated for an hour at room temperature, after which the absorbance at 725 nm was measured in comparison to a blank. The total phenol content was calculated using the Gallic acid (12.5 µg/mL

- 500 µg/mL) standard instead of test solution to draw a calibration curve and was represented as milligrams of Gallic acid equivalent per gram dry extract.

Antioxidant activity

A 0.1 mM DPPH (2,2-diphenyl-1-picrylhydrazyl) solution was freshly prepared by dissolving DPPH in methanol to evaluate the free-radical scavenging activity of the extracts. The plant extracts were diluted in methanol to concentrations ranging from 1.56 to 25 µg/mL, and ascorbic acid was used as a positive control within the same concentration range. In the assay procedure, 1 mL of each extract or control solution was mixed with 1 mL of the DPPH solution in a cuvette. The mixture was then incubated in the dark at room temperature for 30 minutes to facilitate the reaction. Following incubation, the absorbance of the solution was measured at 517 nm using a UV-visible spectrophotometer to determine the scavenging activity (15).

Scavenging activity (%) = $\frac{A-B}{A} \times 100\%$

Where A represents the absorbance of the control (DPPH solution without the sample) and B represents the absorbance of the DPPH solution in the presence of the sample (extracts/ascorbic acid).

Statistical analysis

Antioxidant activity was assessed in triplicate for accuracy and reproducibility. Data analysis using Microsoft Excel 2021 included calculating the mean and standard deviation. Results were reported as mean ± standard deviation to facilitate comparisons and validate findings.

RESULTS

Extraction Yield Value

The extractive values (% yield) of plant species using 70 % ethanol was found to be higher in bulbs of CA followed by leaves of AB and rhizomes of DC yielded the least amount of extract (Table 2).

Table 2. % Yield of extract in 70% ethanol solvent

Plant species	Parts used	% Yield by solvent
AB	Leaves	**11.23%**
CA	Bulbs	**13.55%**
DC	Rhizomes	**5.22%**

TLC Profiling

Figure 1 and Figure 2 show the TLC profiling of three separate extracts using three different sprays in the solvent system of chloroform, methanol, and water (6:4:1). The spot codes for the three different species are depicted as 1 (CA bulbs), 2 (AB leaves), and 3 (DC rhizomes).

$CHCl_3$: MeOH: H_2O (6:4:1)

From the Figure 1of the TLC profile, spots can be detected in the upper layer of the TLC plate as a result of the solvents being more polar, when observed at 254 nm and 365 nm.

10% (v/v) H_2SO_4 / heat

The majority of the plant extract demonstrated the presence of flavonoids (yellow-colored spots) and saccharides (black colored spots) on the TLC plate after spraying 10% $H_2SO_4$and heating. (Figure 2).

10% (w/v) aqueous Ferric chloride

The pre Figure 1 sence of phenols was detected as brown colored spots on TLC plate after spraying with 10% $FeCl_3$ (Figure 2).

DPPH Solution

After dipping in DPPH solution in the dark, observation of pale yellow or no colour on the TLC plate suggested the presence of potent antioxidants in AB leaves extract, CA bulb extract as represented by Figure 2.

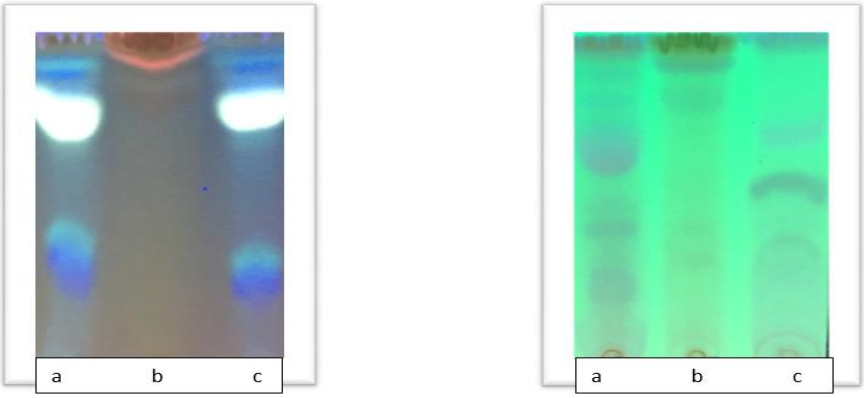

Figure 1. TLC plate with three different spots from three different species a) CA bulbs b) AB leaves c) DC rhizome observed in UV 365 nm and s UV 254 nm respectively

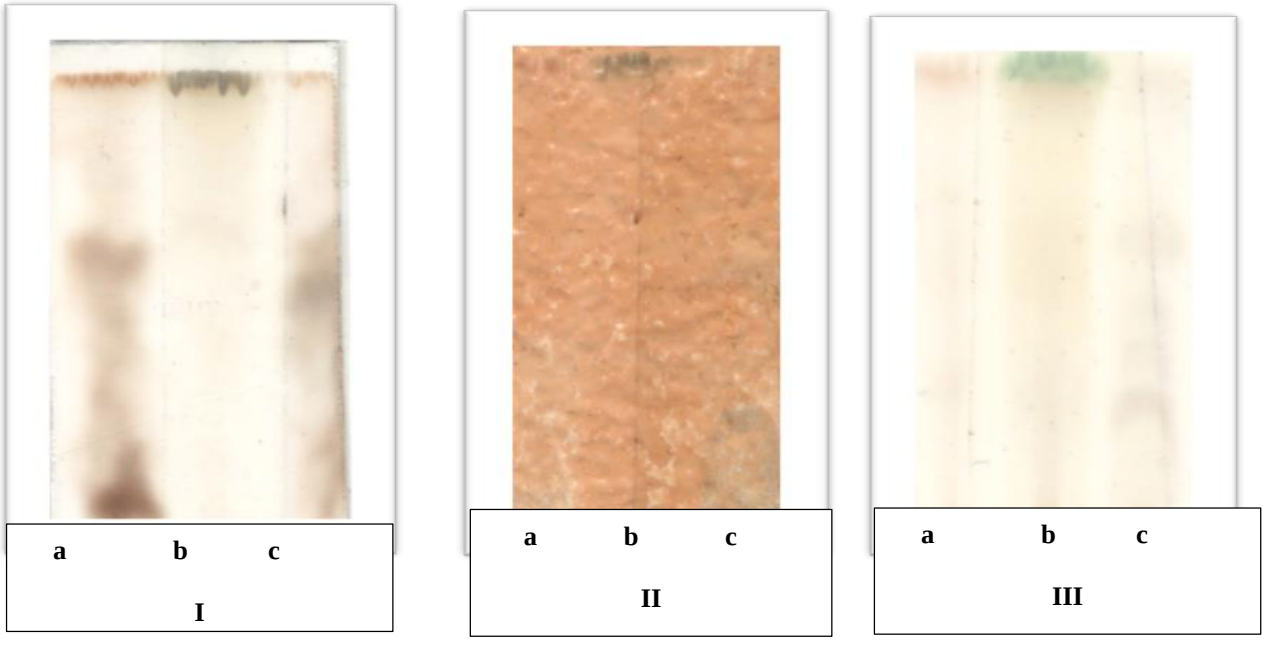

Figure 2. TLC plate with three different spots from three different species a) CA, bulbs b) AB, leaves c) DC, rhizomes sprayed with (I) 10% H_2SO_4 /Heat, (II) 10% $FeCl_3$, (III) DPPH

Phytochemical test

The extracts from the plant species AB, CA, and DC were subjected to various phytochemical tests and the tests revelaed the presence of flavonoids, phenols, saponins, and tannins as indicated in Table 3.

Table 3. Qualitative phytochemical data of plant species extract

Phytochemical Constituent	Test Conducted	Results of Different Plant species extract		
		AB leaves	CA bulbs	DC rhizomes
Alkaloids	Wagner test	-	+	+
Flavonoids	Alkaline reagent test	+	+	+
Phenols	Sodium hydroxide test	+	+	+
Tannins	Ferric chloride test	+	+	+
Glycosides	Salkowski's test	-	+	+
Saponins	Lead acetate test	+	+	+
Carbohydrates	Fehling test	-	+	+

Note: + present, - absent

Total flavonoids content

The total amount of flavonoids, which is expressed as mg quercetin equivalent (GAE)/g of dry weight of extract, was calculated using Quercetin. The table provides quantitative data regarding the flavonoids content in the extract of AB leaves, CA bulbs, and DC rhizomes. The results show that the leaves of AB had the highest content of flavonoids in the ethanolic extract as shown in Table 4.

Table 4. Total flavonoids content expressed as mg Quercetin equivalents per gram dry weight of extract

	Sample Plant	Parts used	Total flavonoid content mg QE/g dry weight of extract
1	Aleuritopteris bicolor	Leaves	398.86±6.94
2	Crinum amoenum	Bulbs	260.16±5.05
3	Drynaria coronans	Rhizomes	178.60±0.57

Note: Data expressed as mean ± standard deviation (n=3).

Total Phenolic Content

The total amount of phenol, which is expressed as mg Gallic acid equivalent (GAE)/g of dry weight of extract, was calculated using Gallic acid. The Table 5 provides quantitative data regarding the phenol content in the extract of AB leaves, CA bulbs, and DC rhizomes in which the ethanol extract from CA bulb was found to have the highest phenol content.

Table 5. Total phenol content expressed as mg phenol equivalent per gram dry weight of extract

	Sample Plant	Parts used	Total phenol content mg GAE/g dry weight of extract
1	Aleuritopteris bicolor	Leaves	28.13±0.57
2	Crinum amoenum	Bulbs	172.9± 1.77
3	Drynaria coronans	Rhizomes	52.41± 0.47

Note: Data expressed as mean ± standard deviation (n=3).

DPPH Free Radical Scavenging Analysis

Through DPPH scavenging activity, the antioxidant activity of the plant extract was assessed. Five concentrations (25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.125 µg/ml, and 1.56 µg/ml) were used for the computation of the IC_{50} value of the extract and ascorbic acid, as shown in Table 6. When compared to other extracts (CA bulbs extract 21.3 µg/ml, DC rhizomes extract.>25 µg/ml), the extract of AB leaves was shown to have stronger antioxidant activity with an IC_{50} value of 3.233 µg/ml, which was even potent than ascorbic acid (8.94 µg/ml).

Table 6. DPPH radical scavenging activity of the extract and ascorbic acid at different concentration

(%) DPPH Scavenging Activity of Ethanol extract						IC_{50} value
Sample	1.56 µg/ml	3.125 µg/ml	6.25 µg/ml	12.5 µg/ml	25 µg/ml	**µg/ml**
Ascorbic acid	12.26±0.049	21.93±0.2	40.27±1.36	66.05±0.48	81.51±1.51	8.94
AB leaves extract	41.26±0.20	50.79±0.178	63.80±0.54	85.95±0.05	88.78±0.21	3.23
CA bulbs extract	29.27±0.129	32.97±0.32	36.71±0.34	42.53±0.17	52.79±0.16	21.33
DC rhizomes extract	3.59±0.13	8.33±0.045	14.60±0.182	23.37±0.09	34.37±0.16	>25

Note: Data expressed as mean ± standard deviation (n=3)

DISCUSSION

In the present investigation, qualitative phytochemical test, total flavonoid and phenolic content, antioxidant activity of the chosen plant extracts was evaluated. Due to their low cost, easy accessibility, and few side effects, herbal plants have played a significant role as alternate sources for the management of numerous illnesses and conditions (16). The pharmacological effectiveness of many of these plants has not been scientifically studied, despite the widespread use of herbal plants for the treatment of various illness problems (5).

Secondary plant metabolites play a crucial role in defensive mechanisms of plants against microbes, prey, stress, and interspecies protection. Saponins, flavonoids, tannins, terpenoids, steroids, and alkaloids are secondary metabolites present in plants, that give herbal medicines their anti-diabetic, anti-parasitic, anti-diarrheal, anti-inflammatory, anti-ulcer, anti-malarial, anticancer, nephroprotective, and hepatoprotective activities (17).

The presence of components in the mixture, their purity, and the identity of the compounds are all revealed by the TLC profile of the herbal species (18). Similarly, phytochemical tests determine the types of secondary metabolite found in a species (12). Figure 1 Figure 1 of the extract and their numerous colored spots showed the presence of a variety of compounds like presence of saccharides, represented by black spot, brown and light yellow represent presence of phenol and flavonoids respectively (11). Similarly the current study on CA bulb and DC rhizome extract shows black spot that represent the saccharides whereas the leaves of AB shows the light yellow which represent the flavonoids in Figure 1 and Figure 2. Additional phytochemical testing reveals presence of phenols, flavonoids, alkaloids, tannins, and saponins in all of species extract and the absence of carbohydrates in the leaves of AB Table 3. These findings are consistent with previous reports and demonstrate the diversity of secondary metabolites presents in these plants extracts, thus indicating the widespread occurrence of these bioactive compounds in medicinal plants (19) (20) (21). The presence of phenols, flavonoids, alkaloids, tannins and saponins in the extracts underscores their potential pharmacological activities, including antioxidant, anti-inflammatory and antimicrobial properties (22) (2). These finding further support the traditional use of herbal plants for medicinal purposes and highlight the importance of exploring their therapeutic through scientific investigation. Quantitative analysis of flavonoids and phenols content in selected extract of plant shows that AB has the highest flavonoid content Table 4 consistent with previous report (21) 429.16 ± 7.21 µg QE/mg of extract, signifying its potential therapeutic significance and this continuity in flavonoid content underscores the reliability and reproducibility of our analytical methods . However, the phenol content of CA extract in present study Table 5, significantly contrasts with previous finding (21) 25.22 ± 2.41 µg GAE /mg of extract indicating a notable increase.

The ability of plant extract to scavenge free radicals was evaluated using the DPPH model. This is a common method used to assess the antioxidant activity of plant extracts, especially when phenolic chemicals are included (22). Half-maximal inhibitory concentration, or IC_{50}, is one of the parameters used to interpret the results of DPPH

scavenging activity. This is described as the amount of medication or inhibitor required to completely stop a biological response (23).

In the current investigation, the IC50 was determined by plotting the percentage of radical scavenging activity against the concentration of the investigated plant extract using the linear regression approach. As shown in Table 6, selected plant extracts demonstrated positive response as antioxidant activity against free radical. The leaves extract of AB demonstrated the highest DPPH scavenging activity when compared to other plant extracts which was greater than that Ascorbic acid (8.94 µg/ml). This highlights the potent antioxidant potential of AB leaves, which could be attributed to the presence of high levels of flavonoids, as indicated in our previous discussion. Comparing our present results with previous studies (21) (20), significant variations emerge. In the earlier studies, AB demonstrated an IC_{50} value of 46.76 µg/ml, significantly lower than the current finding. Additionally, CA exhibited a substantially lower IC_{50} value of 661.76 µg/ml in the previous study, contrasting with present observations as shown in Table 6. Remarkably, DC displayed the lowest antioxidant activity in present study: > 25µg/ml and previous study: 93.30± 5.19 µg/ml (21) when compared with others species. These differences may arise from several factors, including differences in plant material preparations, extraction technique, geographical variations and assay conditions.

The potent antioxidant activity of AB leaves in our present study underscores their potential for pharmaceutical and nutraceutical applications, particularly in combating oxidative stress-related diseases. As reported flavonoids, flavonol O-glycosides, petrosins, sitosterols are present in Pteris family which shows the potent role as antioxidant activity(24) and might be there is also a presence of some flavonoid compound along with some other secondary metabolites in AB like *Cheilanthes tenuifolia* which consists of quercetin and rutin that is also responsible to show higher DPPH scavenging activity.(25) The finding of this study align with previous study (26) introducing new insights and emphasizing the need for further research to explore the therapeutic application of these plant species.

CONCLUSION

Each of the three extracts from AB leaves, CA bulbs, and DC rhizomes shows the presence of different phytochemical components as well as potent DPPH radical scavenging activity. Among them, AB leaves extract exhibited the highest flavonoid content which might correlate with the highest antioxidant activity. Additionally, more in vivo research is needed to confirm the extract's precise efficacy of these species. Therefore, additional isolation and purification of the extract's constituents is needed in order to analyze the chemical that is in charge of the biological effects.

Abbreviations

AB - *Aleuritopteris bicolor*
CA - Crinum amoenum
DC - Drynaria coronans
TLC - Thin Layer Chromatography
UV - Ultraviolet
FeCl3 - Ferric Chloride
DPPH - 2,2-diphenyl-1-picrylhydrazyl
GAE - Gallic Acid Equivalent
QE - Quercetin Equivalent
IC50 - Half-maximal inhibitory concentration

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Conflict of Interest

We declare that we don't have conflict of interest.

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تقييم المكونات الكيميائية النباتية النوعية والكمية والنشاط المضاد للأكسدة في المختبر لمستخلصات أوراق Aleuritopteris bicolor ودرنات Crinum amoenum وجذور Drynaria coronans

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ملخص

نظرًا للإمكانات العلاجية لمركبات النباتات كمضادات للأكسدة، هناك اهتمام متزايد باستخدامها في علاج أو الوقاية من الأمراض. يتم استخدام نباتات مثل *Aleuritopteris bicolor* (AB)، و*Crinum amoenum* (CA)، و *Drynaria coronans* (DC)في الطب العشبي التقليدي في نيبال. تهدف هذه الدراسة إلى تقييم المكونات الكيميائية النباتية النوعية والكمية إلى جانب فعالية مضادات الأكسدة في مستخلصات أوراق AB ودرنات CA وجذور DC. تم تقييم التحليل الكيميائي النوعي باستخدام تقنية كروماتوغرافيا الطبقة الرقيقة (TLC) والاختبارات الكيميائية القياسية، وتم إجراء TLC على صفائح سيليكا جل F254 باستخدام نظام مذيب من الكلوروفورم:ميثانول:ماء (6:4:1)، وتمت ملاحظة الصفائح تحت الأشعة فوق البنفسجية (254 و365 نانومتر) وتطويرها باستخدام كلوريد الحديديك 1%، حمض الكبريتيك 10%، ومحلول DPPHلنشاط مضادات الأكسدة. أُجري التحليل الكمي لمحتوى الفلافونويد والفينولات باستخدام كاشف كلوريد الألمنيوم وكاشف فولين–سيوكالتي، على التوالي. تم قياس نشاط مضادات الأكسدة باستخدام اختبار DPPH للجذور الحرة. أظهرت النتائج أن الأنواع المختارة تحتوي على مركبات الفلافونويد والفينولات والصابونين والعفص، وكان لأوراق ABأعلى محتوى من الفلافونويد (398.861 ± 6.94 ملغم كيرسيتين/غرام من المستخلص الجاف) وأظهرت أيضًا أقوى نشاط مضاد للأكسدة (IC50 = 3.233 ميكروغرام/مل).تُبرز النتائج إمكانات مستخلص أوراق ABللاستخدام الطبي المستقبلي.

الكلمات الدالة: تحليل كيميائي نباتي، مضادات الأكسدة، أوراق Aleuritopteris bicolor، درنات Crinum amoenum، جذور Drynaria coronans.

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