

Phytochemicals detected in *Lysiphyllum strychnifolium* (Craib) A. Schmitz stem extracts and their log-patterns of ABTS radical scavenging activities

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ABSTRACT

Lysiphyllum strychnifolium (W. G. Craib) A. Schmitz is a plant with various medicinal properties, including antioxidant activity and detoxification. In this study, we initially screened the phytochemical compounds in methanolic (MeOH) extract by gas chromatography-mass spectrometry (GC-MS) technique and blasted them with NIST17 library. After screening, some bioactive compounds, such as 1,2-Benzenediol (3.68%); 1,2,3-Benzenetriol (12.16%); 1,3,5-Benzenetriol (24.30%); 13-Docosenamido, (Z)-(3.41%); and D-allose (24.46%) with reported antioxidant properties were identified. Then the next analysis of phytochemicals; including catechin and quercetin in MeOH, ethanolic (EtOH), and aqueous extracts by the high performance liquid chromatography (HPLC). The results revealed that catechin contents in all extracts fell within the range of 3.46–9.55 mg/g DW. However, quercetin is figured out in all extracts except in the EtOH extract, which may be due to its low content and the limitation of detection. Subsequently, we tested the free radical scavenging activities of all extracts using the ABTS method. It is noteworthy that all extracts exhibited significant antioxidant activities, showing a logarithmic pattern when plotting the graph of decolorization versus concentrations, unlike the linear pattern observed with the standard Trolox. Our study confirms that *L. strychnifolium* extracts have excellent antioxidant activities and represents a potential candidate for alternative source of antioxidant agents in medicinal application.

INTRODUCTION

Lysiphyllum strychnifolium (W. G. Craib) A. Schmitz (synonym-*Bauhinia strychnifolia* Craib) belongs to family Fabaceae. *L. strychnifolium* has been used in traditional remedies for treating fever, stomach disorders, and skin infections [1]. It has several biological properties such as anti-inflammatory [2], antimicrobial properties [3], antihyperglycemic effects [4], antioxidant activity [5,6], and detoxification [7]. Regarding antioxidant activity, it plays an important role in inhibiting or delaying the oxidation mechanisms involving the production of free radicals, which are harmful to human cells [8]. Although the antioxidant activities of *L. strychnifolium* were examined

in the leaves extract [5,6], this activity is rarely referred to in stems, especially with a comparison of different concentrations of extracts. Previous studies reported that phytochemical constituents such as kaempferol, ascorbic acid, α -tocopherol, and cyanidin exert antioxidant compounds by GC-MS, HPLC, and liquid chromatography—mass spectrometry (LC-MS) analyzed methods [9–12].

In this study, it is interesting to clarify the components and antioxidant activities in *L. strychnifolium* stem extract for further medicinal application. Previous studies of phytochemicals in this plant described some compounds such as gallic acid, quercetin, resveratrol, epicatechin, catechin, and astilbin [2,13,14]. Various compounds in ethanolic (EtOH) extract of *L. strychnifolium* stem and leaf, as exposed by GC-MS, were reported in previous studies [3]. However, the screening of phytochemicals in methanolic (MeOH) extract blasted with NIST17 libraries was rarely mentioned. Therefore, the present study aimed to investigate the phytochemicals

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composition in MeOH extract of *L. strychnifolium* stem by GC-MS. As well, catechin and quercetin in extracts were analyzed by HPLC. Moreover, the antioxidant activities of various stem extracts derived from different solvents used for extraction were determined.

MATERIALS AND METHODS

Plant materials and extracts preparation

The stems with leaves and flowers of *L. strychnifolium* collected at Yasothon Province, Thailand were identified and the plant reference (BKF No. 197208) was prepared by the Forest and Plant Conservation Research Office, Department of National Parks, Wildlife and Plant Conservation, Thailand.

The stems were collected, washed to remove dust and impurities, dried in a shaded area, cut into small pieces, and then, ground using a grinder to obtain the powder. The extracts were prepared by soaking the stem powder with MeOH, EtOH, or water at a 1:10 ratio (stem powder: solvent), and shaking the mixtures for 3 days. The residues were removed from the extracts by filtration. Consequently, the solvents were evaporated using a vacuum rotary evaporator. All the extracts including MeOH, EtOH, and aqueous extracts, were stored at -20°C before preceding the further analysis.

Screening the phytochemical compounds by GC-MS

The phytochemical compounds in *L. strychnifolium* were initially screened by extracting the stem powder with MeOH and analyzed using GC-MS technique. The MeOH extract of *L. strychnifolium* was MeOH with MeOH and the final concentration was adjusted to 10 mg/ml. The extract solution was filtered through nylon membranes before analysis by GC-MS using the following conditions: 70°C, 4 min of oven temperature; increasing the temperature 15°C/min to 180°C, 5 min hold; increased 10°C/min to 200°C, 5 min hold; and finally increased 10°C/min to 250°C, 1 min hold; total runtime as 29.33 min. The chemical profiles were compared with the NIST17 library database. The quantities of the detected compounds were compared with the internal standard, heptadecanoic acid methyl ester, and reported as a percentage of content.

Analysis the catechin and quercetin contents in *L. strychnifolium* stems by HPLC

After primary screening of the phytochemical compounds in the MeOH extract by GC-MS, all the extracts (MeOH, EtOH, and aqueous extracts) were analyzed by HPLC to determine the catechin and quercetin contents. The extracts were diluted with MeOH (HPLC grade) and filtrated through 0.45 µm nylon filter membranes. The HPLC analysis was performed using a WATERS 2690 separation module with a photodiode array detector. The equipment and conditions were set as follows; a reverse-phase C18 column; a mixture of MeOH, acetonitrile, and water (40:15:45, v/v/v) with the addition of 1.0% acetic acid as a mobile phase; detected wavelength at 259 and 279 nm for catechin and quercetin analysis, respectively [15]. The standard stock solutions at 100 mg/ml of catechin (Sigma-Aldrich) and quercetin (Sigma-Aldrich) were prepared by weighing 10 mg of standard powders and dissolving them

in 100 µL HPLC-grade MeOH. The stock solutions were then diluted with HPLC-grade MeOH to obtain a final concentration of 0.0625, 0.125, 0.25, and 0.5 mg/ml, which were used for standard curve calculation.

Antioxidant activity analysis by ABTS radical scavenging assay

The ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] radical scavenging assay is commonly used to measure antioxidant activity. The ABTS radical solution (0.6–0.7 OD₇₃₄) was prepared as previously described by Re *et al.* [16]. The analysis was conducted by pipetting 190 µl of the ABTS radical solution into each well of 96-well plate, then adding 10 µl of each extract to the wells, and incubating in the dark for 5 minutes before measuring the absorbance of the ABTS radical at 734 nm with multichannel micro-plates reader.

Each treatment was tested in triplicate. The Trolox 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as the standard solution. This stock solution, 100 mg/ml, was prepared by weighing 10 mg of standard powder, dissolving with 100 µL gradient grade EtOH, and then diluting to obtain final concentration of 0.0625, 0.125, 0.25, and 0.5 mg/ml. Gradient-grade EtOH, without the extract, was set as a control for all treatments.

Then, the decolorization effect detected in each sample was calculated using the following equation: Decolorization (%) = $\frac{\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Sample}}}{\text{Absorbance}_{\text{Control}}} \times 100$

RESULTS AND DISCUSSIONS

Screening compounds in MeOH stem extract of *L. strychnifolium*

After analyzing the MeOH extract by GC-MS technique and matching it with NIST17, various compounds were detected and are presented in Table 1. It has been discovered that some bioactive compounds including 1,2-Benzenediol (3.68%); 1,2,3-Benzenetriol (12.16%); 1,3,5-Benzenetriol (24.30%); 13-Docosenamamide, (Z)-(3.41%); and D-allose (24.46%), were identified.

The 1,2-benzenediol (pyrocatechol or catechol) has been reported to possess antioxidant property that can decrease the absorbance of 2,2-diphenyl-1-picrylhydrazyl (DPPH). Additionally, it can prevent autoxidation and slow down lipid oxidation by breaking down lipid hydroperoxides [17,18]. The role of the catechol group in antioxidant and neuroprotective effects in rat brain tissue has been determined [19]. The hydroxyl (OH) groups in the molecule of these phenolic compounds are one of the factors contributing to their antioxidant effect. Mechanically, they prevent oxidative stress-induced apoptosis by activating glutathione peroxidase 4 (GPX4) to reduce the accumulation of reactive oxygen species (ROS) [20]. Moreover, it has repelling activity due to the two hydroxyl groups in the ortho-position [21]. Of note, high concentration of 1,2-benzenediol in rats lead to cytotoxicity. The oral route uptake of this compound has a median lethal dose at 300 mg/kg BW [22]

Table 1. Phytochemical compounds in MeOH extract of *L. strychnifolium* stems blasted with NIST17 library.

RT ^{1/} (minute)	Library searched compounds	Qual ^{2/} (%)	Content (%)
9.72	1,2-Benzenediol	93	3.68
10.76	4,6(1H,5H)-Pyrimidinedione, dihydro-2-thioxo-	38	2.17
10.90	Valine, N-methyl-N- methoxycarbonyl-, pentadecyl ester	25	2.62
11.52	1,2,3-Benzenetriol	97	10.30
11.68	1,2,3-Benzenetriol	97	1.86
12.69	D-Allose	87	24.46
13.43	2-Butanone, 4-(4-hydroxyphenyl)-	70	1.72
13.88	1,3,5-Benzenetriol	97	24.30
14.27	1,3,5-Benzenetriol	38	4.43
14.39	13-Docosamide, (Z)-	38	0.30
14.48	13-Docosamide, (Z)-	60	1.78
15.02	Ethane, isothiocyanato-	43	10.89
15.62	13-Docosamide, (Z)-	89	0.55
15.90	13-Docosamide, (Z)-	91	2.73
15.98	13-Docosamide, (Z)-	94	0.53
16.07	13-Docosamide, (Z)-	93	0.15
18.40	2-Piperidinone, 1-(3,4,5,6-tetrahydro- 2-pyridinyl)-	49	1.37
18.48	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, (1.alpha.,2.beta.,5. alpha.)-	55	3.51
18.94	2-Methyl-6-propylpyridine	27	1.70
19.31	Dodeca-1,6-dien-12-ol, 6,10-dimethyl-	50	0.97

^{1/}RT is retention time.

^{2/}Qual is the similarity blasted with NIST17 library and calculated as the percentage value.

Both 1,2,3-Benzenetriol (pyrogallol or pyrophosphoric acid or pyrogallic acid) and 1,3,5-Benzenetriol (phloroglucinol) are isomers of benzenetriol, and have been studied for their potent antioxidant properties. The isolated seed protein derived from Pumpkin (*Cucurbita* sp.) and its covalent conjugation with 1,2,3-benzenetriol result in an increased number of bound equivalents as the polyphenols concentration increases [23]. This results in the promotion of antioxidant activity. In addition, both compounds have previously been reported to exhibit anti-inflammatory and antimicrobial activities [24,25]. With regard to 1,3,5-Benzenetriol, it is considered as a reducing agent because of its readily donate electrons ability [26]. The antioxidant properties of this compound would have a beneficial effect on cosmetics and skincare because it provides protective activity against oxidative stress and antioxidant activity against DPPH (50% effective concentration values: 12–26 μ M) in skin aging [27].

Besides the aforementioned compounds, another interesting compound is D-allose-, which is a rare naturally

compound occurring monosaccharide belonging to the group of aldohexoses. It has been proposed to play a crucial role in - anti-diabetic properties. In animal studies, it has shown the ability to improve glucose metabolism and enhance insulin sensitivity [28]. Additionally, D-allose has been found to possess antioxidant and anti-inflammatory properties [29–31]. Likewise; 13-Docosamide, (Z)-; also known as erucamide, is a long-chain unsaturated fatty acid amide. It has been reported for antinociceptive and anti-inflammatory [32].

Catechin and quercetin contents in *L. strychnifolium* stem extract

Different extracts of *L. strychnifolium* stems were subjected to determine the quantities of catechin and quercetin using HPLC. The retention times of catechin and quercetin are 2.477 and 4.894 minutes, respectively (Fig. 1). The catechin and quercetin standard curves were plotted, with both R^2 values equal to one. As observation in stem extracts, catechin can be found in all samples (Fig. 1 and Table 2), including MeOH (9.55 mg/gDW), EtOH (7.90 mg/gDW), and aqueous (3.46 mg/gDW) extracts. However, quercetin is found in MeOH (0.09 mg/gDW) and aqueous extracts (0.04 mg/gDW), but was not detected in the EtOH extract. In a previous report, quercetin was mentioned in the EtOH stem extract [7], but not in this study. It may be due to the low quercetin content that is out of the limit concentration of LOD used in this study.

Free radical scavenging activity of *L. strychnifolium* extracts.

The ABTS test was conducted in all extracts; MeOH, EtOH, and aqueous extracts, in order to investigate the effect of different extractive solvents on antioxidant activities. The standard Trolox was used as the reference for antioxidant capacity. The decolorization (%) values of Trolox were plotted versus concentration (mg/ml) ranging from 0 to 0.25 mg/ml. The curve of standard Trolox presents the linear pattern with the equation; $y = 297.28x - 0.6755$, $R^2 = 0.9998$ (Fig. 2). In contrast, all stem extracts provide the logarithm patterns (Fig. 2). The ABTS radical scavenging assay of different extracts shows a strong inhibitory effect at low concentration as compared to standard Trolox. The Minimal Inhibitory Concentrations requiring for inhibiting the antioxidant activity of 50% (MIC_{50}) of *L. strychnifolium* extracts were calculated by substituting the 50% value in the equation that obtained from Figure 2. The statistical analysis was performed using a *t*-test to compare each extract with Trolox. The results show that the radical scavenging activities of all extracts were significantly higher than those of standard Trolox, ranging from 4.95 to 5.26 times (Fig. 3). However, there was no significant difference among the three extracts (data not shown).

Moreover, the relationship of the decolorization of each extract shows logarithm curve, even at the low concentrations (Fig. 2). This implies that the inhibitory potencies of *L. strychnifolium* extracts are extremely higher than that of standard Trolox. The antioxidant activities observed in this study are possibly the results of 1,2-benzenediol; 1,2,3-Benzenetriol (pyrogallol); 1,3,5-Benzenetriol; D-allose; catechin; and quercetin. In a previous

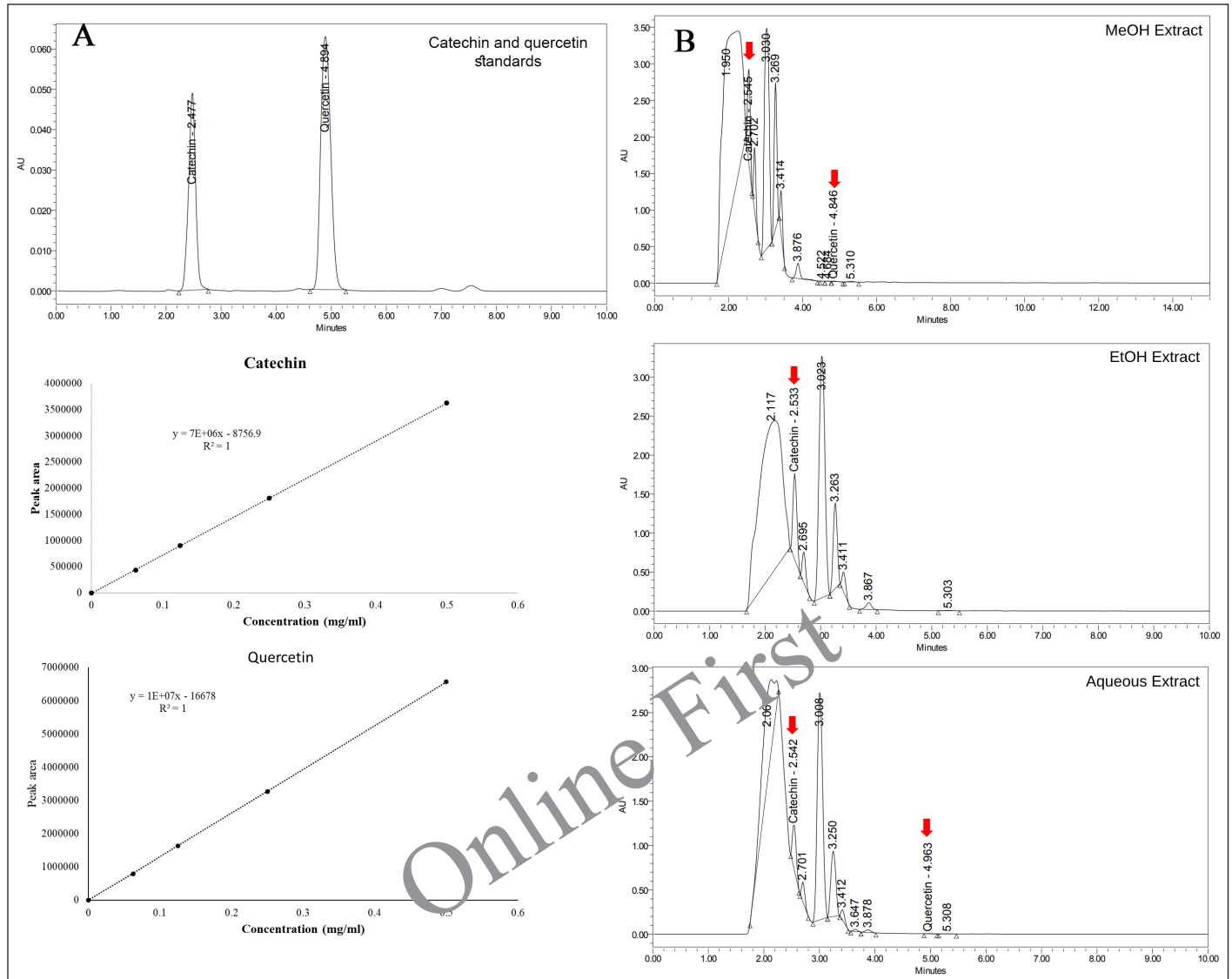


Figure 1. HPLC chromatogram and standard curves of catechin and quercetin (A) and *L. strychnifolium* MeOH, EtOH, and aqueous extracts (B).

Table 2. Catechin and quercetin contents in *L. strychnifolium* stem extracted with different solvents.

Extraction Solvent	Content (mg/gDW)	
	Catechin	Quercetin
Methanol	9.55	0.09
Ethanol	7.90	ND
Distilled Water	3.46	0.04

ND: not detected.

report, catechin and quercetin, known as strong antioxidant agents, were identified in *L. strychnifolium*[33]. In this study, both compounds were also detected in the stem; hence, these greatly exhibited radical scavenging activities are perhaps the results of synergistic effects of catechin, quercetin, and other detected compounds.

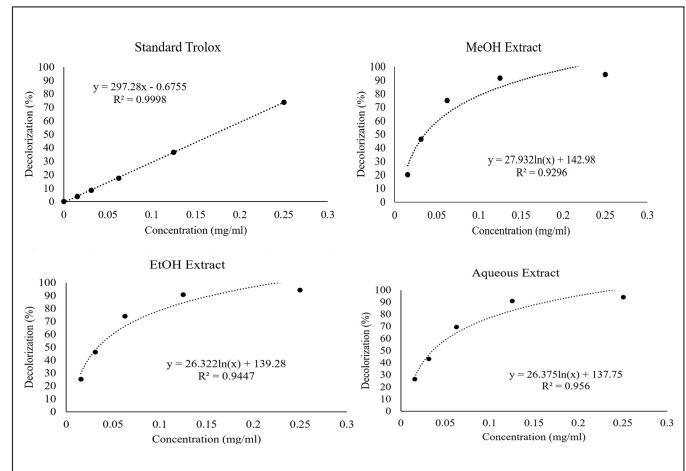


Figure 2. ABTS radical scavenging assays of standard Trolox and *L. strychnifolium* stem MeOH, EtOH, and aqueous extracts.

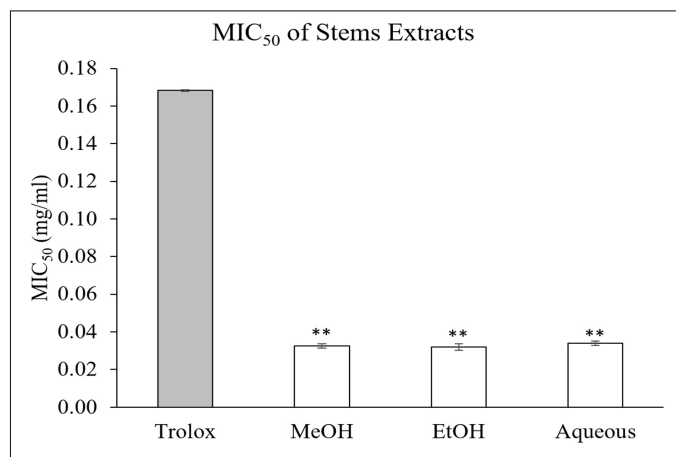


Figure 3. Minimal inhibitory concentration required to inhibit the antioxidant activity of 50% (MIC₅₀) of different *L. strychnifolium* extracts by ABTS radical scavenging assays. The statistical analysis, *t*-test, was compared between each of stem extracts and Trolox at *p* value < 0.01.

CONCLUSION

Based on the results obtained in the present study, it can be concluded that *L. strychnifolium* stem extracts exhibit remarkable antioxidant activities. This is attributed to the presence of several antioxidant agents, including catechin, quercetin, 1,2-Benzenediol, 1,2,3-Benzenetriol, 1,3,5-Benzenetriol, 13-Docosenamamide (Z-), and D-allose. These findings support previous research on the use of *L. strychnifolium* in treating diseases, particularly its detoxification properties. Furthermore, these findings provide strong evidence for the potential application of *L. strychnifolium* stems as a source of bioactive compounds.

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AUTHOR CONTRIBUTIONS

NK conducted the conceptualization, methodology; resources; complete the experiments; data collections and analysis; interpret the data; and writing the original draft and review manuscript. KP managed the conceptualization; funding recruitment; resources preparation; statistical analysis; interpret the data; writing the original draft; proof, review and editing, and finalization the manuscript; and project administration.

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CONFLICT OF INTEREST

All authors declare that they have no conflict of interest.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

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