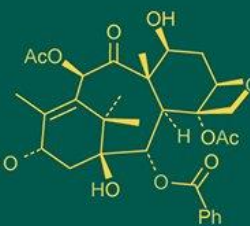
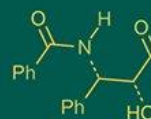
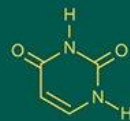


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; SP-10(7): 1328-1333
www.biochemjournal.com
Received: 16-05-2026
Accepted: 20-05-2026

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Assessment of free radical scavenging activity of hydromethanolic leaf extract of *Saussurea costus*

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i7Sp.9231>

Abstract

Saussurea costus (Falc.) Lipsch. is a critically endangered Himalayan medicinal plant valued for its rich phytochemical composition and diverse therapeutic properties. The present study evaluated the antioxidant potential of the hydromethanolic leaf extract of *S. costus* collected from the Deoban region of Uttarakhand, India. Dried leaf powder was extracted using 80% methanol, yielding 10.9% (w/w) extract. Antioxidant activity was assessed through DPPH and ABTS radical scavenging assays using ascorbic acid and quercetin as reference standards, respectively. The leaf extract exhibited concentration-dependent radical scavenging activity in both assays. In the DPPH assay, the extract showed an IC₅₀ value of 74.02 µg/mL, with scavenging activity increasing from 13.56% to 61.69% across the tested concentrations. Similarly, the ABTS assay recorded an IC₅₀ value of 83.88 µg/mL, with inhibition increasing from 8.46% to 63.12%. Although the antioxidant activity was lower than that of the standard antioxidants, the results demonstrate that *S. costus* leaves possess appreciable free radical scavenging capacity, likely due to their phenolic and flavonoid constituents. These findings support the medicinal value of *S. costus* leaves and suggest their potential as a sustainable source of natural antioxidants, reducing dependence on destructive harvesting of underground plant parts and contributing to the conservation of this endangered species.

Keywords: *Saussurea costus*, antioxidant activity, DPPH, ABTS, costunolide

1. Introduction

Medicinal plants have long served as an indispensable source of healthcare, providing numerous bioactive compounds that contribute to the prevention and treatment of human diseases. Their extensive use in traditional medical systems, coupled with increasing scientific validation of their pharmacological properties, has renewed global interest in plant-derived therapeutics [1, 2]. According to the World Health Organization, nearly 80% of the world's population continues to depend on herbal medicines for primary healthcare, particularly in developing countries where medicinal plants remain affordable, accessible, and culturally accepted. These natural resources possess diverse secondary metabolites that exhibit antioxidant, antimicrobial, anti-inflammatory, anticancer, hepatoprotective, and immunomodulatory activities, making them valuable candidates for modern drug discovery [3].

For centuries, the roots of *Saussurea costus* have been extensively utilized in Ayurveda, Unani, Tibetan, and Chinese systems of medicine for the management of respiratory disorders, gastrointestinal ailments, rheumatism, skin diseases, inflammation, fever, ulcers, and infectious diseases. Modern pharmacological investigations have largely substantiated these traditional claims by demonstrating that extracts and purified compounds from *S. costus* possess broad-spectrum biological activities, including antioxidant, antimicrobial, anti-inflammatory, antiviral, hepatoprotective, antidiabetic, immunomodulatory, and anticancer effects [4]. These therapeutic properties are primarily attributed to its diverse phytochemical constituents, particularly sesquiterpene lactones such as costunolide, dehydrocostus lactone, cynaropicrin, and santamarin, together with flavonoids, phenolic acids, alkaloids, tannins, terpenoids, and essential oils [5].

Among the numerous pharmacological attributes of *S. costus*, its antioxidant potential has attracted considerable scientific attention.

Reactive oxygen species (ROS) generated during normal cellular metabolism or under environmental stress can cause oxidative damage to lipids, proteins, and nucleic acids, thereby contributing to ageing and the development of chronic disorders such as cancer, diabetes, cardiovascular diseases, and neurodegenerative conditions. Plant-derived antioxidants neutralize these reactive species, reducing oxidative stress and protecting cellular integrity. The high concentrations of phenolic compounds, flavonoids, and sesquiterpene lactones present in *S. costus* have been reported to exhibit significant free radical scavenging activity and contribute substantially to its protective biological effects. Consequently, *S. costus* has emerged as a promising natural source of antioxidant molecules with potential pharmaceutical and nutraceutical applications^[4, 6]. *S. costus* (Falc.) Lipsch., a perennial medicinal herb belonging to the family Asteraceae, is one of the most valuable and commercially important medicinal plants of the Himalayan region. The species is indigenous to the temperate alpine zones of the northwestern Himalayas, particularly Jammu & Kashmir and Himachal Pradesh, where it naturally occurs at altitudes ranging from approximately 2, 500 to 3, 500 m above sea level^[7]. Commonly known as Kuth, it has been extensively used for centuries in traditional systems of medicine, including Ayurveda, Unani, Tibetan, and Chinese medicine, for the treatment of respiratory disorders, digestive ailments, skin diseases, rheumatism, fever, asthma, ulcers, inflammation, and various infectious conditions^[8, 9]. Owing to its remarkable therapeutic properties and increasing commercial demand, *S. costus* has gained considerable attention from researchers worldwide. The medicinal value of this species is primarily attributed to its rich phytochemical composition, which includes sesquiterpene lactones, flavonoids, phenolic compounds, alkaloids, tannins, terpenoids, lignans, and essential oils. Among these constituents, costunolide and dehydrocostus lactone are recognized as the principal bioactive sesquiterpene lactones responsible for many of the plant's pharmacological activities^[5].

The roots of *S. costus* constitute the principal medicinally important plant parts and are widely utilized in both traditional and modern herbal formulations. Numerous pharmacological investigations have demonstrated that extracts and purified compounds isolated from the roots exhibit broad-spectrum biological activities, including antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, antidiabetic, antiviral, immunomodulatory, gastroprotective, and anticancer effects^[7]. Costunolide and dehydrocostus lactone have been reported to suppress inflammatory mediators, induce apoptosis in various cancer cell lines, inhibit microbial growth, and effectively scavenge reactive oxygen species (ROS). These findings highlight the significant therapeutic potential of *S. costus* as a valuable source of natural bioactive molecules for pharmaceutical and nutraceutical applications^[5, 10].

Despite its immense medicinal importance, the natural populations of *S. costus* have declined drastically because of indiscriminate harvesting of roots, habitat degradation, poor seed viability, and slow natural regeneration. Consequently, the species has been categorized as critically endangered within its native range and is listed in Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), imposing strict regulations

on its international trade. Increasing market demand, coupled with limited cultivation practices, has further intensified pressure on wild populations, emphasizing the urgent need for effective conservation strategies and sustainable propagation techniques^[11, 12].

Phenolic compounds, flavonoids, and sesquiterpene lactones present in the plant contribute significantly to its antioxidant capacity by neutralizing free radicals and reducing oxidative stress, which is implicated in the development of numerous chronic disorders, including cancer, diabetes, cardiovascular diseases, and neurodegenerative conditions. Therefore, evaluating the antioxidant potential of *S. costus* extracts provides important evidence supporting its traditional medicinal uses and facilitates the identification of valuable natural antioxidant compounds. In view of these considerations, the present study was undertaken to assess the antioxidant activity of hydromethanolic extracts obtained from leaves of *Saussurea costus*, thereby contributing to the scientific validation of its therapeutic potential while supporting future conservation and sustainable utilization of this endangered Himalayan medicinal herb^[13].

2. Materials and Methods

2.1 Plant selection and collection

Leaves of *S. costus* were collected in January 2024 from Deoban, a high-altitude region of Uttarakhand, India. The collection site is situated at an elevation of approximately 2, 800-2, 900 m above sea level.

2.2 Preparation of hydromethanolic extract

Hydromethanolic extracts of *S. costus* leaves were prepared following the method described by Deb *et al.*^[14] with slight modifications. Freshly collected leaves were thoroughly washed under running water to remove adhering soil and other extraneous materials, followed by shade drying until a constant weight was achieved. The dried leaves were ground into a fine powder using a mortar and pestle and used for subsequent extraction.

For extraction, 10 g of the powdered leaves was mixed with 100 mL of 80% (v/v) methanol, which was selected because of its high efficiency in extracting a broad spectrum of polar and moderately polar phytochemicals. The mixture was incubated in an orbital shaker at 100 rpm for 48 h to facilitate efficient extraction of bioactive constituents. Following extraction, the suspension was initially filtered through muslin cloth to remove coarse plant debris and subsequently passed through Whatman No. 1 filter paper to obtain a clear filtrate. The filtrate was concentrated under reduced pressure at 45 °C using a rotary vacuum evaporator. The concentrated extract was then freeze-dried by lyophilization to obtain a dry extract. Finally, the dried extract was weighed to determine the extraction yield and stored at -20 °C until further phytochemical and antioxidant analyses.

2.3 Yield

The dried or concentrated extracts obtained after solvent evaporation and lyophilization were weighed to determine the extraction yield on a dry weight basis. For each extraction, 10 g of dried leaves powder was extracted with 100 mL of 80% (v/v) methanol. The extraction yield was expressed as a percentage and calculated using the following equation:

$$Y (\%) = Z2/Z1 \times 100$$

where (Z1) represents the initial weight (g) of the dried plant powder used for extraction, and (Z2) denotes the final dry weight (g) of the recovered extract after solvent removal.

2.4 Antioxidant assay *S. costus*

2.4.1 DPPH radical scavenging assay

The antioxidant activity of the extracts was determined using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay following the method of Bhandari *et al.* [15] with slight modifications. Stock solutions of the extracts and L-ascorbic acid (standard) were prepared at a concentration of 1 mg mL⁻¹. Serial dilutions (10-100 µg mL⁻¹) were prepared, and 150 µL of each dilution was mixed with 1.5 mL of DPPH solution. The reaction mixtures were incubated in the dark at room temperature for 30 min, after which the absorbance was measured at 517 nm using a UV-Visible spectrophotometer (Eppendorf Bio Spectrophotometer). Methanol containing DPPH served as the control, while L-ascorbic acid was used as the reference antioxidant. The IC₅₀ value, representing the concentration required to scavenge 50% of DPPH radicals, was determined from the dose-response curve using non-linear regression analysis. All measurements were performed in triplicate. The free radical scavenging activity was expressed as percentage inhibition and calculated using the following equation:

$$\% \text{Scavenged DPPH radical} = [(A0-A1) / A0] \times 100$$

where (A0) is the absorbance of the control and (A1) is the absorbance of the sample or standard.

2.4.2 ABTS radical scavenging assay

The ABTS radical scavenging assay was performed according to the method of Lee *et al.* [16] with slight modifications. The ABTS radical solution was prepared by mixing 7 mM ABTS with 140 mM potassium persulfate and incubating the mixture in the dark at room temperature for 16 h. Prior to analysis, the solution was diluted with methanol to obtain an absorbance at 734 nm. Stock solutions of the hydro-methanolic extracts and quercetin (1 mg/mL) were prepared, and 100 µL of each sample at concentrations ranging from 10-100 µg/mL was mixed with 100 µL of the ABTS working solution. After incubation in the dark for 6 min at room temperature, the absorbance was measured at 734 nm using a spectrophotometer. A reaction mixture containing the ABTS solution and methanol served as the control. All assays were performed in triplicate and repeated independently three times. The ABTS radical scavenging activity was calculated using the following equation:

$$\text{ABTS radical scavenging activity (\%)} = [(A0-A1) / A0] \times 100$$

where (A0) is the absorbance of the control and (A1) is the absorbance of the sample or quercetin standard.

2.5 Statistical analysis

All data were analysed using Origin software and are presented as mean ± standard deviation (SD). Statistical

differences among treatments were determined using one-way analysis of variance (ANOVA), with significance accepted at $p < 0.05$.

3. Results

3.1 Extraction yield of hydro-methanolic extract of *S. costus*

The hydromethanolic extraction of *S. costus* leaves resulted in an extraction yield of 10.9% (w/w). From 10 g of dried leaves powder, 1.09 g of dried extract was recovered, corresponding to an extraction efficiency of 10.9% on a dry weight basis (Table 1, Figure 1).

3.2 DPPH radical scavenging assay

The DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay is widely used to assess the free radical scavenging activity of plant extracts. In this assay, antioxidants donate hydrogen atoms or electrons to the stable DPPH radical, causing a decrease in absorbance at 517 nm. Antioxidant activity is expressed as the IC₅₀ value, which represents the concentration required to scavenge 50% of DPPH radicals; lower IC₅₀ values indicate stronger antioxidant potential. The antioxidant capacity of plant extracts is largely influenced by their phenolic and flavonoid contents.

In the present study, the DPPH radical scavenging activity of hydromethanolic leaf extracts of *S. costus* collected from the Deoban population was evaluated using ascorbic acid as the reference standard (IC₅₀ = 37.75 µg/mL). The extract showed an IC₅₀ value of 74.02 µg/mL, indicating moderate free radical scavenging capacity (Table 2). A clear concentration-dependent increase in antioxidant activity was observed, with DPPH radical scavenging increasing from 13.56 ± 1.02% at 10 µg/mL to 61.69 ± 0.54% at 100 µg/mL. This gradual increase demonstrates that the antioxidant potential of the leaf extract improved with increasing concentration. The results are shown in Figure 2.

3.3 ABTS radical scavenging assay

The ABTS [2, 2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid)] assay was used to evaluate the antioxidant potential of the extracts by measuring their ability to scavenge the ABTS radical cation. Antioxidant activity was determined by the decrease in absorbance at 734 nm and expressed as the IC₅₀ value, where lower IC₅₀ values indicate greater radical scavenging activity.

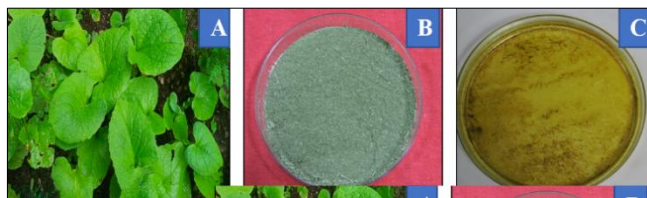
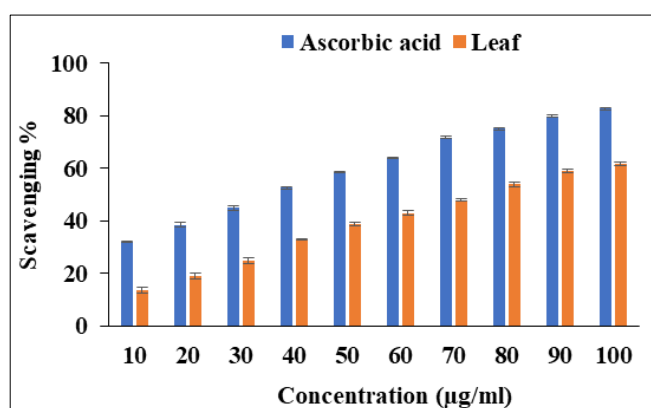
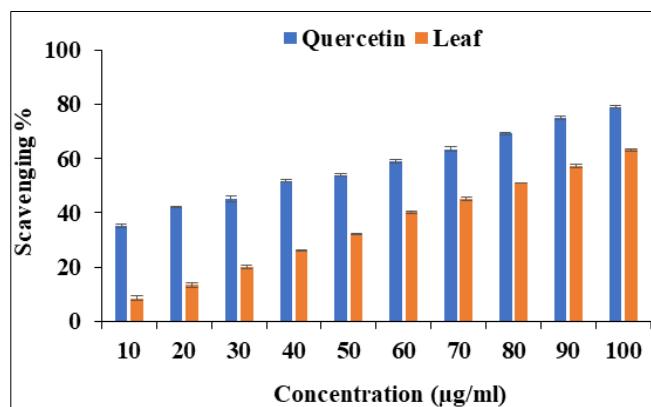
In the present study, the ABTS radical scavenging activity of the hydromethanolic leaf extract of *S. costus* from the Deoban population was evaluated using quercetin as the reference standard (IC₅₀ = 39.53 µg/mL). The leaf extract exhibited moderate ABTS radical scavenging activity with an IC₅₀ value of 83.88 µg/mL, indicating lower antioxidant potential than the reference standard (Table 2). A concentration-dependent increase in radical scavenging activity was observed, with percentage inhibition increasing from 8.46 ± 0.77% at 10 µg/mL to 63.12 ± 0.26% at 100 µg/mL. This progressive increase demonstrates that the antioxidant capacity of the leaf extract improved with increasing concentration, reflecting its ability to effectively scavenge ABTS radicals. The results are shown in Figure 3.

Table 1: Percentage yield of hydromethanolic leaf extract of *S. costus*

S. No.	Plant	Weight of Dried Leaf Powder (g)	Weight of Hydromethanolic Extract Obtained (g)	Percent Yield (%)
1	<i>S. costus</i>	10	1.09	10.9

Table 2. Antioxidant Potential of Hydromethanolic Leaf Extract

S. No.	Assay	IC ₅₀ (µg/mL) Standard	IC ₅₀ (µg/mL) Extract
1	DPPH Radical Scavenging Activity	37.75	74.02
2	ABTS Radical Scavenging Activity	39.53	83.88

**Fig 1:** Sequential representation of *S. costus* showing the plant (A), leaf powder (B) and hydromethanolic leaf extract (C) utilized for phytochemical characterization and antioxidant studies.**Fig 2:** DPPH Radical Scavenging Activity of *S. costus* Leaf Extract**Fig 3:** ABTS Radical Scavenging Activity of *S. costus* Leaf Extract

4. Discussion

Reactive oxygen species (ROS) are continuously generated during normal cellular metabolism and play important roles in cell signalling and defence. However, excessive accumulation of ROS can disturb cellular redox homeostasis, resulting in oxidative damage to lipids, proteins and nucleic acids. Such oxidative stress has been implicated in the development of numerous chronic disorders, including cardiovascular diseases, diabetes, neurodegenerative diseases and cancer. Consequently, there has been increasing interest in identifying natural

antioxidants from medicinal plants as safer alternatives to synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), whose prolonged use has raised toxicological concerns [17, 18].

The DPPH assay is one of the most reliable and widely adopted methods for evaluating the free radical scavenging potential of plant extracts because it measures the ability of antioxidant compounds to donate hydrogen atoms or electrons to neutralize stable DPPH radicals [19]. In the present investigation, the hydromethanolic leaf extract of *S. costus* collected from the Deoban population exhibited a clear concentration-dependent increase in DPPH radical scavenging activity, with inhibition increasing from $13.56 \pm 1.02\%$ at 10 µg/ml to $61.69 \pm 0.54\%$ at 100 µg/ml. The extract recorded an IC₅₀ value of 74.02 µg/ml, demonstrating moderate antioxidant activity when compared with the reference antioxidant ascorbic acid (IC₅₀ = 37.75 µg/ml). The concentration-dependent increase in DPPH scavenging activity indicates the effective free radical neutralizing ability of the leaf extract.

The antioxidant activity observed in the present study is closely associated with the phytochemical composition of the leaf extract. Previous investigations have consistently demonstrated that phenolic compounds and flavonoids represent the principal contributors to the antioxidant capacity of medicinal plants because of their ability to donate hydrogen atoms, stabilize free radicals through resonance, and interrupt oxidative chain reactions [20, 17]. The Deoban leaf extract contained appreciable amounts of total phenolics (85.85 mg GAE/g extract) and total flavonoids (75.66 mg QE/g extract), suggesting that these compounds substantially contributed to the observed DPPH radical scavenging activity. Phenolic hydroxyl groups readily react with free radicals to produce relatively stable phenoxyl radicals, thereby preventing further propagation of oxidative reactions. Similarly, flavonoids possess multiple hydroxyl substitutions that enhance their hydrogen-donating capacity and improve their effectiveness as natural antioxidants. Besides acting as direct free radical scavengers, these compounds may also function as metal chelators and inhibitors of lipid peroxidation, thereby providing multiple mechanisms of antioxidant defence.

The moderate antioxidant activity of the leaf extract obtained in the present study is in agreement with previous reports on *S. costus*. [5] demonstrated that extracts of *S. costus* possess considerable free radical scavenging activity, which was largely attributed to the presence of phenolic constituents and sesquiterpene lactones. Differences in antioxidant activity among published studies may also arise from variations in solvent polarity and extraction efficiency. Hydromethanol is widely recognized as an effective solvent for extracting phenolic compounds because its intermediate polarity facilitates the recovery of both hydrophilic and moderately lipophilic antioxidants. Besides phenolic acids and flavonoids, *S. costus* is known to contain bioactive

sesquiterpene lactones, including costunolide and dehydrocostus lactone, which have been reported to exhibit antioxidant, anti-inflammatory and pharmacological activities [5]. The synergistic interaction among these metabolites may have enhanced the overall radical scavenging capacity of the leaf extract. In one study it was reported that *S. costus* is a rich source of biologically active secondary metabolites, including phenolic compounds and sesquiterpene lactones, that contribute significantly to its antioxidant potential. Although direct comparison of IC₅₀ values among studies is often difficult because extraction solvents, analytical protocols, plant parts and environmental conditions differ considerably, the available literature consistently supports the antioxidant potential of this medicinal species [5].

Several studies have demonstrated a strong positive relationship between total phenolic content and DPPH radical scavenging activity in medicinal plants [17, 20]. The findings of the present study are consistent with this well-established relationship, suggesting that the antioxidant capacity of the Deoban leaf extract is largely determined by its phenolic and flavonoid composition.

The observed antioxidant activity also highlights the potential of *S. costus* leaves as an alternative source of natural antioxidants. Traditionally, the medicinal value of this species has been largely associated with its roots and rhizomes, resulting in destructive harvesting of wild populations. Since *S. costus* is an endangered Himalayan medicinal plant, excessive exploitation of underground plant parts poses a serious threat to its long-term survival. The demonstration that leaf extracts possess appreciable free radical scavenging activity suggests that aerial plant parts could be utilized for antioxidant extraction, thereby reducing harvesting pressure on roots and contributing to more sustainable utilization of this valuable species.

The ABTS radical scavenging assay is a widely accepted method for evaluating the antioxidant potential of plant extracts because it measures the ability of antioxidant molecules to quench the ABTS•⁺ radical cation through electron or hydrogen atom transfer. Unlike the DPPH assay, the ABTS method is applicable to both hydrophilic and lipophilic antioxidant compounds, thereby providing a broader assessment of the antioxidant capacity of plant extracts [18, 21]. In the present study, the hydromethanolic leaf extract of *S. costus* collected from the Deoban population exhibited a concentration-dependent increase in ABTS radical scavenging activity, with percentage inhibition increasing from 8.46 ± 0.77% at 10 µg/ml to 63.12 ± 0.26% at 100 µg/ml. The extract recorded an IC₅₀ value of 83.88 µg/ml, whereas quercetin, used as the reference standard, exhibited an IC₅₀ value of 39.53 µg/ml. These findings indicate that although the antioxidant activity of the leaf extract was lower than that of the standard antioxidant, it possessed a considerable ability to neutralize ABTS radicals.

The progressive increase in radical scavenging activity with increasing extract concentration suggests that the antioxidant constituents present in the leaf extract effectively participated in electron transfer reactions responsible for ABTS radical reduction [18, 21]. The results therefore confirm that the antioxidant activity of the *S. costus* leaf extract is dose dependent and increases proportionally with extract concentration.

The ABTS assay confirmed that the hydromethanolic leaf

extract of *S. costus* from the Deoban population possesses appreciable antioxidant potential through efficient scavenging of ABTS radicals. The concentration-dependent increase in radical scavenging activity, together with the presence of substantial phenolic and flavonoid contents, indicates that these phytochemicals play a central role in the antioxidant properties of the leaf extract. Although the activity was lower than that of quercetin, the findings demonstrate that *S. costus* leaves represent a promising and sustainable source of natural antioxidants. The close agreement between the DPPH and ABTS assays further supports the reliability of the observed antioxidant activity and highlights the potential of leaf biomass for future pharmaceutical, nutraceutical and functional food applications.

The agreement between the DPPH and ABTS assays observed in the present study further strengthens the reliability of the antioxidant evaluation. Both assays demonstrated a concentration-dependent increase in radical scavenging activity, indicating that the leaf extract contains compounds capable of participating in both hydrogen atom transfer and electron transfer reactions. Since the ABTS assay measures the activity of a broader range of antioxidant molecules than the DPPH assay, the similar trend obtained in both assays suggests that the antioxidant constituents of the leaf extract are effective against different types of free radicals. Such consistency among antioxidant assays has been widely regarded as a reliable indicator of the antioxidant potential of plant extracts [18].

The antioxidant capacity observed in the present investigation may also be influenced by the diverse phytochemical profile of *S. costus*. Besides phenolic acids and flavonoids, the species contains sesquiterpene lactones such as costunolide and dehydrocostus lactone, along with essential oils and other secondary metabolites that have been reported to possess antioxidant and pharmacological activities [5]. The interaction among these bioactive constituents may produce synergistic effects that enhance the overall radical scavenging capacity of the extract. Such synergism is frequently observed in medicinal plants, where the combined activity of multiple compounds contributes more effectively to antioxidant defence than individual constituents alone. The ABTS assay confirmed that the hydromethanolic leaf extract of *S. costus* from the Deoban population possesses appreciable antioxidant potential through efficient scavenging of ABTS radicals. The concentration-dependent increase in radical scavenging activity, together with the presence of substantial phenolic and flavonoid contents, indicates that these phytochemicals play a central role in the antioxidant properties of the leaf extract. Although the activity was lower than that of quercetin, the findings demonstrate that *S. costus* leaves represent a promising and sustainable source of natural antioxidants. The close agreement between the DPPH and ABTS assays further supports the reliability of the observed antioxidant activity and highlights the potential of leaf biomass for future pharmaceutical, nutraceutical and functional food applications.

5. Conclusion

The present study demonstrates that the hydromethanolic leaf extract of *S. costus* collected from the Deoban population possesses appreciable antioxidant activity, as evidenced by its concentration-dependent radical

scavenging effects in both DPPH and ABTS assays. The observed antioxidant potential is likely associated with the presence of phenolic and flavonoid compounds, which are known to play an important role in neutralizing free radicals. Although the antioxidant activity was lower than that of the reference standards, the findings confirm that *S. costus* leaves represent a valuable natural source of antioxidant phytochemicals. Importantly, the use of leaves rather than roots offers a sustainable approach for the utilization of this endangered Himalayan medicinal plant by reducing destructive harvesting. These findings provide scientific support for the traditional medicinal value of *S. costus* and highlight the potential of its leaf extract for future pharmaceutical, nutraceutical and functional food applications. Further studies involving the isolation and characterization of individual bioactive compounds and their biological evaluation are warranted to better understand the therapeutic potential of this species.

6. Acknowledgement

We sincerely acknowledge the research facilities provided for this study by the Dean of the College of Basic Sciences and Humanities, Director of the Experiment Station at G. B. Pant University of Agriculture and Technology, Pantnagar-263145, Uttarakhand, India.

7. Competing Interests

Authors have declared that no competing interests exist.

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