RESEARCH ARTICLE

Exploring the Therapeutic Potential of *Sedum lineare* Thunb: Phytochemical Analysis and Identification of Active Bioactive Compounds

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ABSTRACT

Objectives: This research aimed to explore the therapeutic potential of *Sedum lineare* Thunb by conducting phytochemical analysis and identifying active bioactive compounds.

Materials and Methods: *S. lineare* Thunb samples were collected from Pune district, Maharashtra, India in December 2022. Preparation of extracts using a hydroalcoholic solvent (ethanol: water, 70:30 v/v). Phytochemical screening using established methods to determine the presence of alkaloids, flavonoids, glycosides, diterpenes, carbohydrates, saponins, and tannins. Quantitative determination of total phenol and flavonoid contents in the hydroalcoholic extract. Identification of the marker compound (quercetin) in the *S. lineare* Thunb extract using HPLC.

Results and Discussion: Alkaloids, glycosides, flavonoids, diterpenes, carbohydrates, saponins, and tannins were all found in the crude extracts by phytochemical analysis. The hydroalcoholic extract exhibited total phenol and flavonoid contents of 2.75 and 1.452 mg/100 mg, respectively. Quantitative estimation of quercetin in the hydroalcoholic extract was determined to be 0.0148%.

Conclusion: The study demonstrated that *S. lineare* Thunb extracts contain various secondary metabolites, including phenolic compounds, flavonoids, and other bioactive compounds. The hydroalcoholic extract showed significant total phenol and flavonoid contents, indicating its potential therapeutic value. Further research is warranted to explore the specific health benefits and therapeutic applications of the identified bioactive compounds in *S. lineare* Thunb.

Keywords: Phytochemical screening, *Sedum lineare* Thunb, Total flavonoid content, Total flavonoid content.

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INTRODUCTION

Sedi Linear Traditional Chinese Medicine (TCM) has long utilised the complete *S. lineare* Thunb plant (Figure 1), known in Chinese as Herba, to cure a number of ailments including hepatitis, throat swelling, diarrhoea, dermatitis rhus, burns, scalds, and traumatic bleeding.[1] Particularly in the Tujia minority region in western Hubei, China, there has been a recent uptick in interest in the potential of Sedi linearis Herba in suppressing the development of cancerous cells.[2]

Positive findings from pharmacological investigations in mice with experimental acute liver damage. *Sedi linearis* Herba demonstrated liver-protective effects, as evidenced by reduced alanine aminotransferase (ALT) activity and alleviating jaundice symptoms.[3] It exhibited a significant reduction in MDA levels in both blood and hepatic tissue, indicating its potential antioxidant activity. Moreover, the plant extract increased the activity of the superoxide dismutase enzyme, further contributing to its hepatoprotective effects.[4]

Furthermore, ethyl acetate extracted from *Sedi linearis* Herba showed notable anticancer effects on various types of cancer cells. This suggests its potential as a valuable resource for developing new anticancer therapies. It is worth noting that *S. linearis* Herba has also been traditionally consumed as a vegetable in certain regions of China, suggesting its safety profile and further supporting its potential for medicinal applications.[5]

Overall, the traditional use of *S. linearis* Herba in TCM and the findings from pharmacological studies indicate its therapeutic potential in treating various ailments and inhibiting malignant cell formation. However, further research and

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development are necessary to explore its active components mechanisms of action, and optimize its application in clinical settings.[6]

The inclusion of a wide variety of ingredients is a well-known characteristic of crude drugs. As a result, conventional quality control methods that concentrate on measuring a single active component fall short of providing a thorough assessment of the quality of the raw medicine.[7] Crude drugs contain numerous chemical constituents that work together synergistically to contribute to their overall therapeutic effects. To obtain a more comprehensive assessment of the quality of raw drugs, it is essential to employ modern quality control approaches that consider the diverse array of constituents present in crude drugs and their interactions. Such approaches enable a more accurate and reliable evaluation, ensuring the safety and efficacy of herbal medicines and traditional remedies.[8]

MATERIALS AND MATHODS

Plant Material Acquisition and Identification

In December 2022, *S. lineare* Thunb plants were collected from Pune district, Maharashtra, India. Dr. Gaurav Nigam conducted the authentication of the plant from the Botany Department of the Institute of Basic Science at Bundelkhand University. The reference voucher for the plant is designated as *S. lineare* Thunb (Voucher sample number: B.U./Bot./PhD./2022/001), and a specimen of the plant has been deposited in the respective department. Following the collection of the plant, the whole plant material underwent a cleaning process to prevent the corrosion of phytochemicals present in the plant (Figure 2).

Procedure for Extraction

The extraction procedure for preparing the extract from the shade-dried and powdered *S. lineare* Thunb herbs involved the following steps. Firstly, the harvested plants were carefully dried in a shaded area to remove excess moisture. Once dried, the plants were ground into a fine powder using a suitable grinding apparatus. Subsequently, the powdered herbs were extracted using a proper solvent, such as ethanol, methanol, or a solvent mixture like ethanol-water. The choice of solvent depended on the desired phytochemicals to be extracted. Various extraction techniques, including maceration, reflux, or sonication, could be employed based on extraction efficiency and time requirements. The resulting extract was then filtered

Figure 1: *S. lineare Thunb*. plant

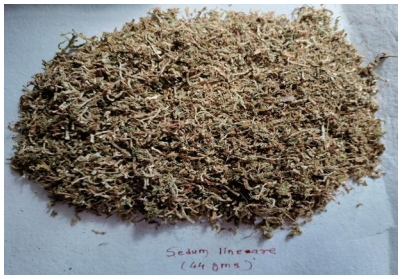

Figure 2: Collection of *S. lineare Thunb*

to eliminate any solid particles or impurities, ensuring a clear extract. If desired, the extract could be concentrated through techniques like rotary evaporation or freeze-drying to remove the solvent and obtain a concentrated extract. Finally, the prepared extract was stored in suitable containers, such as amber glass bottles, in a cool and dry location, protected from light and heat, to maintain the stability and quality of the phytochemicals. It is important to note that specific variations in the extraction procedure may occur based on the intended application or research requirements.[9]

Defatting of plant material

A total of 44 grams of whole plant material from *S. lineare* Thunb was subjected to the maceration method using petroleum ether as the solvent. The maceration process involved soaking the plant material in the solvent to facilitate extraction. Extraction continued until the defatting of the material was achieved, indicating the removal of fats, lipids, and non-polar compounds. The maceration process's duration may vary depending on factors such as extraction efficiency and plant material properties. Once defatting was completed, the extraction process was considered successful, signifying the extraction of non-polar components into the petroleum ether. Further analysis or processing of the obtained extract can be conducted based on specific research objectives or intended applications.[10]

Maceration-based extraction using a single solvent (hydroalcoholic)

The air-dried and powdered defatted marc of *S. lineare* Thunb was utilized for extraction using a hydroalcoholic solvent, specifically a combination of ethanol and water in a ratio of 70:30 v/v. The resulting extract was then filtered using Whatman filter paper no. 1 to remove any solid particles or impurities.[11] Subsequently, the solvent was evaporated from the filtrate to obtain a dry and concentrated extract. The extractive yield was determined by weighing the dried, concentrated crude extract. The extract was then placed in 6 x 2 cm glass vials and kept in a refrigerator at 4°C until further analysis could be performed.[12]

Determination of Extractive Value (%YIELD)

Each extract's percentage yield was determined using the following formula:

Percentage Yield = Weight of extract x 100% / Weight of powdered drug taken

Qualitative Phytochemical Analysis

Extracts must be tested for their individual phytochemical contents, a preliminary phytochemical screening was conducted following established protocols. The extract was put through a battery of tests meant to identify any components it could contain.[13]

Studies on the Quantity of Phytoconstituents

Calculating the overall phenol concentration

The extract's total phenol content was calculated using a Folin-Ciocalteu technique modification. A standard solution was prepared by softening 10 mg of Gallic acid into 10 mL of methanol. Different aliquots ranging from 10 to 50 µg/mL were prepared by diluting the Gallic acid solution in methanol. The powdered extract was then reconstituted in 10 mL of methanol after being dissolved in water. This filtered extract used 2 mL (1-mg/mL) for the phenol assessment.[14]

The phenol concentration was determined by combining 2 mL of the extract or the standard solution with 1-mL of Folin-Ciocalteu reagent (1:10 v/v dilution in distilled water) and 1-mL of sodium carbonate solution (7.5 g/l). After standing for 10 minutes to develop color, the resultant mixture was given a quick 15 second spin in a vortex.

A spectrophotometer was used to determine that the produced colour had an absorption of 765 nm.[15]

Flavonoid content approximation

Using the aluminium chloride technique, the total flavonoid content of the extract was calculated. At first, 10 mg of quercetin was dissolved in 10 mL of methanol to make a standard solution. Concentrations ranging from 5 to 25 g/mL were obtained by diluting the reference solution in methanol.

Ten milligrammes of the dried extract was reconstituted in 10 mL of methanol and then filtered to produce the extract. The amount of flavonoids was calculated using 3 mL (1-mg/mL) of the filtered extract.

The flavonoid concentration was determined by adding 1 mL of a 2% aluminium chloride solution to each 3 mL of extract or standard solution. After combining the ingredients, we let the concoctions 15 minutes to sit at room temperature. The produced color was then analyzed by determining its absorbance at 420 nm in a spectrophotometer.[16]

Identification of Marker Compound (Quercetin) in *S. Lineare Thunb* Extract by HPLC

Reagents and chemicals

Scan Research Laboratories of Bhopal, India, generously supplied quercetin for the research. In New Delhi, India, we shopped at Merck Ltd. for HPLC-grade methanol and acetonitrile. All of the HPLC quality water used in the studies came from Merck Ltd. in New Delhi, India.[17]

The λ_{max} was determined using a LABINDIA Series 3000 UV-vis Spectrophotometer equipped with a thermospectronic model and 1-cm matched quartz cells. For the HPLC analysis, a Waters HPLC system was utilized. The system comprised a pump, a UV-visible detector, and a Thermo C18 column (250 × 4.6 mm, 5 µm). The chromatographic data acquisition and processing were performed using Data Ace software.[18]

Table 1: Choice of separation parameters

Variable	*Condition*
Column	Dimension: 250 × 4.60 mm
Particle size	5 µm
Bonded phase	Octadecylsilane (C18)
Mobile phase	Acetonitrile 50%, Methanol 50%
Flow rate	1-mL/min
Temperature	Room temperature
Sample Size	20 mL
Detection wavelength	256 nm
Retention time	2.7 ± 0.5 min

The selection of separation variables for the analysis is presented in Table 1. The column dimensions were 250 × 4.60 mm, with a particle size of 5 µm. Octadecylsilane was used as the bound phase (C18). Acetonitrile (50%) and methanol were used as the chromatographic mobile phase (50%). A constant 1-mL/min flow rate and ambient temperature were used. A sample volume of 20 mL was used for the analysis. The detection wavelength was configured at 256 nm, and the retention time was observed to be 2.7 ± 0.5 minutes. These variables were carefully chosen to ensure optimal separation and accurate analysis of the sample.

Preparation of standard solution

A precise amount of Quercetin, 10 mg, was correctly weighed and moved into a 10 mL volumetric flask to make the stock solution. The stock solution was formulated by adding methanol to the flask up to the calibration mark, resulting in a concentration of 1000 ppm.

From the stock solution, 1-mL was taken and diluted with a suitable solvent to reach a final volume of 10 mL. This diluted solution was utilized as the primary standard solution for subsequent analyses.

Aliquots of 0.5, 1.0, 1.5, 2.0, and 2.5 mL were taken from the main standard solution and placed in individual 10 ml volumetric flasks to make a series of standard drug solutions. The volume of each flask was adjusted to 10 mL using the mobile phase. The concentrations obtained in the flasks were 5, 10, 15, 20, and 25 g/mL, which are considered as the standard concentrations for drug solutions.

Analysis of Extract

To prepare the extract solution, the extract was weighed out and located in a 10 mL volumetric flask to ensure accuracy. Subsequently, the flask was occupied to the mark with methanol, yielding a solution with a 1000 µg/mL concentration.

The solution was subsequently filtered through a Whatman filter paper to remove any solid particles or impurities. After filtration, the volume was adjusted to the mark using the same solvent, ensuring a consistent concentration of 1000 µg/mL.

To further refine the solution, it was filtered once again using a 0.45 µ membrane filter to achieve additional clarity

Table 2: Percentage yield of *S. lineare* Thunb

Extract	*Percentage yield (w/w) (%)*
Petroleum ether	1.68
Hydroalcoholic	9.41

and purity. Following filtration, the solution was subjected to sonication for 10 minutes to ensure proper mixing and dissolution of the extract components.

RESULTS AND DISCUSSION

Percentage Yield

Table 2 presents the percentage yields of Sedum lineare Thunb extracts obtained using different solvents. The petroleum ether extract yielded 1.68% (w/w), indicating that 1.68 grams of extract were obtained from every 100 grams of plant material processed using petroleum ether as the solvent. In contrast, the hydroalcoholic extract exhibited a higher yield at 9.41% (w/w), signifying that 9.41 grams of extract were obtained from every 100 grams of the plant material when a hydroalcoholic solvent was employed. These findings illustrate the varying efficiencies of solvents in extracting compounds from Sedum lineare Thunb, a crucial consideration in the isolation of bioactive compounds for further analysis and therapeutic applications.

Phytochemical screening of extracts of *S. lineare* thunb

Table 3 outlines the phytochemical screening results of the hydroalcoholic extract of Sedum lineare Thunb obtained through maceration. The analysis revealed the presence of several important constituents, including alkaloids (positive in Hager's and Wagner's tests), glycosides (positive), flavonoids (positive in Shinoda, Lead acetate, and Alkaline tests), diterpenes (positive in Copper acetate test), phenolic compounds (positive in Ferric Chloride Test), carbohydrates (positive in Fehling's and Benedicts Tests), saponins (positive in Froth Test), and tannins (positive in Gelatin test). Notably, proteins were absent (negative in Xanthoproteic and Biuret tests), as were oil and fats (negative in Stain/spot test and Solubility test). These findings shed light on the phytochemical profile of the extract, providing valuable insights into its potential therapeutic and medicinal properties.

Phenol and Flavonoid Content Estimation of Extract

Phenolic content prediction

The extract sample's total phenolic content (TPC) was determined and expressed as mg per 100 mg of gallic acid equivalent (mg/100 mg GAE). The calibration curve equation, obtained from the standard curve analysis, was utilized for this calculation. The equation is represented as y = 0.014x - 0.013, where x represents the gallic acid equivalent (GAE) and y represents the absorbance (Figure 3) (Table 4).

The coefficient of determination (R^2) for the calibration curve was determined to be 0.999, indicating a strong correlation between the absorbance and the concentration of gallic acid equivalent.

Using this equation, the TPC of the extract sample was calculated by substituting the absorbance value (y) obtained from the experimental analysis into the equation. The resulting value represents the dry extract sample's TPC expressed as mg/100 mg GAE.

Table 3: Phytochemical screening of extract of *S. lineare* Thunb using maceration method

S. No.	*Constituents*	*Hydroalcoholic extract*
1.	Alkaloids	Negative (-ve)
	- Mayer's test	Negative (-ve)
	- Hager's test	Positive (+ve)
	- Wagner's test	Positive (+ve)
2.	Glycosides	Positive (+ve)
	- Legal's test	
	- Baljet test	
3.	Flavonoids	Positive (+ve)
	- Shinoda test	
	- Lead acetate Test	
	- Alkaline test	
4.	Diterpenes	Positive (+ve)
	- Copper acetate Test	
5.	Phenol	Positive (+ve)
	- Ferric Chloride Test	
6.	Proteins	Negative (-ve)
	- Xanthoproteic Test	
	- Biuret test	
7.	Carbohydrate	Positive (+ve)
	- Fehling's Test	
	- Benedicts Test	
8.	Saponins	Positive (+ve)
	- Froth Test	
9.	Tannins	Positive (+ve)
	- Gelatin test	
10.	Oil and Fats	Negative (-ve)
	- Stain/spot test	
	- Solubility test	

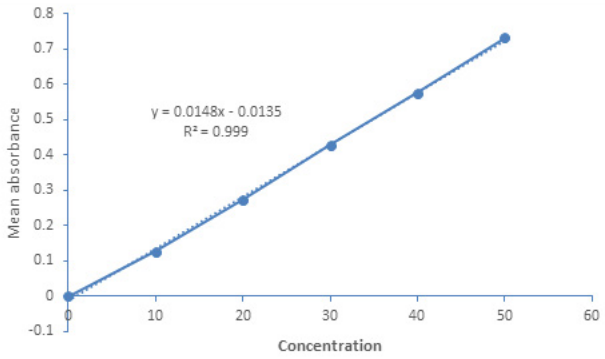

Figure 3: Gallic acid's calibration curve graph

Estimation of total flavonoids content (TFC)

The total flavonoid content of the extract sample was determined and expressed as milligrams per 100 milligrams

Table 4: Gallic acid calibration curve development

S. no.	*Concentration (µg/mL)*	*Mean absorbance*
1	10	0.125 ± 0.001
2	20	0.273 ± 0.03
3	30	0.428 ± 0.004
4	40	0.576 ± 0.002
5	50	0.732 ± 0.005

(n=3, Mean ± SD)

Table 5: Quercetin calibration curve preparation

S. no.	*Concentration (µg/mL)*	*Mean absorbance*
1	5	0.211 ± 0.005
2	10	0.395 ± 0.003
3	15	0.572 ± 0.002
4	20	0.754 ± 0.004
5	25	0.931 ± 0.002

Table 6: The phenolic and flavonoid content of *S. lineare* Thunb. is approximatively calculated

S. No.	*Extract*	*Total phenol content*	*Total flavonoids content*
		mg/ 100 mg	*mg/ 100 mg*
1.	Hydroalcoholic	2.75	1.452

Table 7: Preparation of flavonoid calibration curve (Quercetin)

S. no.	*Concentration (µg/mL) n = 3*	*Mean AUC*
1.	5	503.24 ± 12
2.	10	892.62 ± 35
3.	15	1332.75 ± 19
4.	20	1747.26 ± 15
5.	25	2157.51 ± 11

Table 8: Hydroalcoholic extract of *S. lineare* Thunb for quantification of Quercetin content

S. no.	*Extract*	*RT*	*Area*	*%Assay*
1.	Hydroalcoholic extract	2.679	49.667	0.0148%

of quercetin equivalent (mg/100 mg QE). This calculation was based on the calibration curve equation obtained from the standard curve analysis. The equation is represented as y = 0.036x + 0.015, where x represents the Quercetin equivalent (QE) and y represents the absorbance.

The coefficient of determination (R^2) for the calibration curve was found to be 0.999 (Table 5), representing a strong correlation between the absorbance and the concentration of quercetin equivalent (Figure 4).

By substituting the absorbance value (y) obtained from the experimental analysis into the equation, the total flavonoid content of the extract was calculated as quercetin equivalent (mg/100 mg QE). This provides an estimation of the concentration of flavonoids present in the extract sample relative to the equivalent amount of quercetin (Table 6).

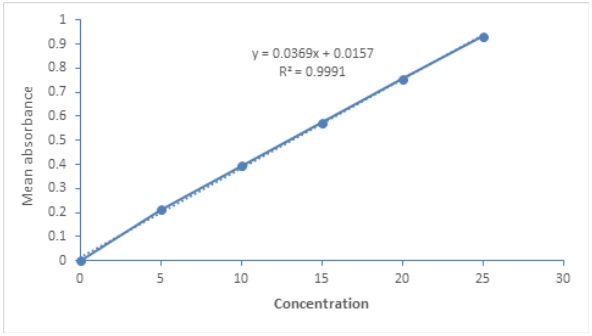

Figure 4: Calibration curve of quercetin graph

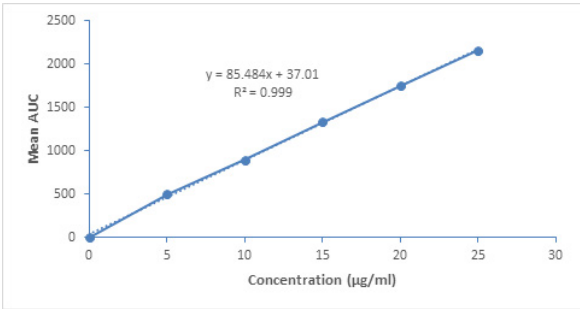

Figure 5: Calibration curve of flavonoid (Quercetin)

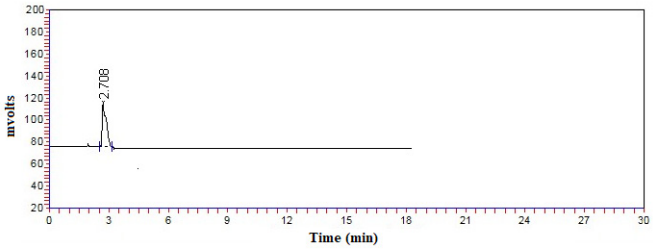

Figure 6: Chromatogram of standardflavonoid (Quercetin)

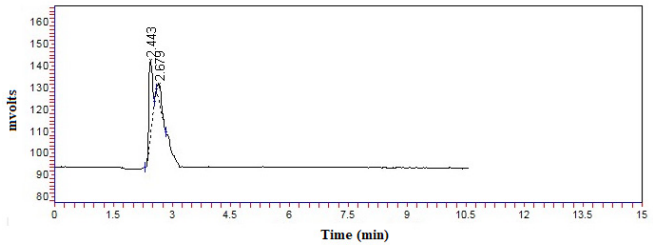

Figure 7: *S. lineare* Thunb. hydroalcoholic extract chromatogram

RESULTS OF HPLC

In the HPLC results, Table 7 presents the preparation of a flavonoid calibration curve using quercetin as the reference compound. The table shows the concentrations (in µg/ml) of quercetin at n = 3 replicates and their respective mean area under the curve (AUC) values. Figure 5 illustrates the resulting calibration curve, while Figure 6 displays the chromatogram of the standard Flavonoid (Quercetin). Furthermore, Figure 7 provides the chromatogram for the hydroalcoholic extract of Sedum lineare Thunb. Lastly, Table 8 reports the HPLC analysis results for the hydroalcoholic extract, indicating a retention time (RT) of 2.679 minutes, an area of 49.667, and a

Quercetin content of 0.0148%. These findings demonstrate the quantification of Quercetin in the extract, which is a significant component with potential therapeutic implications.

DISCUSSION

The extraction process involved defatting the plant material using petroleum ether, followed by extraction with a hydroalcoholic solvent using the maceration method. The percentage yields of the extracts were determined, with the hydroalcoholic extract yielding a higher percentage (9.41%) compared to the petroleum ether extract (1.68%).

Alkaloids, diterpenes, glycosides, flavonoids, phenols, carbohydrates, saponins, and tannins were all found in the hydroalcoholic extract during phytochemical analysis. These findings suggest that *S. lineare* Thunb contains a diverse range of phytoconstituents, which may contribute to its potential medicinal properties.

The study also quantitatively determined the hydroalcoholic extract's total phenol and flavonoid contents. Both the total phenolic and flavonoid contents were measured to be 2.75–100 mg and 1.452–100 mg, respectively. Phenols and flavonoids are known for their antioxidant properties and potential health benefits, indicating that the extract of *S. lineare* Thunb may possess antioxidant activity.

Furthermore, HPLC analysis was conducted to identify and quantify the marker compound, quercetin, in the hydroalcoholic extract. Quercetin is a flavonoid with various pharmacological properties. The results of the investigation showed that quercetin was indeed present in the extract, with a retention time of 2.679 minutes and a percentage assay of 0.0148%.

The findings of this research provide light on the phytochemical profile of *S. lineare* Thunb. The presence of bioactive compounds such as alkaloids, glycosides, flavonoids, diterpenes, phenols, carbohydrates, saponins, and tannins indicates therapeutic potential. The significant levels of total phenols and flavonoids suggest that the extract may exhibit antioxidant activity, which is beneficial for human health.

It is worth noting that the identification and quantification of quercetin, a known bioactive compound, further supports the potential pharmacological significance of *S. lineare* Thunb. Quercetin has been associated with various health benefits, including anti-inflammatory, antioxidant, and anticancer properties.

Overall, this study contributes to understanding *S. lineare* Thunb as a probable source of bioactive compounds, particularly phenols, flavonoids, and quercetin. Further research and investigation are warranted to explore the specific biological activities and potential therapeutic applications of these phytochemicals and their synergistic effects within the plant extract.

CONCLUSION

In conclusion, the study conducted on *S. lineare* Thunb has provided significant insights into its phytochemical composition and potential medicinal properties. The researchers obtained a hydroalcoholic extract with a notable yield through careful procurement, authentication, and extraction processes. The phytochemical analysis of *S. lineare* Thunb showed a wide variety of bioactive components, including alkaloids, glycosides, flavonoids, diterpenes, phenols, polysaccharides, saponins, and tannins. The quantitative analysis of total phenols and flavonoids demonstrated substantial levels of these compounds in the extract, indicating its potential antioxidant activity. The antioxidant properties of phenols and flavonoids, such as their capacity to neutralize harmful free radicals and provide resistance to the damaging effects of oxidative stress, have long been recognized as important to human health. Moreover, the identification and quantification of quercetin, a well-studied flavonoid, further underscored the pharmacological significance of *S. lineare* Thunb. Quercetin's presence in the extract suggests additional therapeutic potential, as it has been associated with various health benefits such as anti-inflammatory, antioxidant, and anticancer effects.

In summary, the study's findings highlight the significance of *S. lineare* Thunb as a potential source of bioactive compounds. The presence of diverse phytoconstituents, significant levels of phenols and flavonoids, and the identification of quercetin offer promising avenues for future research and the development of natural therapeutics.

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