



***In Vitro* Antioxidant and Bioactive Component Analysis of Himalayan Spice Faran (*Allium Stracheyi*)**

Anjali Gusain¹ • Nisha Singh^{1*}

¹Molecular Immunology Laboratory, Department of Biochemistry, Hemvati Nandan Bahuguna Garhwal University (A Central University), Srinagar (Garhwal), Uttarakhand-246174, India.

*Corresponding Author Email: nishasingh0711@gmail.com

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Abstract: Reactive oxygen species (ROS), a group of highly reactive molecules derived from oxygen metabolism, have been implicated in numerous health disorders. As a result, there has been growing interest in the potential of antioxidant-based drugs and natural antioxidants for treating complex diseases. This study aimed to assess the antioxidant potential of the Himalayan spice Faran (*Allium Stracheyi*) by determining the presence of phytochemicals such as phenols and flavonoids and evaluating its *in vitro* antioxidant activity using various assays. Chloroform, petroleum ether, and aqueous extracts of *Allium Stracheyi* leaves were tested using DPPH radical scavenging assays, ferric reducing antioxidant power, total antioxidant capacity and reducing power assay. The Chloroform extracts demonstrated significantly higher ($p \leq 0.05$) levels of phytochemicals and superior antioxidant activity compared to the other extracts. These findings suggest that Faran could be a promising candidate for developing nutraceuticals to treat diseases such as diabetes and cancer.

Keywords: Antioxidant activity • plant extracts • DPPH • FRAP • phenolics • flavonoids.

Introduction

For millennia, nature has served as a rich source of medicinal agents, with a remarkable number of contemporary drugs derived from natural origins. In fact, over half of all modern clinical drugs can be traced back to natural products, which continue to play a significant role in the pharmaceutical industry's drug development efforts. Isolating and identifying a drug's active principles, as well as elucidating its mechanism of action, are of utmost importance (Koparde et al 2019). As such, research on both traditional medicine mixtures and single active compounds is essential.

Oxidative stress occurs when there is an imbalance between reactive oxygen species (ROS) generation and the body's endogenous antioxidant systems. ROS are primarily responsible for initiating oxidation both *in vivo* and *in vitro*, leading to oxidative stress. This excessive stress, in turn, contributes to a plethora of diseases and disorders, including but not

limited to cancer, cardiovascular disease, atherosclerosis, neurological disorders such as Alzheimer's and Parkinson's disease and aging (Forman and Zhang 2021).

Plant leaves have been the focus of numerous studies due to their abundant antioxidant content, such as vitamins A, C, and E, carotenoids, phenols, and flavonoids. These antioxidants are known for their multiple biological effects, including their antioxidant and free radical scavenging abilities, as well as anti-inflammatory and anti-carcinogenic properties (Akbari et al 2022). Flavonoids and phenolic compounds, which are widely distributed in plants, have been shown to exhibit these beneficial effects.

In addition to their direct antioxidant properties, these plant-derived compounds may also interact with and modulate various signaling pathways, transcription factors, and enzyme activities involved in the cellular response to oxidative stress. This multi-targeted action could provide a more comprehensive approach to counteracting



oxidative stress and its associated pathologies (Shiau et al 2022). Furthermore, the synergistic effects of multiple compounds within a plant extract may enhance the overall antioxidant potential and therapeutic efficacy, highlighting the importance of investigating both whole plant extracts and individual compounds.

To harness the full potential of natural antioxidants, further research is needed to understand their bioavailability, metabolism, and optimal dosages, as well as any potential side effects or interactions with other medications. Ultimately, the integration of traditional knowledge with modern scientific approaches could lead to the discovery of novel therapeutic agents, paving the way for improved prevention and treatment strategies for oxidative stress-related diseases.

Allium stracheyi, commonly known as Faran or Himalayan Spice, is a perennial herb belonging to the Allium genus and the Amaryllidaceae family. This plant species is native to the alpine regions of the Himalayas, predominantly found in areas of India, Nepal, Bhutan, and China at elevations ranging from 3,000 to 5,000 meters (Tiwari et al 2014). *Allium stracheyi* is known for its strong, characteristic fragrance and is often used as a spice or flavoring agent in traditional cuisine. Faran, or *Allium stracheyi*, has significant medicinal importance among local tribes. It is used to treat various ailments such as aiding digestion, relieving respiratory issues, reducing inflammation and pain, promoting wound healing and antimicrobial activity, and acting as a diuretic (Mohan et al 2019).

Although the traditional uses of *Allium stracheyi* are well-documented, more scientific research is needed to validate and understand the full scope of its medicinal properties. Studies on the plant's bioactive compounds, their mechanisms of action, and potential synergistic effects will help establish its therapeutic potential and guide the development of novel, plant-based treatments for various health conditions.

Materials and methods

Plant Material Collection: *Allium stracheyi* plant samples were collected from the village of Malari, Chamoli district, Uttarakhand (Fig.1). Plant verification (Ref no: BSI/NRC Herb (Ident.)/2022-23/1038) was done by Botanical survey of India, Dehradun, Uttarakhand.

Sample Preparation:

The leaves were washed with distilled water, shade-dried for three weeks, and ground to create a crude powder. Soxhlet apparatus was employed for sequential extraction using various solvents such as petroleum ether, chloroform, and distilled water. 25 grams of the ground leaf powder was weighed, packed, and loaded into the Soxhlet extractor with a sample-to-solvent ratio of 1:10. The extraction continued until the solvent in the thimble turned transparent, with temperatures based on the boiling points of the solvents. The extracts were then recovered using a rotary evaporator under vacuum. The dried extracts were stored at -20°C. Prior to bioactivity assessments, the dried extract was reconstituted in DMSO.

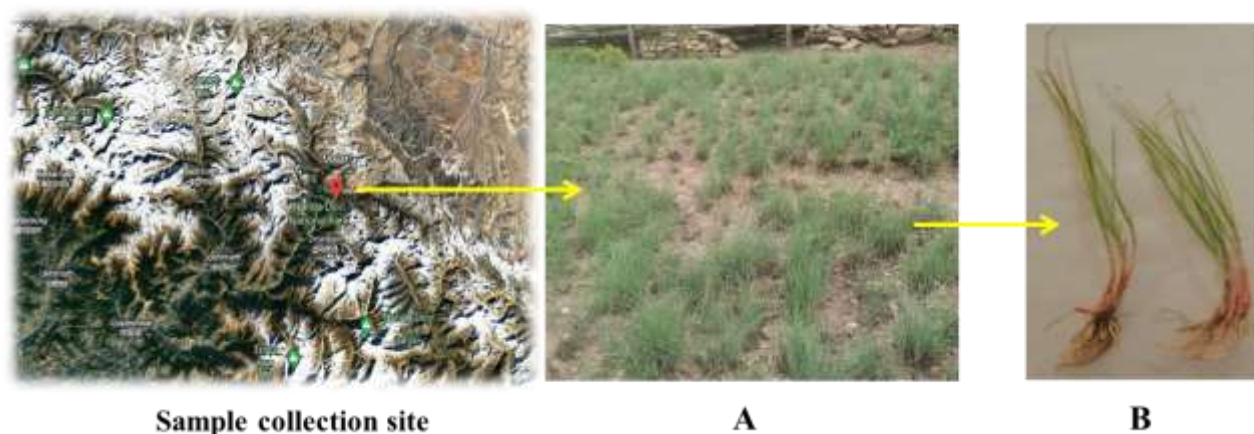


Fig 1. A *Allium stracheyi* grown in hilly field of Malari village, Chamoli district, Uttarakhand. **B.** Whole plant of *Allium stracheyi*.

Bioactive component Determination

Bioactive component determination was conducted on petroleum ether (APE), chloroform (ACE), and aqueous extracts (AAE) using standard methods (Yadav et al 2014, Aziz et al 2015). *A. stracheyi* extracts were tested for the presence of carbohydrates, proteins, phenolics, flavonoids, alkaloids, tannins, terpenoids, saponins, anthocyanins, and coumarins.

Total Phenolic Content

Phenolic content was estimated using the Folin-Ciocalteu method, with gallic acid as the standard (Sen et al 2013). To obtain a final concentration of 1.0 mg/mL, each plant extract was dissolved in DMSO. Subsequently, 0.5 mL of both the samples and standards were combined with 2.5 mL of Folin-Ciocalteu reagent diluted 10-fold and 4 mL of 7.5% sodium carbonate in separate test tubes. The tubes were then covered and left undisturbed at room temperature for 60 minutes. Sample absorbance was measured using a UV-visible spectrophotometer at 760 nm. The total phenolic content was quantified and reported as milligrams of gallic acid equivalents (GAEs) per milligram of extract.

Total Flavonoid Content

The evaluation of total flavonoid content was conducted through the utilization of the aluminum chloride colorimetric method, employing quercetin as the reference standard (Sen et al 2013). Each plant extract was dissolved in DMSO to reach a 1.0 mg/mL final concentration. In different test tubes, 0.5 mL of each standard and extract solution was mixed with 1 mL of 10% $AlCl_3$, incubated for 6 minutes, and then combined with 1 mL of 1M potassium acetate solution. Subsequently, 2 mL of a 4% NaOH solution was introduced, and the volume was made up to 5 mL with distilled water. The absorbance at 520 nm was then measured using a UV-vis spectrophotometer. The total flavonoid content was expressed as milligrams of quercetin equivalents (QEs) per milligram of extract.

Antioxidant Activity

DPPH Radical Scavenging Activity: The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of plant extracts was determined as Saxena et al (2010) with slight modification (Hazra et al 2008). Each extract was dissolved in methanol to yield a 1 mg/mL final concentration, as were ascorbic acid standard solutions. Serial dilution was made for different extracts and standard ascorbic acid



(1mg/ml to 0.015mg/ml) in a in a 96-well microtiter. In each well, 0.1 mL of different samples and standard was mixed with 0.2 mL of 0.1 mmol/L DPPH and incubated at 37°C in dark for 30 min. After incubation, decrease in absorption for each solution was measured at 490 nm using a microplate reader. Results were expressed as IC₅₀ in mg/ml.

FRAP Assay: The antioxidative activity of plant extracts was determined by the ferric reducing antioxidant power (FRAP) assay with some modification (Sen et al 2013). The stock solutions utilized in the experiment consisted of a 300 µM acetate buffer with a pH of 3.6, a 10 µM solution of 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 µM HCl, and a 20 µM solution of FeCl₃. The FRAP reagent was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ, and 2.5 mL FeCl₃. For antioxidant activity determination, 0.2 mL FRAP reagent was mixed with 0.1mL of 1 mg/mL sample extracts microtiter plates. Plates were covered and incubated in dark for 30 minutes. Absorbance was taken at 570 nm in a microplate reader. Ferrous Sulphate (FeSO₄) was used for standard curve preparation. FRAP assay results were expressed as mM Fe (II)/mg extract.

Reducing Power Assay: The reducing power of extracts was determined following the method described by Sen et al (2013). Each extract was dissolved in DMSO to achieve a 1 mg/mL final concentration, as were ascorbic acid standard solutions. Briefly, 1 mL aliquot of the plant extract was combined with 2.5 mL of phosphate buffer (pH 6.6) and 2.5 mL of potassium ferricyanide (1%). The resulting mixture was incubated at a temperature of 50°C for a duration of 20 minutes, subsequently cooled, and then mixed with 2.5 mL of trichloroacetic acid (10%). Following this, the mixture was subjected to centrifugation at 3000 rpm for a

period of 10 minutes. The upper layer (2.5 mL) was further mixed with 2.5 mL of distilled water and 0.5 mL of freshly prepared ferric chloride (0.1%). The absorbance of the resulting solution was measured at a wavelength of 700 nm using a UV spectrometer. A blank sample was concurrently prepared without the inclusion of the plant extract. An increase in absorbance indicates increased reducing power. The results were expressed as mg of Ascorbic acid equivalent (AAEs)/mg of extract.

Total antioxidant capacity Assay: The phosphor-molybdenum complex assay was used to determine the total antioxidant capacity in leaf extract (Hazra et al 2008). In summary, a 1 mL volume of the sample extract was combined with 3.3 mL of a freshly prepared reagent solution consisting of 28 mM/L sodium phosphate, 0.6 M/L sulfuric acid, and 4 mM/L ammonium molybdate. The resulting mixture was then subjected to incubation in a water bath maintained at 95°C for a duration of 90 minutes and measured after cooling at 695 nm against a blank. Inhibition of molybdate ions was expressed as milligrams of Ascorbic acid equivalent (AAEs)/mg of extract for triplicate analysis, and data were presented as mean ± standard deviation (SD).

Results

Bioactive component determination: The bioactive components of *Allium stracheyi* leaf extract showed the presence of various bioactive compounds, including tannins, phenols, flavanols, flavonoids, anthocyanins, and saponins in different extracts (Table 1). The medicinal value of many plants is attributed to these phytochemicals, which have been reported to possess significant therapeutic properties. Consequently, the presence of these compounds in *Allium stracheyi* contributes to its use in treating various diseases.



Table 1. Qualitative screening of bioactive compounds.

Tests	Petroleum Ether Extract (APE)	Chloroform Extract (ACE)	Aqueous Extract (AAE)
Detection of steroids and terpenoids			
Liebermann Burchard test	+	++	+++
Salkowski's test	+	++	+++
Detection of phenolics and tannins			
Ferric chloride test	+	++	+
Shinoda's test (for flavonoids)	++	++	+
Lead acetate test (for flavonoids)	++	+	+
Alkaline reagent test (for flavonoids)	+	++	+
Test for anthocyanins	++	+	-
Test for coumarins	+	+	+
Detection of alkaloids			
Mayer's test	-	-	-
Wagner's test	-	-	-
Dragendorff's test	-	-	+
Detection of saponins			
Forth test	-	+	-
Detection of carbohydrates			
Molisch's test	+	++	+
Benedict's test	-	-	-
Anthrone test	+	+	+
Detection of proteins and amino acids			
Millons test	-	-	-
Ninhydrin test	-	-	-

(+) presence, (-) absence.

Total Phenolic Content

The present study aimed to investigate the phenolic content of different extracts obtained from a leaf sample using various solvents. The findings revealed that the presence of phenols was evident in all extracts, albeit in varying quantities depending on the solvent employed for extraction Fig. 2(A). Notably, the chloroform extract had the highest phenolic content (0.85 ± 0.03 mg GAEs/mg of extract) which differed significantly from the other extracts ($p < 0.05$). On the other hand, the petroleum ether extract (APE) had a phenolic content of 0.47 ± 0.02 mg GAEs/mg of extract, which was significantly lower than the chloroform extract but higher than the aqueous extract that had a

phenolic content of 0.36 ± 0.008 mg GAEs/mg of extract.

Total flavonoid content: Fig. 2(B) displays the results of the flavonoid content of different extracts obtained from *Allium stracheyi* using different solvents, including petroleum ether, chloroform and water. The results indicate that the Chloroform Extract has the highest flavonoid content with a mean value of 0.150692 ± 0.006526 mg QEs/mg of extract, followed by Petroleum Ether Extract with a mean value of 0.099942 ± 0.005897 mg QEs/mg of extract. The Aqueous Extract has the lowest flavonoid content with a mean value of 0.081858 ± 0.004964 mg QEs/mg of extract. The differences in flavonoid content between the



three types of extracts are statistically significant ($p < 0.05$).

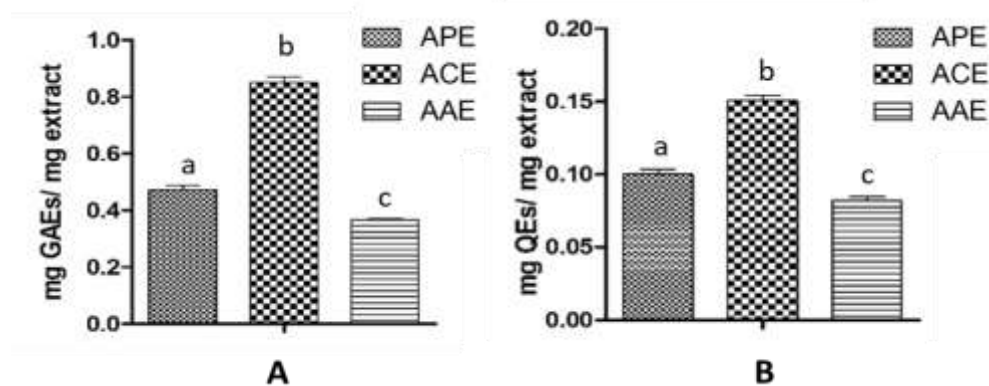


Fig. 2 (A) Showed total Phenolic content (TPC) in mg GAEs/mg of extract and (B) showed total flavonoid content (TFC) in mg QEs/mg of extracts. Values are represented as mean \pm standard deviation of three replicates. Different small letters indicate significant differences ($p < 0.05$).

Antioxidant assay:

DPPH Radical Scavenging Activity: The DPPH Radical Scavenging Activity of three different extracts, viz. APE, ACE, and AAE were assessed in this study (Table 2). Ascorbic acid was used as the positive control. The results demonstrated that the IC_{50} values, which represent the concentration of the sample required to scavenge 50% of the DPPH free

radicals, varied significantly ($p < 0.05$) among the tested samples. The mean IC_{50} value for ACE (1.13 ± 0.10 mg/ml) was significantly lower ($p < 0.05$) than that of APE (1.68 ± 0.02 mg/ml) and AAE (2.38 ± 0.14 mg/ml). The mean IC_{50} value for ascorbic acid (0.36 ± 0.03 mg/ml) was significantly lower ($p < 0.05$) than that of all three plant extracts.

Table 2: DPPH radical scavenging activity of *Allium stracheyi* leaf extracts

Sample Extract	IC_{50} ; mg/ml ¹
APE	$1.68 \pm 0.02d$
ACE	$1.13 \pm 0.10c$
AAE	$2.38 \pm 0.14b$
Ascorbic acid ²	$0.36 \pm 0.03a$

1) IC_{50} (mg/mL), concentration for scavenging 50% of DPPH radicals.

2) Ascorbic acid was used as positive control.

3) Extracts from *Allium stracheyi* leaf: APE Petroleum ether extract; ACE Chloroform extract; and AAE Aqueous extract.

4) Measurements were done in triplicate and values represent mean \pm SD.

FRAP Assay: In this study, we evaluated the Ferric Reducing Antioxidant Power (FRAP) of three different plant extracts, specifically APE, ACE, and AAE, and used ascorbic acid as a positive control (Table 3). The FRAP values,

which reflect the antioxidant capacity of the samples to reduce ferric ions to ferrous ions, exhibited significant variation ($p < 0.05$) among the tested samples. The mean FRAP value for ACE (0.374 ± 0.002 mM Fe (II)/mg extract) was



significantly higher ($p < 0.05$) than that of APE (0.312 ± 0.023 mM Fe (II)/mg extract) and AAE (0.110 ± 0.007 mM Fe (II)/mg extract). Furthermore, the mean FRAP value for ascorbic acid (0.899 ± 0.002 mM Fe (II)/mg extract) was

significantly higher ($p < 0.05$) than that of all three plant extracts. These results demonstrate that the tested plant extracts possess antioxidant properties.

Table 3. FRAP assay of *Allium stracheyi* leaf extracts

Sample Extract	mM Fe (II)/mg extract ¹
APE	0.312 ± 0.023 d
ACE	0.374 ± 0.0024 c
AAE	0.110 ± 0.0070 b
Ascorbic acid ²	0.899 ± 0.0022 a

1) Concentration of substance having ferric-TPTZ reducing ability expressed as mM Fe (II) equivalents/ mg extract.

2) Ascorbic acid was used as positive control

3) Measurements were done in triplicate and values represent mean \pm SD.

Reducing Power Assay: The chloroform extract (ACE) exhibited the highest reducing power (0.0247 ± 0.0026 mg AAE/mg Extract) and was significantly higher ($p < 0.05$) from the other two extracts (Fig.3A). The petroleum ether extract (APE) showed the second-highest reducing power (0.0106 ± 0.0034 mg AAE/mg Extract), while the aqueous extract (AAE) had

the lowest reducing power (0.0083 ± 0.0022 mg AAE/mg Extract). The results suggest that the chloroform extract (ACE) may have the highest antioxidant potential among the extracts from *Allium stracheyi*. These findings are consistent with the results of the DPPH assay, which also showed that the chloroform extract had the highest % inhibition.

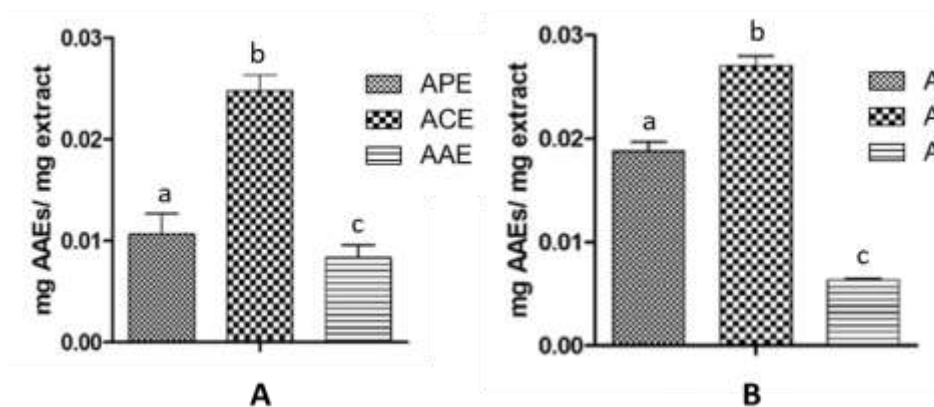


Fig 3: (A) Reducing power assay and (B) total antioxidant capacity assay of *Allium stracheyi* leaf extracts. Values are represented as mean \pm standard deviation of three replicates. Different small letters indicate significant differences ($p < 0.05$).

Total antioxidant capacity Assay: The total antioxidant assay was conducted to determine

the antioxidant potential of the extracts from *Allium stracheyi* (Fig. 3B). The results showed



that the chloroform extract (ACE) had the highest total antioxidant activity (0.0270 ± 0.0016 mg AAE/mg of Extract), which was significantly different from the other two extracts ($p < 0.05$). The petroleum ether extract (APE) demonstrated the second-highest antioxidant activity (0.0187 ± 0.0015 mg AAE/mg Extract), while the aqueous extract (AAE) had the lowest total antioxidant activity (0.0063 ± 0.0000 mg AAE/mg of Extract).

Discussion

Plants have been widely used in traditional medicine across the world, and many modern medicines have their origins in natural products. These plants are a rich source of bioactive compounds such as flavonoids, phenols, and other antioxidants, which have been found to have a range of therapeutic benefits including antimicrobial, anti-inflammatory, and anti-cancer activities (Koparde et al 2019, Forman and Zhang 2021). This study investigated the bioactive compounds present in the petroleum ether extract (APE), chloroform extract (ACE), and aqueous extract (AAE) of *Allium stracheyi* leaf. The extracts were tested for their total phenol content, flavonoid content, and antioxidant activities using various assays.

The results of the study revealed that the chloroform extract (ACE) had the highest phenolic and flavonoid contents, followed by the petroleum ether extract (APE) and aqueous extract (AAE). Phenolic and flavonoid compounds are well known for their antioxidant properties, which could be due to their ability to donate hydrogen atoms to free radicals, making them less reactive and less damaging to cells (Huyut et al 2017).

The extracts were also tested for their antioxidant activities using various assays, including the DPPH assay, FRAP assay, reducing power assay, total antioxidant assay, and hydrogen peroxide assay. The highest

antioxidant activity was found in the chloroform extract (ACE), followed by the petroleum ether extract (APE), aqueous extract (AAE), respectively (Argolo et al 2004). The DPPH and FRAP assays showed that the high antioxidant activity of the chloroform extract (ACE) could be attributed to the presence of flavonoids and phenolic compounds, which have been reported to possess potent antioxidant properties.

The reducing power assay revealed that the highest reducing power was exhibited by the chloroform extract (ACE), which could be attributed to the presence of high levels of phenolic compounds and flavonoids (Do et al 2014). In contrast, the aqueous extract exhibited the lowest reducing power among the three extracts tested, likely due to the lower solubility of phenolic compounds and flavonoids in water compared to organic solvents. These findings are consistent with previous studies that have found organic solvents like chloroform to be an effective solvent for extracting phenolic compounds and flavonoids from plant materials as compared to water (Vuong et al 2014, Zhao et al 2019).

Overall, the chloroform extract (ACE) exhibited the highest antioxidant activity and total phenol and flavonoid contents, indicating that it may be a potential source of natural antioxidants with therapeutic applications. However, further studies are needed to fully elucidate the bioactive compounds responsible for the observed effects and to determine their potential therapeutic uses.

Conclusion

This study evaluated bioactive components and the antioxidant potential of different extracts of *Allium stracheyi* leaf using various assays. The results showed that the *Allium stracheyi* leaf had a significant antioxidant potential, and the chloroform extract was the most potent in all assays. The phenolic and flavonoid contents of



the extracts were found to vary significantly, with the chloroform extract having the highest flavonoid content and the aqueous extract having the highest phenolic content.

Overall, the findings of this study suggest that the *Allium stracheyi* leaf has promising antioxidant activity, which may be attributed to its high phenolic and flavonoid contents. The chloroform extract, in particular, showed strong antioxidant potential and may be a potential source of natural antioxidants. However, further studies are needed to identify the active compounds responsible for the observed antioxidant activity and to determine their mechanisms of action.

The results of this study have important implications for the development of new natural antioxidant agents, which can be used as functional ingredients in food, pharmaceutical and cosmetic industries. Moreover, the use of plant extracts as a source of natural antioxidants can provide a sustainable and eco-friendly alternative to synthetic antioxidants.

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Conflict of Interest: None

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