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Research Article

Comprehensive Antioxidant Evaluation of *Tiliacora triandra* Extracts: Assays of Leaf, Stem, and Root

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ABSTRACT

This study evaluates the antioxidant potential of *Tiliacora triandra* (locally known as "Yanang") extracts from the leaves, stems, and roots using three established assays: DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)), and FRAP (ferric reducing antioxidant power). The extracts were prepared using 95% ethanol, and their antioxidant activities were assessed in terms of IC50 and Trolox equivalents. The DPPH assay yielded IC50 values of 187.91±28.09 µg/ml, 85.93±10.91 µg/ml, and 71.31±11.29 µg/ml for the leaf, stem, and root extracts, respectively. Similarly, the ABTS assay showed IC50 values of 181.78±22.96 µg/ml for the leaf extract, 70.07±6.40 µg/ml for the stem extract, and 48.09±8.77 µg/ml for the root extract. In both assays, the root and stem extracts exhibited comparable antioxidant activity, whereas the leaf extract showed significantly lower activity ($p < 0.05$). The FRAP assay revealed no significant differences among the extracts, with Trolox equivalent values ranging from 190 to 211 mg TE/g extract. These findings suggest that the root and stem extracts possess strong antioxidant activity and may be used interchangeably in applications requiring such properties, whereas the leaf extract has comparatively lower potential. Further studies are recommended to explore the therapeutic properties and potential health benefits of these extracts.

Keywords: Antioxidant, DPPH, *Tiliacora triandra*, Yanang, Five roots.

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INTRODUCTION

Antioxidants are crucial in combating oxidative stress, which is implicated in the development of various health conditions, such as cardiovascular diseases, cancer, and neurodegenerative disorders[1]. Recently, there has been growing interest in natural antioxidants[2], particularly those derived from plant sources, leading to the study of medicinal plants with potent antioxidant properties[3]. *Tiliacora triandra* (locally known as "Yanang"), a plant native to Southeast Asia, has long been traditionally used in folk medicine for its medicinal benefits, including its anti-inflammatory, antimicrobial, and antioxidant effects[4,5,6,7].

Additionally, *Tiliacora triandra* is an important component in the traditional Thai herbal remedy known as "Five Roots", which consists of equal parts of the roots from five medicinal plants: *Harrisonia perforata*, *Tiliacora triandra*, *Clerodendrum petasites*, *Ficus racemosa*, and *Capparis micracantha*. This formulation is traditionally used as an antipyretic to reduce fever[8,9,10,11].

Various parts of *Tiliacora triandra*, including its leaves, stems, and roots, are believed to contain bioactive compounds with free radical-scavenging activity and antioxidant potential[4,6]. Among the most commonly used methods for evaluating antioxidant activity are the DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)), and FRAP (ferric reducing antioxidant power) assays[2,12,13,14]. These analytical methods are widely applied to assess the capability of plant extracts to neutralize harmful free radicals, which are unstable molecules that can cause cellular damage through oxidative stress. By mitigating such oxidative damage, these extracts demonstrate their protective properties, which are essential for preventing various degenerative diseases linked to oxidative stress. The results of these assays provide valuable insights into the antioxidant potential of the extracts, offering a quantitative measure of their ability to combat oxidative damage and maintain cellular health[15].

This research aims to assess the antioxidant properties of *Tiliacora triandra* extracts from its leaves, stems, and roots using established methods such as DPPH, ABTS, and FRAP assays. The primary objective is to evaluate and compare the antioxidant activity of these plant parts to identify differences or similarities in their potential. This comparison will provide information on whether the leaves or stems can serve as viable substitutes for the roots, which are traditionally used in Thai herbal medicine, particularly in the preparation of the "Five Roots Remedy," which incorporates *Tiliacora triandra* root as ingredient.

Understanding these differences is crucial for optimizing the use of *Tiliacora triandra* in both traditional and present applications. If the leaves or stems are found to have comparable or superior antioxidant activity, they could offer a sustainable alternative to root harvesting, preserving the plant's ecological balance. By exploring the antioxidant capacity of each part, this study aims to expand the therapeutic potential of *Tiliacora triandra* and inform future applications in pharmaceutical and nutraceutical products targeting oxidative stress and related diseases.

MATERIAL AND METHOD

DPPH, ABTS, Potassium persulfate, TPTZ (Ferric tripyridyl triazine), HCL (Hydrochloric acid), $FeCl_3$

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(Ferric chloride), Sodium acetate-3-hydrate, Ascorbic acid, Ethanol, L-ascorbic acid, Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), Freeze dryer (Lyophilization), Ultrasonic Ultrasonic Extractor; Kudos, UV-VIS spectrophotometer; Genesys 10 UV, Super line Grinder, type SCL – KR, TH class B.

Preparation of Herbal Samples

The preparation of the sample was performed according to Makinde EA with some modification[5]. Briefly, the leaves, stems, and roots of *Tiliacora triandra* were subjected to maceration with 95% ethanol for 3 days. After maceration, the mixture was shaken using a temperature-controlled ultrasonic extraction, maintaining a temperature of 50°C and a frequency of 60 kHz for 3 cycles, each lasting 30 minutes. The extract was then filtered using No. 1 filter paper. After filtration, the solvent was removed using a rotary evaporator under reduced pressure. The resulting extract was collected in a flask, sealed tightly, and stored at -20°C for 24 hours. The extract was then freeze-dried for 72 hours to remove any remaining solvent, resulting in a concentrated extract for further analysis.

To calculate the percentage yield of the dried herbal powder, the following formula was used:

% Yield (dry weight basis) = (W1 × 100) / W2

Where: W1 = weight (grams) of the extract after solvent evaporation and freeze-drying

W2 = dry weight (grams) of the herbal powder.

Antioxidant Analysis Using the DPPH Method

The antioxidant properties of *Tiliacora triandra* extracts from the leaf, stem, and root were evaluated at different concentrations, using ethanol as the solvent. Trolox and ascorbic acid served as positive controls for comparison. The method involved dispensing 100 µL of a 0.1 mM DPPH solution into a 96-well plate, followed by adding 100 µL of each extract, ascorbic acid, and Trolox standard at varying concentrations. The mixtures were thoroughly shaken and left to incubate at room temperature in the dark for 30 minutes. Absorbance readings were then recorded at 517 nm using a spectrophotometer. Each test was conducted in quadruplicate to ensure accuracy[7,9,15].

The percentage of DPPH radical scavenging activity was calculated using the formula:

% Radical scavenging = [(A control - A sample) / A control] × 100

Where: A control = absorbance of the control (without extract).

A sample = absorbance of the sample.

The values obtained were used to calculate the Inhibitory Concentration (IC50), which represents the concentration required to reduce 50% of the free radicals. This was determined from a graph showing the relationship between the concentration of the extract and the percentage of radical scavenging.

Antioxidant Analysis Using the ABTS Method

The antioxidant activity of extracts from the leaves, stems, and roots was evaluated, with ascorbic acid and Trolox serving as reference standards. The ABTS reagent was prepared by combining 8 mL of a 7 mM ABTS stock solution with 12 mL of a 2.45 mM potassium persulfate solution, followed by incubation for 18 hours. After preparation, 180 µL of the ABTS reagent was dispensed into each well of a 96-well plate. Subsequently, 20 µL of each extract, along with ascorbic acid and Trolox at various concentrations, was added to the respective wells. The plate was incubated in the dark at room temperature for 120 minutes. Absorbance readings were taken at 734 nm using a spectrophotometer, and the data were recorded. The experiment was conducted in quadruplicate, and the ABTS radical cation scavenging activity was determined using a specific formula[9,15]:

% ABTS Radical Cation Scavenging Activity = [(A control - A std or sam) / A control] × 100

Where: A control = absorbance of the ABTS solution

A std = absorbance of the standard (Ascorbic acid or Trolox)

A sam = absorbance of the plant extract

This method assesses the extract's ability to neutralize ABTS radicals, contributing to the overall understanding of its antioxidant properties.

Antioxidant Analysis Using the FRAP Method

The FRAP reagent was prepared by combining a 300 mM acetate buffer (pH 3.6), a 20 mM ferric chloride solution, and a 10 mM TPTZ solution dissolved in 40 mM HCl in a ratio of 10:1:1. For the assay, 150 µL of the FRAP reagent was added to each well of a 96-well plate, followed by 50 µL of extracts from leaves, stems, and roots. The plate was incubated at room temperature in the dark for 10 minutes. Absorbance was then measured at 593 nm using a spectrophotometer, and the results were recorded. The experiment was performed in quadruplicate to ensure accuracy.

The relative antioxidant activity (FRAP value) was calculated from a standard curve of $FeSO_4$ and compared to the efficacy of Trolox. The result was expressed as Trolox equivalents (TE), providing insights into the extract's antioxidant potential.

This method evaluates the ability of the extracts to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), reflecting their overall antioxidant activity[7,9,10].

Statistical Analysis Used in Data Evaluation

All variables were summarized by calculating the mean, percentage, and standard deviation. To test for differences between sample groups and standard substances, One-Way ANOVA followed by Tukey's Honestly Significant Difference (HSD) test was performed for pairwise comparisons. A significance level of $p < 0.05$ was set to determine statistical significance. The analysis was conducted using R version 4.1.0.

RESULT

Percentage yield of the dried herbal powder

Extraction of various parts of *Tiliacora triandra* using 95% ethanol revealed that the root had the highest extract yield, followed by the leaves, and finally the stems. The %yield values were shown in Table 1, with the average %yield of all three parts being 7.17%.

Antioxidant Analysis Using the DPPH

The antioxidant activity of *Tiliacora triandra* extracts demonstrated significant free radical scavenging activity, indicating that its active compounds interact with DPPH• radicals through electron donation or hydrogen atom transfer. This reaction stabilizes the DPPH• radicals, leading to their reduction and a subsequent decrease in color intensity. The antioxidant activity of the extracts at concentrations of 9.8–156.3 µg/ml, revealed that the root extract exhibited the highest radical scavenging ability, followed by the stem and then the leaf extracts. These results highlight the antioxidant potential of the extract. However, the antioxidant activity of root extract was still lower than that of Vitamin C and Trolox, with their IC50 values approximately 8 times lower than that of the root extract (Figure 2 and Table 2).

Table 1. Show the %yield of *Tiliacora triandra* extract when 100 grams of herbal powder was extracted using 95% ethanol.

Sample	Herbal weight (g)	% yield
Leaves	100	6.988
Stems	100	2.129
Roots	100	12.398

Table 2. Antioxidant inhibition of *Tiliacora triandra* extracts from different plant parts (roots, stems, and leaves) using DPPH, ABTS, and FRAP Assays.

Sample or standard	DPPH IC50 µg/ml	ABTS IC50 µg/ml	FRAP mg.TE/g. extract
Leaves	187.91±28.09^{c}	181.78±22.96^{c}	190.59±25.27^{a}
Stems	85.93±10.91^{a}	70.07±6.40^{a}	195.85±10.06^{a}
Roots	71.31±11.29^{a}	48.09±8.77^{a}	210.95±3.70^{a}
Vit C	9.15±0.43^{b}	15.33±0.98^{b}	-
Trolox	8.29±0.39^{b}	12.10±1.02^{b}	-

Note: Different letters in the column indicate a statistically significant difference ($p < 0.05$).

Figure 1. *Tiliacora triandra* (A) Roots (B) Leaves and stems.

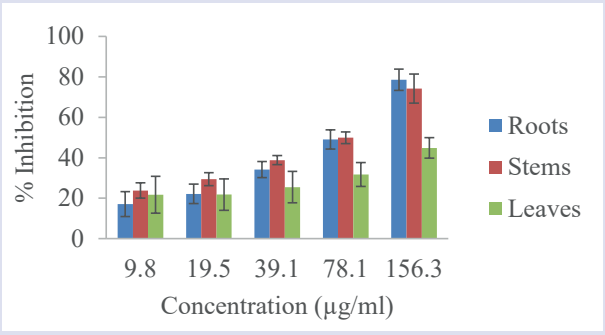

Figure 2. DPPH radical scavenging activity of *Tiliacora triandra* roots, stems, and leaves extracts at various concentrations.

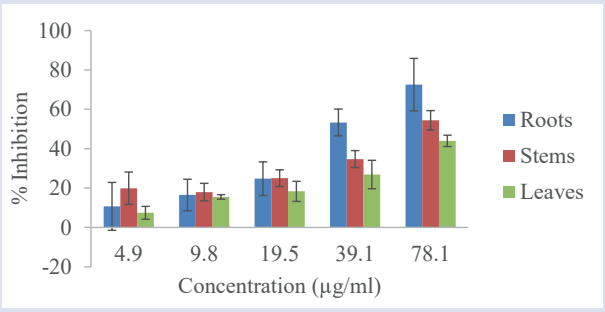

Figure 3. ABTS radical scavenging activity of *Tiliacora triandra* roots, stems, and leaves extracts at various concentrations.

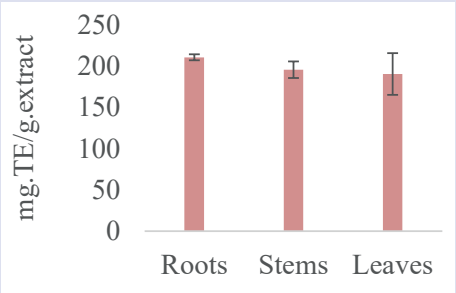

Figure 4. The antioxidant activity of the roots, stems, and leaves extracts of *Tiliacora triandra* were measured and expressed as milligrams of Trolox equivalent using the FRAP assay method. There was no statistically significant difference ($p < 0.05$).

Antioxidant Analysis Using the ABTS

The herbal extracts exhibited notable free radical scavenging activity, indicating that their active compounds interact with $ABTS^{+}$• radicals by donating electrons or hydrogen atoms. This reaction reduces and stabilizes the blue-green $ABTS^{+}$• radicals, leading to a decrease in color intensity. The ABTS assay demonstrated that *Tiliacora triandra* extracts exhibited significant antioxidant activity. The root extract showed a difference of only 3-fold compared to Vitamin C and 4-fold compared to Trolox. When ranking the antioxidant activity of the extracts, the root extract displayed the strongest activity, followed by the stem and then the leaf extracts (Figure 3 and Table 2).

The results of the ABTS assay were consistent with those of the DPPH assay, showing that the root and stem extracts did not differ significantly and demonstrated greater antioxidant activity than the leaf extract in both testing methods.

Antioxidant Analysis Using the FRAP

In the FRAP assay, the *Tiliacora triandra* extracts exhibited free radical scavenging activity, indicating that its active compounds donated electrons to reduce ferric ions (Fe^{3+}) in the Fe^{3+}-TPTZ complex to the more stable ferrous form (Fe^{2+}-TPTZ), which produces a deep blue color. The electron donation from the extracts reduced Fe^{3+} to Fe^{2+}, resulting in a color change to deep blue, thereby demonstrating the antioxidant potential of the extract. Figure 4 accurately describes the data shown for total antioxidant capacity in terms of Trolox equivalent per gram of extract for each part of the plant. The experimental results revealed that the extracts from all parts showed no statistically significant difference in their capacity in terms of Trolox equivalent, with milligrams of Trolox equivalent ranging between 190 and 211 mg TE/g extract.

DISCUSSION

The comparative experiment on the potential of leaf, stem, and root extracts of *Tiliacora triandra* using 95% ethanol showed that the results from each testing method were similar, especially the DPPH and ABTS tests. The results indicated that the root and stem extracts had comparable IC50 values, with the leaf extract showing the lowest antioxidant activity. This study found that the root and stem extracts, when tested using the DPPH method, did not differ significantly ($p < 0.05$). Only the leaf extract exhibited the least antioxidant activity. Furthermore, the ABTS test revealed no significant difference in antioxidant inhibition between the root and stem extracts ($p < 0.05$), and both exhibited better inhibition than the leaf extract. The FRAP test showed no significant statistical difference between all extracts. Based on the experimental results, it was indicated that the electron or hydrogen atom donating abilities of the root and stem extracts for scavenging DPPH• and ABTS• radicals were not significantly different.

Furthermore, the reducing potential of Fe^{3+} to Fe^{2+} among the root, stem, and leaf extracts of *Tiliacora triandra* also showed no significant differences. This evidence indicates that various parts of *Tiliacora triandra* may be used interchangeably in herbal formulations.

The antioxidant activity of the 95% ethanol extract from *Tiliacora triandra* roots in this study was comparable to the finding of the research by Nadee et al. (2024)[10], which also used ethanol for extraction. Additionally, the activity of the leaf extract aligned with the findings of Pradapyut et al. (2024)[2], with the methanol extract exhibiting approximately 20 times lower than that of vitamin C when extracted with ethanol. In contrast, the methanol and water extract of the leaves from the study by Rattana S. (2010)[7] demonstrated higher activity than the findings of our research. Specifically, the methanol extract exhibited activity comparable to that of vitamin C. These results suggest that methanol extraction may enhance the bioactivity of the leaves. Therefore, using methanol or water as the extraction solvents for comparing different parts of *Tiliacora triandra* could yield different results compared to 95% ethanol extraction. Traditionally, *Tiliacora triandra* leaves play a significant role in both culinary and medicinal practices. In cooking, they are commonly used to prepare dishes by extracting their juice, which adds a distinct flavor and is believed to have health benefits. Medicinally, the juice from *Tiliacora triandra* leaves is widely used as a natural remedy for detoxifying the body and reducing fever.

CONCLUSIONS

The antioxidant activity tests of different parts of *Tiliacora triandra* revealed that the root and stem extracts exhibited comparable results across all three testing methods. In contrast, the leaf extract consistently demonstrated the lowest activity in the DPPH and ABTS assays. Meanwhile, the FRAP assay showed no significant differences among the extracts from the three parts. These findings suggest that various parts of *Tiliacora triandra* may serve as substitutes for the root. Specifically, the stem extract appears to be a promising alternative to the root extract. Based on these findings, it can be concluded that the root and stem extracts are likely interchangeable. However, since the antipyretic properties of the root extract are related to inflammatory processes in the body, further studies on its anti-inflammatory effects and other related properties should be conducted to optimize the use of these plant parts. Future research should also explore the broader bioactivities of *Tiliacora triandra* extracts, including their potential applications in pharmaceutical and nutraceutical products.

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