DOMINANT ATTRIBUTIONS PHENOLIC COMPOUNDS IN LEPIDAGSTHIS THORELII EXTRACTS RESPONDING FOR ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES

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Abstract: Extracts from some species in the genus Lepidagsthis have demonstrated biological activities such as antifungal, anti-inflammatory, antioxidant, and insecticidal propertie. *Lepidagsthis thorelii* is a species widely spreading from tropical to subtropical regions, however no research has been published on the antioxidant properties of this plant species until now. This study evaluated the TPC, phenolics, antioxidant, and antimicrobial activity of extracts from two *L. thorelii* parts by four extract solvents (methanol, ethanol, acetone, methylacetate). The methanol extracts obtained the highest TPC. Two methanol *L. thorelii* extracts and two acetone *L. thorelii* extracts with the relevant IC_{50} values (0.35, 0.39, 0.37, and 0.26 mg/mL) possessed remarkable properties of scavenging reactive oxygen species. The antioxidant capacities in three extracts indexed good correlations with their TPC values, except the *L. thorelii* flower. Methanol extracts revealed impacts to the growth of *B. subtilis*, and *E. coli*. The study confirmed extracts from *L. thorelii* provide potential phenolic compounds for antioxidant therapeutic applications or antimicrobial activity of *L. thorelii* extracts. This study first reported the presence of hydroxybenzoic acid (HBA) and ferulic in *L. thorelii* as molecular indicators to understand the antioxidant potential of *L. thorelii*.

Keywords: Antioxidant and antimicrobial activities, ferulic acid, hydroxybenzoic acid (HBA), *Lepidagsthis thorelii* (*L. thorelii*), and the total phenolic content (TPC), IC_{50}.

1 INTRODUCTION

Lepidagsthis is a genus with a population of 100 species classified widely spread from tropical to subtropical regions of Asia and Africa [1]. Extracts from some species in the genus Lepidagsthis have demonstrated biological activities such as antifungal, anti-inflammatory, antioxidant, and insecticidal properties. The prominent constituents have identified many extracts in this genus: alkaloids, essential oils, flavonoids, inorganic minerals, saponin, and phenolic acid [2-5]. These plants with therapeutical qualities could serve as the basic raw materials for complex pharmaceutical formulation [4,6].

Phenolic acids are the leading group widely distributed in plants, vegetables, fruits, grains, and other seeds and comprise possible beneficial effects on human health [7-9]. There are two classes of phenolic acids that can be distinguished depending on their structure: benzoic acid derivatives (hydroxybenzoic acids, C6-C1) and acid derivatives (hydroxycinnamic acids, C6-C3) [10]. A more significant number of phenolic compounds either have free radical scavenger functionalities [11] or play important roles in the prevention or control of many chronic diseases [8]. In benzoic acid derivatives, the bioavailability of gallic acid (3,4,5‑trihydroxybenzoic acid) is a natural compound that has been suggested to possess intense antioxidant activities in many studies in vitro and in vivo. Next, salicylic acid also demonstrates activities in the regulation of plant processes such as seed germination, seedling development, plant vegetative growth, fruit yield, respiration, and response to ultraviolet (UV)‑B radiation [12,13]. Similarly, various compounds in cinnamic acid derivatives such as chlorogenic acid, caffeine, p-coumaric acid, ferulic acid, hydroxybenzoic acid, cinnamic acid, rutin, and quercetin display biological activities consistent with antioxidant, anti-inflammatory as well as anti-tyrosinase properties [14-16].

It was discovered that 87% of therapeutic drugs were made from naturally occurring substances taken from plants. Hence, there is increased attention towards plant-based natural antioxidants [17,18]. *Lepidagsthis thorelii* was discovered in North Vietnam in 1911 and concentrated in the Central and Southern regions [19]. The plant is an herbaceous perennial, with a square stem, reaching a height of 2‑5 cm and covered in

hairs. The leaves are 6–10 cm long and 3–4 cm wide, broadly ovate with obtuse tips. There is 6–8 pairs of lateral veins, the petioles are short, the flowers are solitary in terminal clusters (Figure 1).

Figure 1. Morphology of *L. thorelii* species in Vietnam

However, to date, no research has been published on the antioxidant properties of this plant species. Hence, this study aims to determine the most suitable solvent for extracting the high TPC of *L. thorelii* and evaluate antioxidant capacity from extracts involved in antioxidant components. Additionally, the study seeks to identify the main ingredients present in the solvent extract and calculate the antioxidant capacity of the principal components, thereby laying a foundation for the clinical application of *L. thorelii*. Finally, confirming phenolic compounds in *L. thorelii* extracts in this report could give applications in producing antioxidant agents and developing interventions against bacterial-related disease.

2 MATERIALS AND EXPERIMENTAL METHODS

2.1 Materials

Gallic acid, chlorogenic acid, caffeic acid, p-coumaric acid, ferulic acid, hydroxybenzoic acid (HBA), salicylic acid, cinnamic acid, rutin, and quercetin (standard, ≥98%, Sigma), aluminum chloride (AlCl3) (China), potassium acetate (CH3COOK) (China), sodium carbonate (Na2CO3) (China), Folin-Ciocalteu (Indian), 1,1-diphenyl-2-picrylhydrazyl (DPPH) (standard, ≥97%, Sigma), 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) (standard, ≥98%, Sigma), sodium persulfate (Na2S2O8) (China), Luria-Bertani media (LB) (Himedia), Nutrient Broth media (NB) (Himedia), Dimethyl Sulfoxide (DMSO) (China).

2.2 Collection and preparation of *L. thorelii* extracts

The *Lepidagsthis thorelii* plant was collected at Binh Chau-Phuoc Buu Nature Reserve, Xuyen Moc District, Ba Ria-Vung Tau Province, in October 2022. The Binh Chau-Phuoc Buu Nature Reserve is located south of Hai Van Pass and belongs to the Southern Vietnam Lowland Dry Forest ecoregion. The area's aridity and isolation have led to the development of unique plant communities, including *L. thorelii*, which has been found exclusively in this location [20].

The samples (leaves and flowers of *L. thorelii* plant) was dried for about 24 h at 45ºC to obtain moisture < 5%, Afterwards, each part was finely ground into powder. The dried samples were mixed with solvents, incluce methanol (Me), ethanol (Et), acetone (Ace), and ethyl acetate (Ea) at a ratio of 1 g dry sample/40 mL. After shaking on a shaker for 24 h, the mixture was filtered through Whatman filter paper, and the solvents were removed using a vacuum rotary evaporator to obtain various extracts of each component. These extracts were stored at deep freeze conditions and used for analyses such as TPC, phenolic compounds, antioxidant activity, and antimicrobial assays.

2.3 Determination of total phenolic content

The TPC in the extracts were quantified by the Folin-Ciocalteu method and followed the previous research by Kim (2016) [21]. Add 0.5 mL extract solution into the test tubes containing 2.5 mL of Folin Ciocalteu 10%, shake, and let stand for 3 – 8 mins in the dark. Then add 2 mL Na_2CO_3 7.5%, shake, and incubate in the dark for 60 min. The absorbance was taken by a UV–vis spectrophotometer (Thermo GENESYS 20 UV-Vis) at 765 nm and Gallic acid was used as standard compound.

2.4 Determination of main compounds in *L. thorelii* extracts by HPLC method

The analysis of each of the phenolics was conducted utilizing a Shimadzu LC-2030C HPLC, system paired with a diode-array detector (HPLC-DAD) and furnished with a VertiSep™ GES C18 reverse-phase chromatographic column (dimensions: 250 × 4.6 mm, particle size: 5.0 µm). The mobile phase consisted of methanol (A) and a solution of 1% formic acid in water (B). The composition of the mobile phase changed as follows: from 0 to 3 mins, 25% A; 3 to 5 mins, 25% to 40% A; 5 to 16 mins, 40% to 60% A; 16 to 21 mins, 60% A; 21 to 24 mins, 60% to 80% A; 24 to 27 mins, 80% A; 27 to 35 mins, 80% to 25% A. The elution process operated at a flow rate of 0.8 mL/min, while the column temperature was maintained at 40 ºC. The detection of phenolic acids and flavonoids were set at a wavelength of 295nm, and 360 nm. Subsequent data analysis was performed using LabSolutions software developed by Shimadzu in Kyoto, Japan. The quantification of phenolic acids was carried out based on calibration curves established using five different concentrations (0.1, 0.25, 0.5, 1, 5 µg/mL) [22].

2.5 DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity of *L. thorelii* extracts

The DPPH radical scavenging activity was determined as the method described by Qian et al. (2020) with some modification [23]. In brief, 1.0 mL of extracts with various concentrations mixed with 4.0 mL of 0.076 mM DPPH dissolved in methanol. After vortex, the fluid was kept in the dark at 25ºC for 30 mins and then the absorbance of the resulting solutions measured at 517nm against methanol as a blank. Lower absorbance indicated higher radical-scavenging activity. The DPPH radical-scavenging activity was expressed as: scavenging rates = (A1 – A2)/A1 ×100%, where A1 is the absorbance of the control (DPPH solution without sample); A2 is the absorbance of the test sample. The standard curve was constructed using a series of concentrations of ascorbic acid to replace the tested samples. Three replicates were completed for each test.

2.6 ABTS (2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical antioxidant activity of *L. thorelii* extracts

The antioxidant activity was measured according to the method of Centenaro et al. (2011) [24], with some modifications. The cationic radical ($ABTS^{\bullet+}$) was prepared by mixing two stock solutions (7 mM ABTS•+ dissolved in deionized water and 2.45 mM $Na_2S_2O_8$ in a 1:1 ratio, and the mixture was incubated for 16 h at 25 ºC in the dark. Then the stock solution diluted with deionized water to an absorbance of 0.70 ± 0.02 at 734nm. The reaction begins with 100 µL of extract of various concentrations mixed with 3 mL of $ABTS^{\bullet+}$ solution, shaken and incubated for 6 mins. Absorbance was measured at 734nm. The percentage of ABTS radical scavenging activity is calculated by the formula: Scavenging rates = (A3 – A4)/A3 ×100%, where A3 is the absorbance of the control ($ABTS^{\bullet+}$ solution without sample); A4 is the absorbance of the test sample. The standard curve was constructed using a series of concentrations of ascorbic acid to replace the tested samples. Three replicates were finished for each test.

2.7 Antimicrobial activity of *L. thorelii* extracts

The antimicrobial potency of each plant extract was evaluated using one strain of Gram negative (Escherichia coli) and two strains of Gram positive (*Staphylococcus aureus* and *Bacillus cereus*). Agar well diffusion method was used to screen the antimicrobial activities [25]. The bacteria cultures were grown in 5 mL NB medium at 37ºC. After 6–8 h of growth, bacterial culture was standardized using McFarland standard (106 CFU/mL of 0.5 McFarland standard) and was inoculated on the surface of LB agar plates. Even streaks of the microbial broth suspension with the sterile cotton swab were placed onto the surface of the LB agar plates in 3 planes to cover their whole surface with the microorganism and let it dry for 5 mins after the inoculation. After that, using an Oxford cup (6 mm diameter) to make wells into the agar plates. Each well was filled with 100 µL plant extracts of different concentrations (25, 50, 75 and 100 mg/mL) prepared in DMSO 100%. Gentamicin (10 µg/disc) was used as positive control and negative/solvent control (DMSO), respectively. It was allowed to diffuse for about 1–2 hours at 4ºC and incubated for 18–24 hours at 37ºC. After incubation, plates were observed for the formation of a clear zone around the well which corresponds to the antimicrobial activity of tested compounds. The zone of inhibition (ZOI) was observed and measured in millimeters (mm) and the experiment was repeated in triplicate.

2.8 Statistical Analysis

Measurements were recorded in triplicates for all the analysis. Results were calculated as the mean (n=3) ± SD (standard deviation) for each sample. Two-way ANOVA followed by multiple range test was performed using Stat graphics Centurion XV. $P< 0.05$ were considered significant. The Pearson correlation coefficient (r) was used to study correlations of phenolics and flavonoids with observed antioxidant activity. IC50 values were determined using a nonlinear regression method.

3 RESULT AND DISCUSSION

3.1 The total phenolic contents (TPC)

The pharmacological activities of vascular plants are ascribed to the presence of phenolic compounds [2]. In the present study, the measured TPC of all eight extracts from two parts include leaves and flower of *L. thorelii* in four different solvents: methanol, ethanol, acetone, and ethyl acetate in Table 1 that were highlighted large variations in phenolic content between different plant parts and in each different extraction solvent. In general, the result shows that methanol extracts of two parts of leaves, and flowers exhibit the highest relative TPC value (219.38; 319.04 mgGA/g dw) opposed to all other extracts. In contrast, the ethyl acetate extract of flower part exhibiting exhibits the lowest TPC content (1.417 mgGA/g dw). The high TPC observed in the methanol extracts suggests that methanol is an effective solvent for extracting effectively most phenolic compounds from the leaves, and flowers of the *L. thorelii*. Besides, the TPC from leaves parts in the same methanol extract of *L. thorelii* is higher than that of *L. keralensis*, but the ethyl acetate extracts inherit the lowest TPC indexed that this solvent might not efficiently extract phenolic compounds inheriting in different parts of *L. thorelii* [3].

The polarity of the solvent plays an important role in the extraction of phenolic compounds. Methanol and ethanol are polar solvents widely used in the extraction of natural compounds due to their broad solubility range for both polar and moderately polar compounds. Their effectiveness stems from their ability to break down plant cell walls, facilitating enhanced extraction. Additionally, these solvents are widely used for phenolic extraction due to their ability to preserve the bioactive properties of the compounds [26]. In contrast, ethyl acetate is less polar than methanol and ethanol; however, it is also a commonly used solvent for phenolic extraction, and the lowest total phenolic content (TPC) extraction results align with its polarity.

Table 1. The total phenolic contents of *L. thorelii* extracts

Sample	Unit	Me	Et	Ace	Ea
Leaves	mgGA/g dw	219.38 ±5.009	63.083±0.589	8.979±0.088	7.729±0.147
Flowers		391.04 ±2.062	35.552±0.014	10.271±0.029	1.417±0.059

Me: methanol extract; Et: ethanol extract; Ac: aceton extract; Ea: etylacetate extract.

3.2 Phenolic compounds

The presence and content of several phenolic and flavonoid compounds in four extracts from four different solvents, including methanol, ethanol, acetone, and ethyl acetate, from the leaves and flowers of *L. thorelii* were examined using HPLC, and the data are presented in Table 2. The results showed the presence of 10 compounds, including 8 phenolic compounds and 2 flavonoid compounds in this species. The compound present in the highest amount was gallic acid, with values ranging from 1.26 to 1123.59 mg/g in the leaves and 2.50 to 1340 mg/g in the flowers, with the acetone extract having the highest content in both the leaves and flowers, indicating that acetone is a good solvent for extracting gallic acid. Next was the compound HBA, with high content ranging from 0.59 to 9.78 mg/g in the leaves and 3.16 to 4.44 mg/g in the flowers. The compound present in the smallest amount was quercetin, found only in the methanol extract of the leaves and the ethanol extract of the flowers. For both the leaves and flowers, when extracted with different solvents, the compounds appeared most abundantly and in the highest amounts in the methanol extract, followed by the ethanol extract, and the least in the ethyl acetate extract, corresponding to the TPC content of the extracts shown in Table 1, where the methanol extract had the highest TPC content and the ethyl acetate extract had the least among the four extracts.

When exploring species in the genus Lepidagsthis, it is interesting to note that no studies have yet analyzed the phenolic content present in the species of this genus. Broadening the scope, there are some publications regarding the presence of phenolic compounds in other genera belonging to the Acanthaceae family. For instance, in the species *Dipteracanthus patulus*, *Andrographis paniculata*, and *Rhinacanthus nasutus*, the presence of many phenolic and flavonoid compounds has been found [26, 27]. There is much evidence indicating that phenolic and flavonoid compounds play important roles in various plant functions, such as antioxidant, antibacterial, and hepatoprotective activities. The research results show a correlation between the TPC (Table 1), the phenolic compounds (Table 2), and their antioxidant and antibacterial capabilities [28-31].

According to the result, the optimal under correlation of three factors to extract the highest TPC contents from cashew testa were determined as follows: liquid-solid ratio 42.6 (mL/g), extraction time 97.8 (min), and extraction temperature (48.5^{o}C), the TPC was 375.73 mg GAE/g. The results are shown in Table 3. The result of TPC in Vietnamese cashew testa has a little higher than in Sri Lanka samples [17], and the TPC content is better than the total phenolic that extract from other materials in the previous research [18,19,21].

Table 2. The phenolic compound contents of *L. thorelii* extracts

Parts	Phenolics (mg/g)	Extracts			
		Me	Et	Ac	Ea
Leaves	Gallic acid	1.26 ±0.501	35.90±0.007	1237.59±8.401	169.58±0.141
	Chlorogenic acid	2.29 ±0.135	0.65±0.002	0.88±0.179	0.66±0.014
	Caffeic acid	0.22±0.014	0.37±0.002	0.46±0.070	0.27±0.013
	p-Coumaric acid	0.39±0.037	2.00±0.011	0.45±0.072	0.37±0.018
	Ferulic acid	0.98±0.068	2.64±0.032	0.95±0.005	0.26±0.009
	HBA	1.46±0.036	9.78±0.084	4.97±0.014	0.59±0.030
	Salicylic acid	0.89±0.078	3.62±0.075	Na.	Na.
	Cinnamic acid	0.94±0.006	0.76±0.027	3.59±0.142	Na.
	Rutin	0.62±0.070	0.72±0.011	0.48±0.026	Na.
	Qercetin	0.01±0.006	Na.	Na.	Na.
Flower	Gallic acid	6.55±0.018	2.50±0.008	1340.920±2.150	271.42±0.049
	Chlorogenic acid	2.21±0.136	0.24±0.003	1.454±0.023	2.29±0.000
	Caffeic acid	0.85±0.069	3.13±1.966	0.204±0.014	0.02±0.001
	p-Coumaric acid	0.81±0.008	0.16±0.005	1.342±0.001	0.62±0.011
	Ferulic acid	1.52±0.007	1.02±0.033	2.147±0.085	0.41±0.013
	HBA	4.44±0.147	4.16±0.083	4.333±0.011	3.16±0.014
	Salicylic acid	1.14±0.061	1.13±0.071	4.474±0.004	2.12±0.017
	Cinnamic acid	1.77±0.148	2.12±0.026	2.601±0.053	Na.
	Rutin	0.15±0.011	2.94±0.020	0.147±0.001	Na.
	Qercetin	Na.	0.19±0.010	Na.	Na.

The concentrations of phenolics are calculated in mg/g; Na.: not available

Me: methanol extract; Et: ethanol extract; Ac: aceton extract; Ea: etylacetate extract.

3.3 The total DDPH and ABTS radical scavenging activities

The assessment of the free radical scavenging ability by DPPH and ABTS scavenging assays are widely utilized to evaluate different free radical's capacities. The IC_{50} values (inhibitory concentration, 50%) recorded in Table 3 and Table 4 proven the radical scavenging activities of *L. thorelii* extracts were many fluctuations with dissimilarly trends. Overall, DPPH free radical scavenging activity with IC_{50} values of 0.26 to 0.37 mg/mL in two parts of flower combined flower in acetone extract were determined the three best antioxidant capacities, followed the IC_{50} values from 0.7 to 2.5 mg/mL of leaf ethyl acetate extract and remaining ethanol extracts. Like DPPH method, the highest antioxidant capcity was observed in flower in acetone extracts with respective IC_{50} value of 1.66 mg/mL, followed to two methanol extracts of flower and leaves, about 2.37 and 2.08 mg/mL, to then consequently to lowest capacities or non-identification were in ethanol extracts and ethyl acetate extracts. Furthermore, variations of IC_{50} values indexing for

significant differences of each part in the same solvent ($p < 0.05$) and uppercase letter indexing significant differences in each solvent in the same part of *L. thorelii* ($p < 0.05$) proven clearly about the antioxidant potential of *L. thorelii* extracts were independent of what part of *L. thorelii*. According in these series, antioxidant activities of *L. thorelii* extracts are ranked in as follow decreasing order: acetone > methanol > ethanol > ethyl acetate.

Consequently, a summarized analysis about antioxidant activity between all *L. thorelii* extracts and other extracts from five species in the genus Lepidagsthis (Fig 1S) have demonstrated the ABTS radical scavenging activities of the three best *L. thorelii* extracts (above mentions) were medium levels compared to the lowest IC_{50} values observed in two methanol *L. hyaline* extracts [5] from twig and root (0.189 and 0.125 mg/mL) and ethanol *L. pungens* extract (0.049 mg/mL) [4], contrast to the IC_{50} value of ethyl acetate *L. cuspidate* extract was the highest value (0.928 mg/mL) [33,34]. However, the ABTS radical scavenging activities only determined in *L. thorelii* extracts and *L. prostrate* extracts while others have not investigated. As measured IC_{50} values in Fig 2S revealed the ABTS radical scavenging abilities of three best *L. thorelii* extracts fluctuated from 1.66 to 2.37 mg/mL were higher 100-fold compared to that in two *L. prostrata* extracts in methanol and ethyl acetate just around 0.082 and 0.068 mg/mL. Based on these discussed results may demonstrated that the radical scavenging activities of most extracts are dependent on two effective solvents including methanol and acetone. At the same time, this study indicated that two methanol *L. thorelii* extracts and one acetone flower extract also seemed to be the most effective extracts than that in five remaining *L. thorelii* extracts.

Table 3. DPPH and ABTS free radical scavenging activity of *L. thorelii* extracts

	Parts	IC_{50} (mg/mL)				
		Me	Et	Ace	Ea	Ascorbic acid
DPPH	Leaves	$0.39^{a,A}$	> 1	$0.65^{b,B}$	$0.71^{a,B}$	0.007
	Flower	$0.35^{a,A}$	> 2.5	$0.37^{a,A}$	> 1	
ABTS	Leaves	$2.08^{b,A}$	> 5	> 5	-	0.018
	Flower	$2.37^{a,A}$	-	$1.66^{a,B}$	$4.32^{a,C}$	

Different lowercase letter indicates significant differences in solvents (P<0.05). Different uppercase letter indicates significant differences in solvents (P<0.05).

3.4 Antimicrobial activities of all *L. thorelii* extracts

After petri plates were incubated at 37ºC for 24 h, presences of inhibition zone (mm) of flower and leaf methanolic extracts (Fig 2a, b); and flower acetone extract (Fig 2c) against *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* (10^6 CFU/mL) at different concentrations around the extract in Figure 2 and Table S3 was demonstrated better antimicrobial ability of methanol *L. thorelii* extract ($P < 0.05$). The overall analysed results showed that almost all ZOI appeared at the petri plates cultured Escherichia coli and *Bacilllus subtilis*, contrast not appearance of any ZOI in all treated plates by the growth of *Staphylococcus auerus* remained normally at the petri plates. In addition, changes in ZOI also observed at the petri plates contained high concentrations of extracts, and those were larger span than the well contained low concentrations. Detailly, in the *Escherichia coli* cultured petri plates, only at the wells contained 25 mg/mL of the leave methanol extract got occurrence of ZOI that completely contrast to flower methanol extract. Generally, at the petri plates treated by the high content from 50 to 100 mg/mL of methanol *L. thorelii* extracts showed ZOI occurred in all wells. Particularly to the *Bacilllus subtilis*, that was presences of ZOI in petri plates was treated by methanol *L. thorelii* extracts, and the ZOI with relatively largest diameter from 9.17 mm, 8.73 mm and 7.43 mm obtained in the wells contained the highest extract content (100 mg/mL). Differences in diameters of ZOI in all treated petri plates significantly proven inhibition of these methanol extracts to the moderate growth of bacterial cell. Methanol extracts showed larger inhibited zones against *Bacillus subtilis*, and *Escherichia coli* and did inhibit to *Staphylococcus auerus*. The present

study highlighted the impacts of methanol extracts inhibited normal microbial growth of three experimental bacteria in petri plates contained Luria-Bertani agar medium.

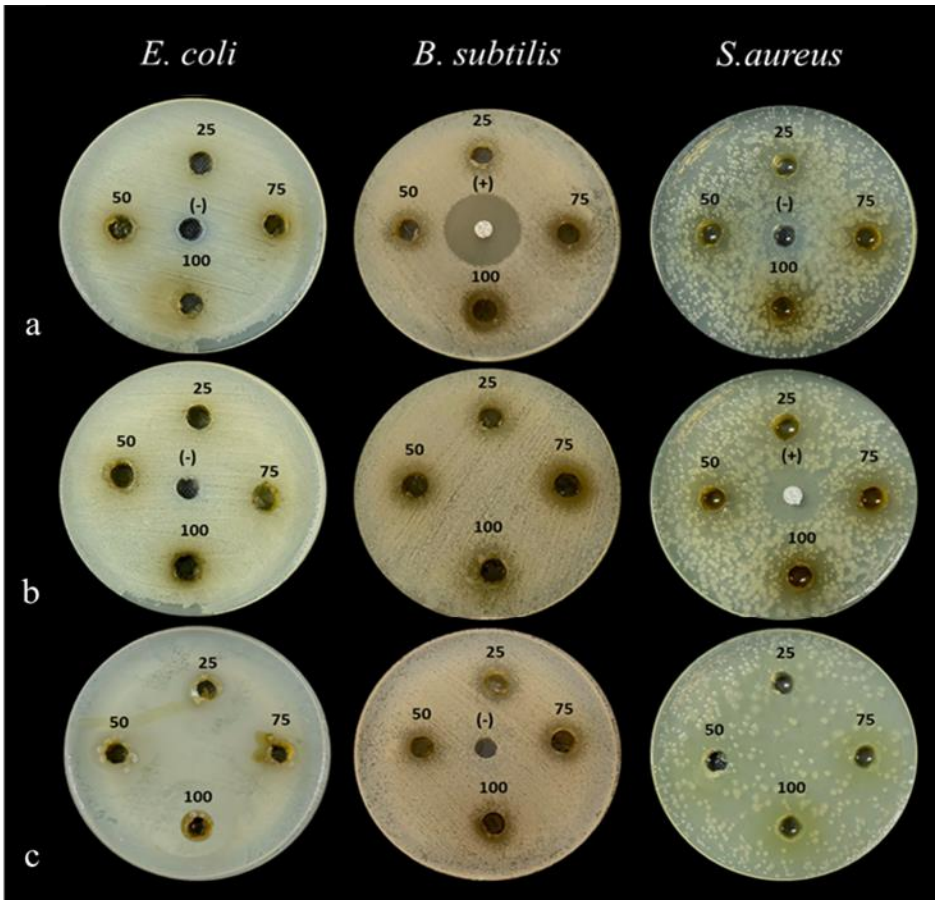

Figure 2: The inhibition zone (mm) of flower and leaf methanolic extracts (a, b); and flower acetone extract (c) against *Escherichia coli, Bacillus subtilis* and *Staphylococcus aureus* (106 CFU/mL) at different concentrations. Negative control (-), DMSO. Positive control, Gentamicin (10 µg/disc).

3.5 Correlation between TPC values with DPPH free radical scavenging activity of *L. thorelii* extracts and the antimicrobial activities of methanol *L. thorelii* extracts.

To analyze differences in the role of five dominant bioactive components relating to free radical scavenging activities, the following Pearson correlation coefficient in Table 4 displayed the best-discovered correlations (r values of 0.998 or 1) between the TPC values in two methanol extracts and one acetone extract with their antioxidant activities. At the same time, the relationship observed in TPC value and the antioxidant activity of flower acetone *L. thorelii* extract were less correlated (0.86) while four remaining extracts were not identified. Furthermore, three distinct solvent-extracted parts of *L. thorelii* involved two compounds with a higher amount, such as HBA and ferulic acid, which can be considered the essential responsibility for the antioxidant activities of leaves acetone extract. More specially, the aspect of the most dominant presence of HBA in leave methanol extract with the best IC_{50} value of 0.26 mg/mL assessed the vital relationship of HBA with its biological activity. Nextly, due to *L. thorelii* belonging to the group of herbs, there is no scientific report about methanol extracts of *L. thorelii* for inhibiting the growth of Gram-negative bacteria. Hence, the Pearson correlation coefficient identified no correlations observed between TPC values with the antimicrobial activities of *L. thorelii*.

Table 4. Pearson correlation coefficient (r) values for correlation between TPC and DPPH free radical scavenging activity of *L. thorelii* extracts

Extracts	Parts	R value
Me	Leaves	-1.00**

	Flower	-1.00**
Et	Leaves	ND
	Flower	ND
Ace	Leaves	0.998*
	Flower	-0.866
Ea	Leaves	ND
	Flower	ND

*Correlation is significant at the 0.05 level (2-tailed)
**Correlation is significant at the 0.01 level (2-tailed)

4 CONCLUSIONS

The natural origin and the diverse biological functions of the phenol acids depend on the structure. Hence, considering the scientific basis for the contribution and content of phenolic compounds in *L. thorelii* is essential for initial knowledge of antioxidant therapeutic applications or antimicrobial activity of *L. thorelii* extracts. In this study, the HPLC analysis of two parts of *L. thorelii* extracted in four solvents confirmed four dominant components were involved in all *L. thorelii* extract consisting of gallic acid, with values ranging from 1.26 to 1123.59 mg/g in the leaves and 2.50 to 1340 mg/g in the flowers, for HBA with high content ranging from 0.59 to 9.78 mg/g in the leaves and 3.16 to 4.44 mg/g in the flowers, and ferulic acid from 0,95 to 2,64 mg/g in the leaves and 1,02 to 2,147 mg/g in the flowers. This is the first study to demonstrate the contributions with the highest contents of ferulic acid and HBA in two parts of *L. thorelii*. Furthermore, the good correlations (r values of 0.998 or 1) between the TPC in two methanol extracts and one acetone-extract with the best antioxidant activities determined that both HBA and ferulic acid are important molecular indicators for the antioxidant potential of *L. thorelii*. Finally, the future direction of this study is to isolate three potential phenolic compounds in *L. thorelii* extracts to investigate deeply more biological functions for applications in developing valuable biological materials.

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CÁC THÀNH PHẦN PHENOLICS CHÍNH TỪ CÁC DỊCH CHIẾT CỦA LOÀI *LEPIDAGSTHIS THORELII* ĐÁP ỨNG HOẠT TÍNH CHỐNG OXI HÓA VÀ KHÁNG KHUẨN

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Tóm tắt: Dịch chiết từ một số loài thuộc chi Lepidagsthis đã cho thấy các hoạt động sinh học như kháng nấm, kháng viêm, chống oxy hóa và tính chất diệt côn trùng. *Lepidagsthis thorelii* là một loài phân bố rộng rãi từ các vùng nhiệt đới đến cận nhiệt đới, tuy nhiên chưa có nghiên cứu nào được công bố về tính chất chống oxy hóa của loài thực vật này cho đến nay. Nghiên cứu này đã đánh giá TPC, phenolics, hoạt động chống oxy hóa và kháng khuẩn của các chiết xuất từ hai bộ phận của *L. thorelii* bằng bốn dung môi chiết xuất (methanol, ethanol, acetone, methylacetate). Chiết xuất methanol đạt được TPC cao nhất. Hai chiết xuất methanol từ *L. thorelii* và hai chiết xuất acetone từ *L. thorelii* với các giá trị IC50 tương ứng (0.35, 0.39, 0.37, và 0.26 mg/mL) có khả năng đáng kể trong việc khử các loại oxy phản ứng. Khả năng chống oxy hóa trong ba chiết xuất có mối tương quan tốt với giá trị TPC của chúng, ngoại trừ hoa *L. thorelii*. Chiết xuất methanol cho thấy tác động đến sự phát triển của *B. subtilis* và *E. coli*. Nghiên cứu đã xác nhận rằng các chiết xuất từ *L. thorelii* cung cấp các hợp chất phenolic tiềm năng cho các ứng dụng điều trị chống oxy hóa hoặc hoạt động kháng khuẩn của các chiết xuất từ *L. thorelii*. Nghiên cứu này lần đầu tiên báo cáo sự hiện diện của các hợp chất phenolic, khả năng kháng oxi hóa cũng như kháng một số chủng vi khuẩn của các dịch chiết của loài *L. thorelii*.

Từ khóa: Antioxidant and antimicrobial activities, ferulic acid, hydroxybenzoic acid (HBA), *Lepidagsthis thorelii* (*L. thorelii*), and the total phenolic content (TPC), IC_{50}.

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