



## Antioxidant Capacity and Phytochemical Analysis of the Traditional Thai Remedy Benchalokawichian

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### ABSTRACT

Benchalokawichian (BLW) is recognized as a medicinal herb in Thailand and is used for its antipyretic properties. This remedy includes five plant roots: *Clerodendrum indicum* (CI), *Capparis micracantha* (CM), *Ficus racemosa* (FR), *Harrisonia perforata* (HP), and *Tiliacora triandra* (TT). The present study investigated the phytochemicals, total phenolic content (TPC), and antioxidant potential of BLW and its herbal components. Phytochemical screening of the six extracts identified flavonoids, phenolics, and terpenoids. TPC quantification using a spectroscopic method indicated that the TT extract had the most quantity (55.97±0.49 mg of gallic acid equivalent/g). Evaluating total antioxidant capacity through the DPPH free radical scavenging assay revealed that the HP extract showed the most antioxidant activity, with an IC<sub>50</sub> value of 23.69±4.05 µg/mL. The FR extract displayed the most active superoxide radical scavenging activity (63.77±1.12% inhibition of superoxide) according to the riboflavin-light-nitroblue tetrazolium assay. Thiobarbituric acid-reactive substances (TBARS) were used to measure lipid peroxidation, indicating the BLW extract was the most potent (75.30±0.47% inhibition of malondialdehyde). The TT extract exhibited the highest reducing power (0.47±0.023 mM Fe<sup>2+</sup>/mg) – measuring the transformation of ferrous to ferric iron in the extract's presence. These preliminary findings suggest that the ethanol root extracts of HP and TT exhibit strong antioxidant activity by scavenging free radicals, and the BLW treatment effectively prevents lipid peroxidation. The use of BLW may be beneficial in preventing chronic, non-communicable diseases that are linked to reactive oxygen species, such as diabetes mellitus.

**Keywords:** Phytochemicals, Phenolic content, Antioxidant, Benchalokawichian remedy.

### Introduction

Researchers are growing increasingly interested in the wealth of natural compounds and pharmacological activities found in medicinal plants, particularly for their potential beneficial effects on health, especially in dealing with chronic non-communicable diseases such as chronic respiratory conditions, cardiovascular issues, cancers, strokes, and diabetes.<sup>1</sup> The prevalence of these chronic diseases is largely connected to excessive free radical or reactive species generation. Consequently, using effective natural sources of antioxidants represents a practical option for both preventative and therapeutic strategies in managing chronic conditions.<sup>2</sup> Polyphenols, found in the diverse category of natural antioxidants, are renowned as significant antioxidant groups, exhibiting various mechanisms of action. These include scavenging and reducing free radicals to curb oxidative damage. In Asian nations, including Thailand, traditional medicines made up of single herbs and herbal concoctions have been extensively used for generations due to their affordability and easy accessibility.

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Nonetheless, more scientific backing is needed to compare the effectiveness of traditional medicine with that of contemporary approaches.

The Benchalokawichian (BLW) remedy, also known as Ya-Ha-Rak, is a renowned ancient polyherbal formula used in traditional Thai medicine. Traditionally, it has been employed as a preferred treatment for fever and skin rashes associated with inflammation.<sup>3</sup> Notably, it has been officially recognized in the National Herbal Medicinal Product of Thailand since 2012.<sup>4</sup> The BLW contains five plant roots, including *Clerodendrum indicum* (CI), *Capparis micracantha* (CM), *Ficus racemosa* (FR), *Harrisonia perforata* (HP), and *Tiliacora triandra* (TT). BLW is prepared by grinding and combining each plant root in equal proportions.<sup>5</sup> The pharmacological properties of the BLW recipe have been documented, including cytotoxic effects on certain cancer cell lines<sup>6,7</sup> and the inhibition of inflammatory processes in Raw 264.7 cell lines.<sup>3</sup> Additionally, BLW has demonstrated inhibitory effects on bacterial growth,<sup>8</sup> antiplasmodial activity,<sup>9</sup> and anti-allergic properties.<sup>10</sup> Clinical studies of this remedy have also been conducted to evaluate its effects on skin irritation and allergic responses in healthy volunteers. Previous research has consistently indicated favorable safety profiles in both rats and humans.<sup>10,11</sup>

While attention has been focused on the characteristics, pharmacological properties, and applications of the BLW formula, there is limited information available about the comparison of phytochemical compounds and antioxidant activity between the BLW remedy and its plant ingredients. Consequently, this present study focuses on evaluating and comparing the phytochemical components and antioxidant capacities of the 80% ethanol extracts of BLW and its plant ingredients. The study aims to provide substantial evidence and insights into the medicinal value of both the BLW remedy and its plant constituents.

### Materials and Methods

#### Plant materials and extraction

Five plant roots used in the BLW recipe were collected in July 2022 from Phon Thong, Roi Et, Thailand (16°18'0.22" N 103°58'40.30" E). A botanist authenticated these plant materials. Voucher samples were safely stored at the Faculty of Pharmacy, Mahasarakham University, and the voucher numbers of the samples are presented in Table 1. The plant materials were cleaned and dried in a hot air oven at a temperature of 70°C for a duration of 72 h, after which they were ground into small pieces. To prepare the ethanol extract of BLW, 500 g (100 g of each) of

the five plant roots were mixed. The samples were macerated at room temperature twice over a 7-day period using 2.5 L of 80% (v/v) ethanol. The resulting solution was filtered through filter paper, and the solvent was removed using a rotary evaporator (Rotavapor R-300, Buchi™, Switzerland). The remaining substance was dried using a freeze-dryer (Scanvac coolsafe 110-4, Labogene™, Denmark). These extracts were then stored at -20°C until further analysis.

**Table 1:** The percent yield and phytochemical constituents of ethanol extracts of the BLW remedy and its plant components.

Phytochemical constituents	Plant extracts*					
	CI	CM	FR	HP	TT	BLW
Anthraquinones	-	-	-	-	-	-
Carbohydrates	+	-	+	+	+	+
Flavonoids	+	+	+	+	+	+
Phenolics	+	+	+	+	+	+
Saponins	-	+	+	+	-	+
Tannins	+	-	+	+	+	+
Terpenoids	+	+	+	+	+	+
% yield	10.36	6.91	5.57	5.50	10.00	7.25
Voucher number	PHCI01	PHCM01	PHFR01	PHHP01	PHTT01	PHBLW01

Key: \*Phytochemical compounds are (-) absence; (+) presence.

Abbreviations: CI = *Clerodendrum indicum*; CM = *Capparis micracantha*; FR = *Ficus racemosa*; HP = *Harrisonia perforata*; TT = *Tiliacora triandra*; BLW = Benchalokawichian

#### Phytochemical analysis

##### Qualitative assessment of chemical components

The qualitative examination of extract samples aimed to identify various chemical groups, including anthraquinones, carbohydrates, flavonoids, phenolics, saponins, tannins, and terpenoids. This identification process involved using color reactions. The solution consisted of plant extracts, with 50 mg of extract dissolved in 10 mL of ethanol, and underwent a phytochemical analysis using established standard methods.

##### Test for anthraquinones (Borntrager's test)<sup>12</sup>

Initially, a volume of 2 mL of chloroform was introduced into 2 mL of the extract solution, and the mixture was filtered. Subsequently, 1 mL of a 10% (w/v) ammonia solution was added to the filtered substance, which was then vigorously agitated for half a minute. The presence of anthraquinones was indicated by the development of a pink, violet, or red color in the ammonia layer.

##### Test for carbohydrates (Molisch's test)<sup>13</sup>

Carbohydrates were identified by observing a purple ring formed at the boundary between the test material and acid. This was achieved after adding two drops of Molisch's reagent [20% (w/v)  $\alpha$ -naphthol dissolved in ethanol] to 2 mL of the extract solution, then adding a small quantity of concentrated sulfuric acid.

##### Test for flavonoids (Shinoda's test)<sup>13</sup>

After a brief period, a mixture of five drops of concentrated hydrochloric acid and 2 mL of the extract solution was combined with five to eight pieces of magnesium. The emergence of a pink or crimson-red color signified the presence of flavonoids.

##### Test for phenolics (Ferric chloride test)<sup>13</sup>

To detect phenolic compounds, 2 mL of the extract solution was mixed with a small amount of neutral 2% (w/v) ferric chloride solution. The emergence of a dark green color was taken as a positive result.

##### Test for saponins (Foam test)<sup>14</sup>

Initially, 50 mg of the extract was dissolved in 10 mL of distilled water, and the mixture was vigorously agitated. This solution was then thoroughly stirred with a small quantity of concentrated hydrochloric acid. The formation of foam confirmed the presence of saponins.

##### Test for tannins (Braymer's test)<sup>13</sup>

Add 2 mL of deionized water and a small amount of 5% (w/v) ferric chloride solution to the 2 mL extract solution. The presence of tannins can be determined by the appearance of a brownish-green or blue-black coloration.

##### Test for terpenoids (Salkowski's test)<sup>13</sup>

To detect terpenoids, 5 mL of the extract solution was combined with 1 mL of chloroform. Then, 0.5 mL of concentrated sulfuric acid was added to 2 mL of this mixture. A reddish-brown coloration at the boundary confirmed the presence of terpenoids.

#### Quantitative phytochemical analysis

##### Evaluation of total phenolic content (TPC)

TPC was quantified using the Folin-Ciocalteu method<sup>15</sup> on a 96-well plate. The reaction mixture was prepared by combining 20  $\mu$ L of the extract solution, 0.1 mL of 10% Folin-Ciocalteu's reagent in distilled water, and 80  $\mu$ L of 7.5% (w/v) sodium carbonate solution. Gallic acid served as the reference phenolic compound. After a 30-min incubation in a light-free environment at room temperature, the optical density of the mixture was measured at a wavelength of 765 nm using a microplate reader (Varioskan LUX, Thermo Scientific™, USA). The TPC was determined via a standard curve established with gallic acid, with results as presented in milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g).

##### DPPH radical scavenging assay

The DPPH method provides a simple, quick, consistent, and responsive approach to evaluating the antioxidant activity of plant extracts. This test involves the DPPH free radical, which accepts hydrogen and undergoes reduction facilitated by an antioxidant. The DPPH radical scavenging ability of the extracts was assessed following established procedures.<sup>16</sup> In brief, 0.1 mL of the extract at varying concentrations was thoroughly combined with 0.1 mL of a 0.15 mM DPPH solution in

95% ethanol. These mixtures were placed in a 96-well plate, shaken, and incubated at room temperature in the dark for 30 min. Subsequently, the optical density of the reaction mixture was measured at 517 nm with a Varioskan LUX microplate reader (Thermo Scientific™, USA). Ascorbic acid was employed as the reference solution. The DPPH radical scavenging potential was evaluated as the percentage of inhibition using the provided formula (equation 1), and the IC<sub>50</sub> values were calculated. Moreover, the DPPH results are presented as vitamin C equivalents, denoted as milligrams per gram of dry extract (mg VCEAC/g), using the ascorbic acid standard curve.

$$\% \text{ inhibition of DPPH radical} = \frac{[A_{\text{control}} - (A_{\text{sample}} - A_{\text{blank}})]}{A_{\text{control}}} \times 100 \dots\dots \text{Eqn. 1}$$

Where A<sub>control</sub> represents the absorbance of the control reaction and contains all reagents excluding the extract; A<sub>sample</sub> represents the absorbance of the sample when the extract is present; and A<sub>blank</sub> represents the absorbance of the sample blank, which consists of absolute ethanol added to the extract without DPPH.

#### Superoxide radical scavenging assay

A modified method was employed to evaluate the scavenging of the superoxide (O<sub>2</sub><sup>-</sup>) anion radical. This approach relied on the extract's potential to inhibit the photochemical reduction of nitroblue tetrazolium (NBT).<sup>17</sup> Specifically, the reaction mixture consisted of 20 μL of 0.75 mM NBT, 20 μL of 1 mM EDTA, 100 μL of 0.266 mM riboflavin, 20 μL of 50 mM potassium phosphate buffer solution (pH 7.4), and a 40 μL of test sample solution (concentration: 1 mg/mL), along with a standard ascorbic acid (concentration: 0.2 mg/mL). These were all set in a 96-well plate. After undergoing a 10-min exposure to fluorescent light, the absorbance readings were taken at 590 nm using a Varioskan LUX microplate reader (Thermo Scientific™, USA). The percent inhibition of superoxide formation was calculated by comparing the absorbance readings of the control to those of the reaction mixture, which contained the test sample (equation 2).

$$\% \text{ inhibition of SO radical} = \frac{[A_{\text{control}} - (A_{\text{sample}} - A_{\text{blank}})]}{A_{\text{control}}} \times 100 \dots\dots (\text{equation 2})$$

where A<sub>control</sub> represents the absorbance of the control reaction and contains all reagents excluding the extract; A<sub>sample</sub> represents the absorbance of the sample when the extract is present; and A<sub>blank</sub> represents the absorbance of the sample blank, which consists of the reaction solution added to the extract without riboflavin.

#### Lipid peroxidation assay

In order to explore the creation of lipid peroxides, a thiobarbituric acid-reactive species (TBARS) assay was employed.<sup>18</sup> Malondialdehyde (MDA), an offshoot of polyunsaturated fatty acid oxidation, reacts with two molecules of thiobarbituric acid (TBA). In an experimental tube, 50 μL of the extract solution (concentration: 1 mg/mL) and a standard ascorbic acid (concentration: 0.2 mg/mL) were mixed with 400 μL of egg yolk homogenate (diluted 1:10 in phosphate saline buffer). Following this, 150 μL of 75 mM FeSO<sub>4</sub> was added to the reaction mixture, and then incubation was carried out at 37°C for 30 min to stimulate lipid peroxidation. To halt the reaction, 200 μL of 15% trichloroacetic acid and 0.5% TBA were included. The ensuing mixture was then vortexed and heated at 95°C in a boiling water bath for 15 min. After a cooling period, centrifugation was carried out at 3,500 rpm for 15 min, and the absorbance of the supernatant was gauged at 532 nm using a Varioskan LUX microplate reader (Thermo Scientific™, USA). The percentage inhibition of lipid peroxidation was computed using the absorbance values (equation 3).

$$\% \text{ inhibition of MDA} = \frac{[A_{\text{control}} - (A_{\text{sample}} - A_{\text{blank}})]}{A_{\text{control}}} \times 100 \dots\dots (\text{equation 3})$$

Where A<sub>control</sub> represents the absorbance of the control reaction, comprises all reagents excluding the extract; A<sub>sample</sub> represents the absorbance of the sample when the extract is present; and A<sub>blank</sub>

represents the absorbance of the sample blank, which consists of the reaction solution added to the extract without TBA.

#### Reducing power assay

The test was conducted with the method outlined by Cushnie *et al.*<sup>16</sup> In this procedure, a volume of 25 μL of the extract solution was carefully combined with 175 μL of the FRAP reagent at varying concentrations in a 96-well plate. After a 4-minute incubation at room temperature, the optical density of the reaction mixture was measured at 593 nm using a microplate reader (Varioskan LUX, Thermo Scientific™, USA). Ascorbic acid was chosen as the reference solution. Ferrous sulfate was used at various concentrations to create a standard curve. The FRAP value was determined and reported as millimoles of Fe<sup>2+</sup> per milligram of dry extract (mM Fe<sup>2+</sup>/mg). Furthermore, it was computed as vitamin C equivalents, appearing as milligrams per gram of dry extract (mg VCEAC/g).

#### Statistical analysis

The data are presented as the mean ± standard error of the mean (SEM), and the IC<sub>50</sub> values are depicted as the mean ± standard deviation (SD) from three independent experiments. The data was subjected to a one-way analysis of variance (ANOVA), which was then followed by Tukey's post-hoc multiple comparison test. The level of significance was set at *p* < 0.05 to determine statistical significance, and the analysis was performed with GraphPad Prism (version 9.0).

## Results and Discussion

The excessive production of oxidants, specifically reactive oxygen species within the human body, is the underlying cause of some disorders. It is believed that eliminating these oxidants can effectively reduce organisms' oxidative stress levels. Studies have indicated an association between the consumption of medicinal plants and a decreased risk of several chronic diseases. This effect is believed to be due to antioxidant phytochemicals present in these plants, which promote health. These antioxidant phytochemicals are found in various medicinal plants and have a significant role in preventing and treating chronic diseases caused by oxidative stress. They often display potent antioxidant and free radical scavenging properties, as well as anti-inflammatory effects. These characteristics form the basis for numerous biological activities and health benefits, including anti-cancer, anti-aging, and protective effects against cardiovascular diseases, diabetes mellitus, obesity, and neurodegenerative diseases.<sup>19</sup> The goal of the present study was to evaluate and compare the phytochemical components and antioxidant capacities of 80% *ethanol* extracts from the traditional Thai remedy BLW and its plant ingredients. Moreover, this study aimed to provide substantial evidence and insights into the medicinal properties of these extracts.

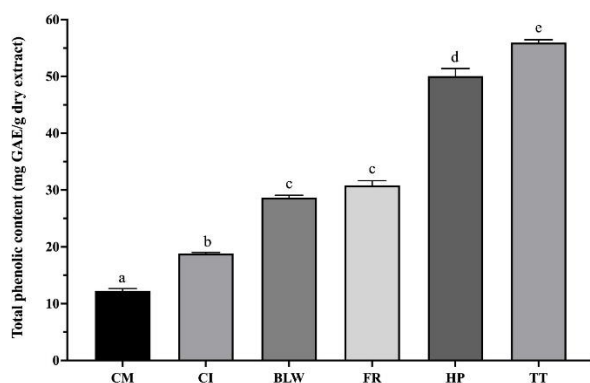
#### Extract yield and phytochemical constituents

The phytochemical screening and percentage yield of 80% *ethanol* extracts procured from the BLW remedy and its corresponding herbal components are depicted in Table 1. All of the plant extracts contained flavonoids, phenolics, and terpenoids; however, no anthraquinones were detected in any of the *ethanol* extracts. These findings align with those of Seubnooch *et al.*, who researched the *methanol* extracts of the BLW formula and its five plant ingredients, identifying phenolic and flavonoid compounds using both positive and negative electrospray ionization modes. Both phenolic acids and glycosides, along with flavonoids like quercetin and kaempferol, are acknowledged for their antioxidant properties.<sup>20</sup> Phenolic and flavonoid compounds have been scrutinized for their capacity to deter free radicals and provide protection against oxidative stress. Lupeol, an active triterpenoid, was detected in the extracts of BLW and its plant constituents. Its quantification was carried out in these extracts, and a correlation between lupeol content and cytotoxic activity was manifested.<sup>7</sup> Lupeol exhibits various biological functions, such as potent antioxidant, antidiabetic, and anti-inflammatory properties.<sup>21</sup> The presence of these phytochemical substances in the BLW remedy and its plant components

suggests potential antioxidant benefits for health and the potential to reduce the incidence of chronic non-communicable diseases.<sup>19</sup>

#### Total phenolic contents

The total phenolic content (TPC) was determined using the Folin-Ciocalteu method and was expressed as milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g), based on the standard curve ( $y = 0.0624x + 0.1027$ ,  $R^2 = 0.999$ ). The TT extract displayed the highest phenolic content ( $55.97 \pm 0.49$  mg GAE/g), while the CM extract had the lowest content ( $12.22 \pm 0.42$  mg GAE/g). The BLW remedy showed an intermediate phenolic content of approximately  $28.67 \pm 0.43$  mg GAE/g (Figure 1). Suwannarat *et al.* evaluated the safety profiles of 95% ethanol extract of the remedy, and five root components against potential skin irritation. They noticed a similar distribution of phenolic compounds in BLW and its herbal constituents. However, these concentrations were about 1.2–3 times higher compared to the 80% ethanol extracts obtained in this study.<sup>22</sup> The findings suggest that TPC is significantly influenced by the concentration of ethanol, where lower water content results in higher TPC levels. However, the optimal ethanol concentration varies based on the type of plant.<sup>23</sup> Notably, no information is available regarding the antioxidant properties of the 95% ethanol extracts. Phenolic compounds' antioxidative properties are deployed through redox reactions, which are crucial for absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, and decomposing peroxides.<sup>24</sup>



**Figure 1:** Total phenolic content (TPC) of the ethanol extract of the BLW remedy and its plant components

TPC is quantified in milligrams of gallic acid equivalent per gram (mg GAE/g of dry extract). Data values are expressed as mean  $\pm$  SEM. Different letters on each bar signify a notable distinction at  $p < 0.05$ .

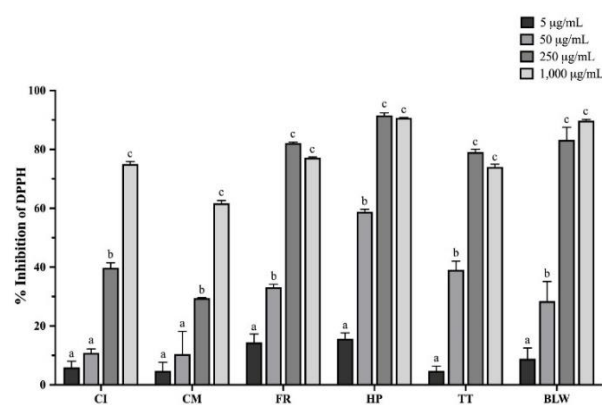
Abbreviations: CI = *Clerodendrum indicum*; CM = *Capparis micracantha*; FR = *Ficus racemosa*; HP = *Harrisonia perforata*; TT = *Tiliacora triandra*; BLW = Benchalokawichian

#### Antioxidant activities

Table 2 presents the antioxidant properties and antioxidant capacity equivalent to vitamin C, as determined by the DPPH and FRAP assays. The HP and TT extracts showed low  $IC_{50}$  values ( $23.69 \pm 4.05$  and  $35.08 \pm 8.53$   $\mu$ g/mL, respectively) in the DPPH assay. These values were similar to the standard ascorbic acid ( $17.98 \pm 8.02$   $\mu$ g/mL) with no significant difference. The BLW extract's potency was lower than that of the HP, TT, and FR extracts, as well as ascorbic acid. However, its potency exceeded that of the CI and CM extracts. As Figure 2 illustrates, all extracts demonstrated effective DPPH radical scavenging activity that increased with concentration. Surprisingly, the HP and BLW extracts displayed the highest DPPH radical inhibition percent at a concentration of 1,000  $\mu$ g/mL, with values of  $90.75 \pm 0.15$  and  $89.72 \pm 0.50$ , respectively. A prior study highlighted the DPPH radical scavenging efficacy of the 95% ethanol extracts of the same formulation, using butylated hydroxytoluene (BHT) as the reference substance. Out of all the extracts, the FR extract displayed the most potent antioxidant activity, while the BLW extract also showed antioxidant capacity, with an  $IC_{50}$  value of 40.9  $\mu$ g/mL.<sup>3</sup> Echoing the

results of the current study, the TT and HP extracts exhibited low  $IC_{50}$  values. Generally, phenolic compounds play a crucial role as antioxidant components by neutralizing free radicals through their capacity to provide hydrogen atoms to these radicals. Various literature sources reveal a direct relationship between the total phenolic content and the antioxidant capacity.<sup>25,26</sup> These findings support the potential therapeutic use of this formulation in conditions related to oxidative stress.

The DPPH assay is merely correlated with other antioxidant assays, indicating that it represents distinct antioxidant characteristics as a result of a different reaction mechanism. The FRAP test for antioxidants is characterized by its simplicity and great reproducibility. The ability of compounds to undergo a conversion from  $Fe^{3+}$  to  $Fe^{2+}$  is a reliable indicator of antioxidant activity.<sup>27</sup> In the FRAP assay, the extracts exhibited a trend consistent with the results obtained using the DPPH method. No significant differences were observed among the extracts. However, it is important to note that all of the extracts showed a diminished ability to exert power compared to ascorbic acid – about 20 times lower – and their VCEAC values were notably low (Table 2). Yet, there is currently no available evidence with which to compare these data. The antioxidant activity of the remedy and its herbal components may not be due to their capacity to reduce power.



**Figure 2:** The percentage inhibition of DPPH radicals by the extracts

The reported % inhibition values represent the mean  $\pm$  SEM, calculated from three independent experiments. Different letters within each group signify a statistically significant difference at  $p$ -values  $< 0.05$ .

Abbreviations: CI = *Clerodendrum indicum*; CM = *Capparis micracantha*; FR = *Ficus racemosa*; HP = *Harrisonia perforata*; TT = *Tiliacora triandra*; BLW = Benchalokawichian

In the assay for superoxide radical scavenging, the FR extract exhibited the highest percentage of inhibition at a concentration of 1 mg/mL ( $63.77 \pm 1.12\%$ ). In contrast, the CM extract showed the least activity ( $11.02 \pm 0.77\%$ ). The BLW extract displayed a moderate inhibition of  $23.56 \pm 2.03\%$ . When compared, the standard ascorbic acid at a concentration of 200  $\mu$ g/mL demonstrated a potent inhibition of  $90.74 \pm 1.19\%$  (Figure 3). Information for a direct comparison between the remedy and the five root plants remains scarce. With regards to the FR extract, a previous study suggested that the 95% ethanol extract of FR stem bark showed a concentration-dependent scavenging ability of superoxide radical anions, presenting an  $IC_{50}$  of 66.88  $\mu$ g/mL.<sup>28</sup> In comparison, *methanol* extracts from the FR leaf and FR fruit displayed  $IC_{50}$  values of 130.104  $\mu$ g/mL and 122.264  $\mu$ g/mL, respectively, similar to the standard ascorbic acid, with an  $IC_{50}$  of 83.003  $\mu$ g/mL.<sup>29</sup> While all extracts studied here showed lower activity than the standard ascorbic acid and revealed significant differences, it is noteworthy that the BLW extract demonstrated moderate inhibition when compared to its plant derivatives.

In the lipid peroxidation assay, the BLW extract exhibited the highest inhibition percentage at a concentration of 1 mg/mL, producing  $75.30 \pm 0.47\%$ . These results were not significantly different from the

inhibition observed with ascorbic acid ( $87.18 \pm 3.30\%$ ). Conversely, the FR extract demonstrated the lowest activity, with an inhibition of  $13.39 \pm 8.10\%$  (Figure 4). This study represents the first examination of the BLW remedy and its botanical components for their ability to inhibit lipid peroxidation. A prior investigation revealed that the standard ascorbic acid at a concentration of  $100 \mu\text{g/mL}$  resulted in a strong inhibition of  $93.57\%$ , closely paralleling the results observed in the current study.<sup>30</sup> Juckmeta *et al.* discovered that pectolinarigenin can be employed as a quality control marker for BLW.<sup>6</sup> Pectolinarigenin is an active flavonoid that possesses a variety of biological activities,

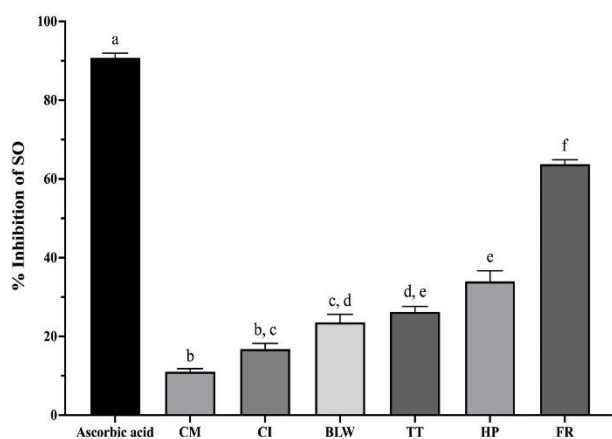
including anticarcinogenic, antidiabetic, and anti-inflammatory activity. Furthermore, it has been reported that pectolinarigenin has the capacity to scavenge radicals and reduce the level of the oxidative stress marker, MDA, in the colons of mice induced by dextran sodium sulfate.<sup>31</sup> This report is consistent with the current study. As previously stated, the BLW remedy is prepared by blending five root plants in equal measures, resulting in a formulation in which each plant constitutes only 20% of the mixture (a 1 in 5 ratio). Notably, when assessing the TPC and antioxidant capacity in comparison to the individual herbal components, the remedy displayed moderate activity.

**Table 2:** Antioxidant activity of the ethanol extracts and ascorbic acid

Sample	DPPH assay (IC <sub>50</sub> in $\mu\text{g/mL}$ )	FRAP assay (mM Fe <sup>2+</sup> /mg)	VCEAC (mg/g)	
			DPPH assay	FRAP assay
CI	$264.24 \pm 53.52^a$	$0.07 \pm 0.008^a$	$37.78 \pm 2.01^a$	$12.74 \pm 0.18^a$
CM	$455.11 \pm 108.84^b$	$0.08 \pm 0.028^a$	$32.04 \pm 0.45^a$	$13.88 \pm 3.14^a$
FR	$66.25 \pm 13.61^c$	$0.14 \pm 0.002^a$	$98.57 \pm 2.64^b$	$19.89 \pm 0.43^{a,c}$
HP	$23.69 \pm 4.05^d$	$0.40 \pm 0.022^a$	$201.16 \pm 2.63^c$	$45.19 \pm 1.62^b$
TT	$35.08 \pm 8.53^d$	$0.47 \pm 0.023^a$	$182.29 \pm 3.16^d$	$52.62 \pm 2.87^b$
BLW remedy	$118.22 \pm 26.26^e$	$0.21 \pm 0.001^a$	$86.05 \pm 5.57^b$	$26.94 \pm 0.61^c$
Ascorbic acid	$17.98 \pm 8.02^d$	$9.67 \pm 0.708^b$	-	-

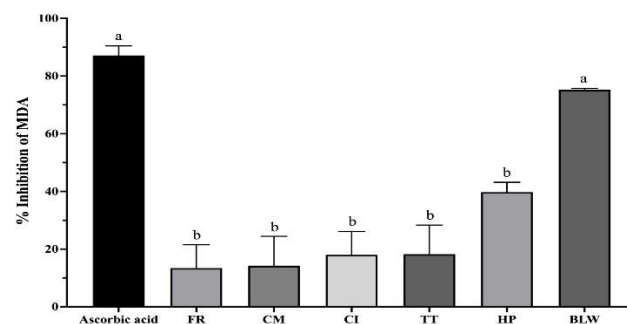
Note: DPPH and FRAP tests were performed using ascorbic acid as the reference standard. The results are expressed as IC<sub>50</sub> values (mean  $\pm$  SD) for DPPH. The mean  $\pm$  SEM values for FRAP and VCEAC are reported based on three independent experiments. The values with different superscript letters (a-e) in a column are significantly different from each other while the same superscript letters mean values that are not significantly different from each other ( $p < 0.05$ ).

Abbreviations: CI = *Clerodendrum indicum*; CM = *Capparis micracantha*; FR = *Ficus racemosa*; HP = *Harrisonia perforata*; TT = *Tiliacora triandra*; BLW = Benchalokawichian



**Figure 3:** The percentage inhibition of superoxide (SO) radicals by the extracts. The reported % inhibition values represent the mean  $\pm$  SEM, calculated from three independent experiments. Different letters denote a statistically significant difference at  $p$ -values  $< 0.05$ .

Abbreviations: CI = *Clerodendrum indicum*; CM = *Capparis micracantha*; FR = *Ficus racemosa*; HP = *Harrisonia perforata*; TT = *Tiliacora triandra*; BLW = Benchalokawichian



**Figure 4:** The percentage inhibition of malondialdehyde (MDA) by the extracts. The reported % inhibition values represent the mean  $\pm$  SEM, calculated from three independent experiments. Distinct letters indicate a statistically significant difference at  $p$ -values  $< 0.05$ .

Abbreviations: CI = *Clerodendrum indicum*; CM = *Capparis micracantha*; FR = *Ficus racemosa*; HP = *Harrisonia perforata*; TT = *Tiliacora triandra*; BLW = Benchalokawichian

## Conclusion

The ethanol root extracts of *H. perforata* and *T. triandra* have shown potent free radical scavenging activity, and the Benchalokawichian remedy, a Thai medicinal plant, effectively inhibits lipid peroxidation. The observed pharmacological effects are likely attributed to the

presence of phenolic, flavonoid, and terpenoid compounds in these plant extracts, with a notable role played by phenolics. These findings suggest that the use of the Benchalokawichian remedy could aid in preventing chronic, non-communicable diseases that are associated with reactive oxygen species, such as diabetes and cardiovascular disease. As a result, further investigations are necessary to characterize the active compounds and explore their antiglycation activity, which is closely associated with oxidative stress. Further isolation and identification of active compounds, the combination of herbal elements, and the chemical interactions are required to evaluate its other pharmacological activities.

### Conflict of Interest

The authors declare no conflict of interest.

### Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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