PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIOXIDANT PROPERTIES OF *LINOSTOMA DECANDRUM*

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Abstract. *Linostoma decandrum* is a rare species, and this study is the first to report its phytochemical screening and antioxidant properties. As a result, the ethanol extracts isolated from the leaves and stems of this plant contain many bioactive components such as phenol, tannin, flavonoid, alkaloid, saponin, coumarin, terpenoid, and steroid. The leaves extract includes the total flavonoid, polyphenol, and triterpene contents of 98.06 mg QE/g DW, 43.28 mg GAE/g DW, and 6.35 mg OAE/g DW, respectively. In comparison, 16.90 mg QE/g DW, 26.03 mg GAE/g DW, and 4.51 OAE/g DW were the total flavonoid, polyphenol, and triterpene contents in the stem extract. The leaves and stem extract showed the DPPH radical-scavenging activities with IC_{50} values of 0.45 and 0.68 mg/mL, respectively.

Keywords. Antioxidant activity, *Linostoma decandrum*, qualitative, quantitative phytochemistry.

1. INTRODUCTION

People have used medicinal plants in folk medicine for thousands of years, with human communities passing down experiential knowledge of their beneficial effects through many generations [1]. Some medicinal plants are now employed in modern pharmacotherapy [2]. The Thymelaeaceae family comprises more than 966 species in 48 genera, with approximately 113 species used in traditional medicine [3]. Almost all members of this family are shrubs or trees, usually annual and rarely perennial. Many species are widely used not only as medicinal plants but also as ornamental plants, raw materials for paper-making, and incense. For instance, the genera Gyrinops and Aquilaria produce agarwood, a non-timber resinous wood used as high-grade incense in Asian countries [4]; *Stellera chamaejasme* and *Daphne genkwa* have long been important in traditional Chinese medicine [5]; and in Japan, *Wikstroemia sikokiana* and *Edgeworthia chrysantha* were used to make high-quality banknotes or paper from their bast fibers [6].

Linostoma is a small genus in the Thymelaeaceae family, distributed across Indo-China, the Malay Peninsula, Myanmar, Thailand, Borneo, India, and Bangladesh [7], and comprises only four accepted species: *Linostoma decandrum* (Roxb.) Steud., *L. longiflorum* Hallier f., *L. pauciflorum* Griff., and *L. persimile* Craib (Royal Botanic Gardens, Kew). *L. decandrum* (Roxb.) Steud. is a rare species found in Assam, Bangladesh, Cambodia, Laos, Myanmar, Thailand, and Vietnam (Royal Botanic Gardens, Kew). To date, studies on the phytochemistry and biological activities of this species are limited. In this study, we provide the first report on the phytochemical screening and antioxidant properties of *L. decandrum*.

2. METHODS AND MATERIALS

2.1. Plants

The leaves and stems of L. decandrum (Roxb.) Meisn were collected from Dinh Mountain, Ba Ria-Vung Tau, Vietnam (10.532210°N, 107.138222°E), GPS coordinates were recorded using a Garmin GPSMAP 64s with an accuracy of ±3 meters. The plant specimen was identified by Le Van Son, an officer of Binh Chau - Phuoc Buu Nature Reserve. The voucher specimens, Le VS401A, were deposited at the Herbarium Binh Chau - Phuoc Buu Nature Reserve (Xuyen Moc district, Ba Ria-Vung Tau province).

2.2. Sample preparation

The leaves and stems of the studied species were dried at 45°C, then ground and passed through a 0.45mm sieve. 0.5g of the prepared samples were extracted with ethanol at a ratio of 1 g of sample to 30 mL of solvent for 8 hours. The resulting supernatant was separated by filtration. The remaining residue was then subjected to two additional extractions, each with ethanol at a ratio of 1 g of sample to 30 mL of solvent for 8 hours. The resulting extracts were designated as solutions 2 and 3. All three extracts (including the

initial supernatant) were combined to obtain the final sample extract [8].

2.3. Qualitative phytochemistry of the studied extract

The qualitative biological activities for the groups of natural compounds that were extracted and identified during this investigation are shown in Table 1. We conducted preliminary experiments to determine potential biological activities associated with coumarins, terpenoids and steroids, alkaloids, saponins, flavonoids, and phenolic and tannin compounds. This qualitative assessment provides an initial overview of the diverse biological potential of these compound groups.

Table 1: Qualitative determination of biological activities

Components	Methods	Results	References
Coumarin	2mL extract + 3mL NaOH (10%)	yellow color	[9]
Terpenoids and steroids	5mL extract + 2mL chloroform + 3mL concentrated H_2SO_4	reddish brown	[10]
Saponin	2mL extract + 10mL distilled water + boil for 2 minutes	form foam	[11]
Flavonoids	2mL extract + 2 mL $Pb(COOH)_2$ (10%)	yellow precipitate	[12]
Alkaloids	2mL of extract + 3-4 drops of Wagner reagent	red brown precipitate	[13]
Phenolic and tannin	2mL of extract + 2mL of H_2O + 2-3 drops of $FeCl_3$ (5%)	blue-black or brown-green precipitate	[14]

2.4. Criteria for qualitative phytochemical intensity scoring

The intensity of phytochemical presence was semi-quantitatively assessed based on color development, turbidity, or precipitate formation during standard qualitative tests, following established phytochemical screening protocols by Harborne (1998) [15]. The grading scale used in this study is as follows:

(+) Trace/Low presence – Weak color change or precipitate observed only faintly or after prolonged reaction time.

(++) Moderate presence – Clear and consistent color development or moderate precipitate formation upon standard reagent addition.

(+++) High/Abundant presence – Intense color change or dense precipitate observed immediately, indicating a strong reaction with high compound concentration.

2.5. Quantitative phytochemistry of the studied extract

2.5.1 Total polyphenol content (TPC)

Preparation of standard curve: A series of quercetin standard solutions (0–10 ppm) was prepared by diluting a 100 ppm stock solution. The standard curve (y = 0.0091x + 0.0148; R2 = 0.9959) was used to quantify the total polyphenol content in the samples, expressed as gallic acid equivalents (GAE).

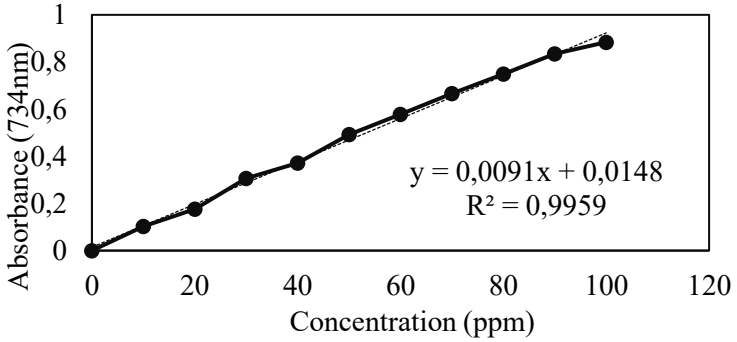

Figure 1: Standard curve of total polyphenol content determination

Add 0.1 mL of the extract and 1.8 mL of Folin-Ciocalteu solution into the tube and leave for 5 minutes after vortexing. Then, 1.2 mL of 15% Na_2CO_3 solution was used to create an alkaline pH, and distilled water was added to make 10 mL. Cover, vortex, and incubate in the dark for 90 minutes, and measure the absorbance at wavelength λ = 734 nm using a spectrophotometer [16]. The TPC was identified using Formula 1.

$$TPC = C_x \times \frac{V}{10^3} \times \frac{100}{a \times (100-W)} \times K \quad (1)$$

Where:
TPC: Total polyphenol content (mg GAE/g DW)
Cx: the total polyphenol concentration in the extract calculated from the standard curve (ppm)
V: sample volume (mL)
a: initial sample mass (g)
W: humidity (%)
K: Dilution Factor
10^3: conversion factor.

2.4.2 Total triterpene content (TTC)

Preparation of standard curve: A series of quercetin standard solutions (0–10 ppm) was prepared by diluting a 100 ppm stock solution. The standard curve (y = 0.0207x – 0.0053; R^2 = 0.9949) was used to quantify the total triterpene content in the samples, expressed as oleanolic acid equivalents (OAE).

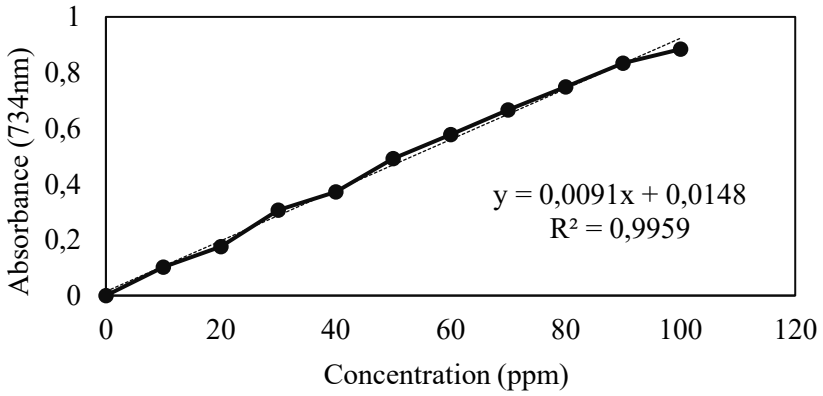

Figure 2: Standard curve of total triterpenen content determination

Add 1 mL of the extract, 0.2 mL of 5% acetic acid, and 1.2 mL of $HClO_4$ into a test tube, mix, and incubate at 70 °C for 15 minutes, then cool for 2 minutes. The mixture was diluted to 5 mL with ethyl acetate. Photometric measurement at 550 nm wavelength [17]. TTC was identified using Formula 2.

$$TTC = C_x \times \frac{V}{10^3} \times \frac{100}{a \times (100-W)} \times K \quad (2)$$

Where:
TTC: Total triterpene content (mg OAE/g DW)
Cx: the total triterpenoid concentration in the extract calculated from the standard curve (ppm)
V: sample volume (mL)
a: initial sample mass (g)
W: humidity (%)
K: Dilution Factor
10^3: conversion factor.

2.4.3 Total flavonoid content (TFC)

Preparation of standard curve: A series of quercetin standard solutions (0–100 ppm) was prepared by diluting a 1000 ppm stock solution. The standard curve (y = 0.0031x + 0.0088; R^2 = 0.9927) was used to quantify the total flavonoid content in the samples, expressed as quercetin equivalents (QE).

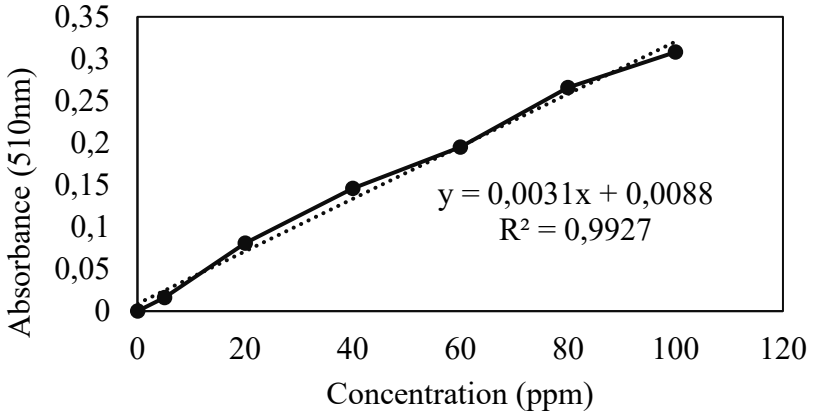

Figure 3: Standard curve of total flavonoid content determination

Add 1 mL of the extract and 0.3 mL of 5% $NaNO_2$ solution into the tube. Vortex, and allow the mixture to stand for 5 minutes. Then, add 0.3 mL of 10% $AlCl_3$ solution, vortex, and let it stand for 5 minutes; add 2 mL of 1M NaOH, vortex again, and add distilled water to make 10 mL. Photometric measurement at wavelength λ=510nm. For the control sample, replace the sample solution with distilled water. Total flavonoid content was expressed as grams of quercetin equivalent QE in Formula 3 [18].

$$TFC = C_x \times \frac{V}{10^3} \times \frac{100}{a \times (100-W)} \times K \quad (3)$$

Where:

TFC: Total flavonoid content (mg QE/g DW)

Cx: the total flavonoid concentration in the extract calculated from the standard curve (ppm)

V: sample volume (mL)

a: initial sample mass (g)

W: humidity (%)

K: Dilution Factor

10^3: conversion factor.

2.6. Determination of the antioxidant activity of the studied extract

Take 0.1 mL of the sample extract into a test tube. In the control sample, the extract was replaced by distilled water. Next, 4 mL of DPPH solution was added to the test tube, and then ethanol was added to the test tube to make 5 mL and incubate in the dark for 30 minutes. Measure optical absorbance at 517 nm. Vitamin C was used as a positive control.

Determine the antioxidant capacity of the sample: take 0.1mL of the extract into a test tube, suck 4mL of 0.1mM DPPH solution into the test tube and add 0.9 mL of ethanol. Leave in the dark for 30 minutes and measure absorbance at 517nm [19]. Based on the vitamin C standard curve equation, calculate the antioxidant capacity according to Formula 4.

$$I = \frac{A_0 - A}{A_0} x100 \quad (4)$$

Where:

I: inhibitory concentration (%)

A: absorbance of sample V: sample volume (mL)

A_0: absorbance of DPPH.

3. RESULTS

3.1. Qualitative phytochemistry of the studied extract

The qualitative tests for preliminary phytochemical screening from the leaves and stems of L. decandrum are shown in Table 2 and Figure 5. The qualitative analysis revealed differences in the abundance of secondary metabolites between the leaf and stem extracts. Leaves showed a high abundance

(+++) of phenolics and tannins, while stems exhibited only moderate levels (++), suggesting that antioxidant activity may be higher in leaf tissues. Both plant parts showed a similar presence of alkaloids (+), indicating a limited yet comparable distribution of these bioactive nitrogen compounds. Flavonoids were highly abundant (+++) in both leaves and stems, supporting their potential role in plant defense and antioxidant properties across tissues. Notably, saponins were more pronounced in the stems (++), compared to a lower presence in the leaves (+), suggesting higher surfactant or antimicrobial potential in the stem extract. Terpenoids and steroids were moderately present (++) in leaves but very abundant (+++) in stems, indicating that the stem extract may offer stronger anti-inflammatory or cytotoxic activity. Coumarins were detected at a low level (+) in the leaves but were very abundant (+++) in the stems, highlighting a significant metabolic divergence in this class of compounds. These variations highlight the importance of considering plant part specificity in phytochemical applications, as each part possesses a distinct profile of bioactive compounds.

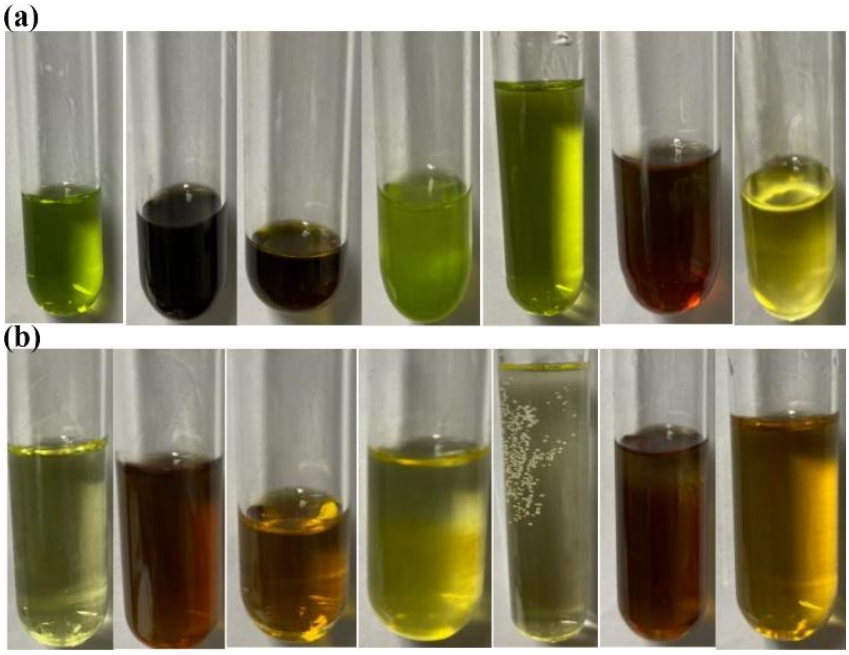

Figure 5: Qualitative phytochemicals of the leaves (a) and stems (b) extracts. From left to right: initial fluid, phenolic and tannins, alkaloids, flavonoids, saponins, terpenoids and steroids, coumarin

Table 2: Qualitative phytochemicals of the leaves (a) and stem (b) extracts

Phytochemicals	Leaves	Stems
Phenolic and tannins	+++	++
Alkaloids	+	+
Flavonoids	+++	+++
Saponins	+	+
Terpenoids and steroids	+++	++
Coumarin	+	+++

Note: (+) less, (++) medium, (+++) very abundant

3.2. Quantitative phytochemistry of the studied extract

The quantitative phytochemical analysis of *L. decandrum* demonstrated significant differences between the leaves and stem extracts in terms of flavonoids, polyphenols, and triterpenes content. Based on the standard calibration curves for total polyphenol (Figure 1), triterpene content (Figure 2), and total flavonoid (Figure 3), the concentrations of these compounds were determined accurately in both plant parts. The leaves extract showed remarkably higher levels of total flavonoids (98.06 mg QE/g DW) compared to the stem extract (16.90 mg QE/g DW), indicating a rich source of antioxidant compounds. Similarly, the total polyphenol content in leaves (43.28 mg GAE/g DW) was significantly greater than that in stems (26.03 mg GAE/g DW), further supporting the antioxidant potential of the leaves extract. Although the difference in

total triterpene content was less pronounced, the leaves still exhibited higher levels (6.35 mg OAE/g DW) than the stems (4.51 mg OAE/g DW). These findings, validated through the use of standard curves, confirm that the leaves extract of *L. decandrum* contains a higher concentration of bioactive phytochemicals than the stem, which may contribute to greater pharmacological activity.

Table 2: Quantitative phytochemistry of the leaves and stem extracts

Constituents	Leaves	Stems
TFC (mg QE/g DW)	98.06 ±2,32	16.90 ±1,06
TPC (mgGAE/g DW)	43.28 ±1,68	26.03 ±0,87
TTC (mg OAE/g DW)	6.35 ±0,29	4.51 ±0,13

TTC: total triterpene content, TPC: total polyphenol content. TFC: total flavonoid content

3.3. Antioxidant activity of the studied extract

Figure 3 presents the DPPH radical-scavenging activities of the leaves and stem extracts. Figure 3 depicts the percentage of DPPH radical-scavenging activity as a function of extract concentration (mg/mL) for both leaf (a) and stem (b) extracts. The inverse relationship between absorbance and concentration indicates that higher extract concentrations correlate with increased radical-scavenging activity. For the leaves extract (a), the absorbance decreases sharply with increasing concentration, reflecting its strong DPPH radical-scavenging potency. The stem extract (b) exhibits a more gradual decline. The results showed that the leaves extract exhibited stronger antioxidant activity than the stem extract with IC_{50} value of 0.45 and 0.68 mg/mL, respectively.

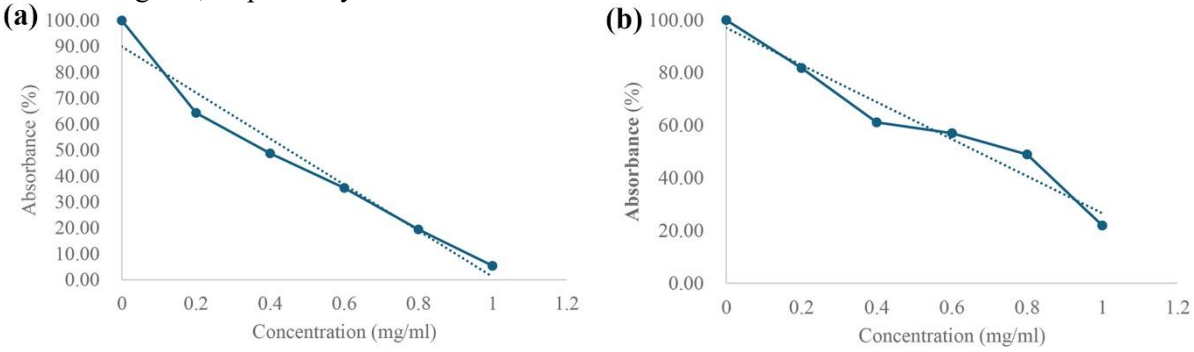

Figure 3: DPPH radical scavenging of leaves (a) and stems (b) extracts

The antioxidant activities observed in L. decandrum extracts suggest their potential for treating oxidative stress-related diseases, including cardiovascular and neurodegenerative disorders. These extracts are capable of neutralizing reactive oxygen species (ROS) and reducing oxidative damage, with the leaves extract demonstrating strong radical scavenging activity. However, further research is needed to explore the full therapeutic potential of this species.

4. DISCUSSION

This study provides the first integrated assessment of the phytochemical profile and antioxidant activity of *L. decandrum*, offering valuable baseline data for this underexplored species. To date, no published research has comprehensively compared the qualitative and quantitative phytochemical composition of different organs in *L. decandrum*, nor within the Linostoma genus. The findings thus fill a critical gap in the phytochemical literature of Thymelaeaceae family.

Qualitative phytochemical screening revealed distinct distributions of secondary metabolites between leaves and stem tissues. Flavonoids were highly abundant in both parts, supporting their core role in antioxidant and protective mechanisms. Phenolics and tannins were especially prominent in the leaves, while saponins, terpenoids, steroids, and coumarins showed higher intensity in the stem. This organ-specific allocation of metabolites suggests a functional division in biochemical roles: leaves likely emphasize antioxidant defense and UV protection, whereas stems may prioritize structural and antimicrobial functions. The strong presence of coumarins and triterpenoids in the stem likely helps the plant defend itself from pests and pathogens [20].

Quantitative data supported these patterns, with the leaves extract showing significantly higher levels of

total flavonoids (98.06 mg QE/g DW), total phenolics (43.28 mg GAE/g DW), and triterpenes (6.35 mg OAE/g DW) than the stem. This biochemical richness aligns with the high antioxidant potential of the leaves observed in DPPH assays. Phenolic compounds are recognized as powerful antioxidants due to their capacity to donate hydrogen atoms and neutralize reactive oxygen species (ROS), thereby reducing cellular oxidative damage [21]. The elevated flavonoid content further enhances this antioxidant profile, given their reported anti-inflammatory, anti-carcinogenic, and cardioprotective effects [22]. Although the difference in triterpene levels between leaves and stem was not very large, these compounds are still important because of their known anti-inflammatory and anticancer properties [23].

The antioxidant activity evaluation confirmed that *L. decandrum* leaves extract had a stronger radical-scavenging capacity, with an IC_{50} value of 0.45 mg/mL compared to 0.68 mg/mL for the stem. These results suggest that the antioxidant potential correlates with the concentration of flavonoids and phenolics, both of which are markedly enriched in the leaves. Previous studies in other species in Thymelaeaceae family, such as *Thymelaea hirsuta*, reported similar trends, where phenolic-rich leaf extracts exhibited strong antioxidant and antimicrobial properties [20].

Additionally, *L. decandrum* has shown larvicidal activity in prior studies, particularly in root extracts against *Aedes aegypti* larvae [24]. Although the present work focuses on leaves and stems, the finding suggests that this species may harbor a broad spectrum of bioactivities across different organs. Taken together, the current results position *L. decandrum* as a promising natural source of phytochemicals with potential applications in pharmaceutical and nutraceutical fields.

As this is the first report to quantify and compare secondary metabolite content and antioxidant capacity across organs of *L. decandrum*, it establishes a scientific foundation for further pharmacological, ecological, and taxonomic studies within the Linostoma genus. Continued exploration, including compound isolation and in vivo assays, will be critical to fully evaluate the therapeutic potential and safety of this species.

5. CONCLUSIONS

This study marks the first investigation into the phytochemical composition and antioxidant properties of *Linostoma decandrum*, a rare species of the Thymelaeaceae family. As demonstrated by both qualitative and quantitative analyses, the ethanol extracts from the leaves and stems revealed a rich profile of bioactive compounds, including phenols, tannins, flavonoids, alkaloids, saponins, coumarins, terpenoids, and steroids, with the leaf extract exhibiting notably higher abundance across most constituents. The superior DPPH radical-scavenging activity observed in the leaf extract likely results from its elevated levels of flavonoids and polyphenols, compounds known for their free radical-neutralizing capacity. These findings underscore the potential of L. decandrum as a promising natural source of antioxidants for pharmaceutical and nutraceutical development. Furthermore, the observed organ-specific differences highlight the importance of tissue-level phytochemical studies in elucidating the therapeutic value of medicinal plants. Future research should focus on isolating and characterizing the active constituents responsible for the antioxidant effects and evaluating their efficacy in biological systems. This pioneering study thus lays the groundwork for future pharmacological exploration of L. decandrum and other members of the Linostoma genus.

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BƯỚC ĐẦU SÀNG LỌC HÓA HỌC THỰC VẬT VÀ ĐẶC TÍNH CHỐNG OXY HÓA CỦA CÂY DÓ MƯỜI NHỊ (*Linostoma decandrum)*

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Tóm tắt. Cây Dó mười nhị (*Linostoma decandrum*) là một loài hiếm và nghiên cứu này là báo cáo đầu tiên về thành phần hóa học cũng như đặc tính chống oxy của loài này. Kết quả cho thấy, chiết xuất ethanol từ lá và thân cây chứa nhiều hợp chất hoạt tính sinh học quan trọng, bao gồm phenol, tannin, flavonoid, alkaloid, saponin, coumarin, terpenoid và steroid. Phân tích thành phần hóa học cho thấy, trong chiết xuất lá, tổng hàm lượng flavonoid, polyphenol và triterpen lần lượt đạt 98,06 mg QE/g DW, 43,28 mg GAE/g DW, và 6,35 mg OAE/g DW. Trong khi đó, chiết xuất thân có tổng hàm lượng flavonoid, polyphenol và triterpen lần lượt là 16,90 mg QE/g DW, 26,03 mg GAE/g DW, và 4,51 mg OAE/g DW. Bên cạnh đó, hoạt tính chống oxy hóa của chiết xuất lá và thân cây được đánh giá thông qua khả năng bắt gốc tự do DPPH, với giá trị IC_{50} lần lượt là 0,45 mg/mL và 0,68 mg/mL, cho thấy tiềm năng chống oxy hóa đáng kể.

Từ khóa. Hoạt động chống oxy hóa, *Linostoma decandrum*, hóa học thực vật định tính, hóa học thực vật định lượng

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