Phytochemical Profile and In Vitro Antioxidant Activity of Ethanolic and Aqueous Extracts of a Kasai Scripture Herbal Compress

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Abstract

This study aimed to investigate the preliminary phytochemical constituents, total phenolic content (TPC), total flavonoid content (TFC), and in vitro antioxidant activity (DPPH and ABTS radical scavenging assays) of the herbal compress formulation described in the Kasai Scripture. A 95% ethanolic extract prepared by maceration was compared with an aqueous extract prepared by decoction. The ethanolic extraction yielded 86.25 g (9.58% w/w), whereas the aqueous extraction yielded 73.27 g (8.14% w/w). All experiments were performed in technical triplicate using extracts prepared from a single extraction batch. Preliminary phytochemical screening revealed that both extracts contained tannins, saponins, and terpenoids, whereas alkaloids and cardiac glycosides were detected only in the ethanolic extract. The ethanolic extract exhibited significantly higher total phenolic content and total flavonoid content than the aqueous extract ($p < 0.05$), with TPC values of 41.06 ± 0.96 and 29.83 ± 0.22 mg GAE/g extract, respectively, and TFC values of 20.28 ± 0.41 and 14.32 ± 0.49 mg CE/g extract, respectively. In the DPPH assay, the aqueous extract demonstrated stronger radical scavenging activity than the ethanolic extract, with IC_{50} values of 0.83 ± 0.09 and 2.15 ± 0.20 mg/mL, respectively. In the ABTS assay, the ethanolic extract exhibited greater radical scavenging activity than the aqueous extract, with IC_{50} values of 2.80 ± 0.28 and 4.33 ± 0.22 mg/mL, respectively. These findings indicate that the extraction solvent influences the phytochemical composition and in vitro antioxidant activity of the herbal compress formulation described in the Kasai Scripture. The results provide preliminary scientific evidence supporting further phytochemical characterization and future quality standardization studies of this traditional Thai herbal formulation. However, additional studies are required to identify bioactive compounds and evaluate biological activities before a quality standard can be established.

Keywords: Antioxidant Activity, Herbal Compress Formulation, Kasai Scripture

Introduction

Medicinal plants have long been used in Thai Traditional Medicine (TTM) for health promotion and disease treatment. Herbal compress therapy is one of the most widely used

TTM modalities for relieving pain, reducing muscle stiffness, and improving blood circulation. The number of herbal compress therapy services in Thailand increased from 611,962 visits in 2022 to 1,464,816 in 2024, reflecting greater integration of this practice into the national healthcare system (Department of Thai Traditional and Alternative Medicine, 2025).

The Kasai Scripture, one of the principal classical texts of TTM, describes a herbal compress formulation to treat Klon Haeng Bang Koet Phuea Patkhat, a condition characterized by musculoskeletal pain, numbness, and stiffness. The formulation consists of nine medicinal plants: *Drypetes roxburghii* (Wall.) Hurusawa, *Ricinus communis* Linn., *Tamarindus indica* Linn., *Acacia concinna* (Willd.) DC., *Crinum asiaticum* Linn., *Zingiber montanum* (J.Koenig) Link ex A.Dietr., *Zingiber zerumbet* (L.) Roscoe ex Sm., *Zingiber officinale* Roscoe, and *Sesamum indicum* L. (Foundation for the Promotion of Thai Traditional Medicine Recovery & Ayurved Thamrong School, 2008).

Although several constituent herbs have demonstrated antioxidant properties, the phytochemical profile and antioxidant activity of the complete formulation remain poorly characterized. Therefore, this study evaluated the preliminary phytochemical constituents, TPC, TFC, and antioxidant activities of ethanolic and aqueous extracts using DPPH and ABTS assays.

Research Objectives

1. To investigate the preliminary phytochemical constituents of the herbal compress formulation described in the Kasai Scripture.
2. To determine the total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activities (DPPH and ABTS radical scavenging assays) of the herbal compress formulation described in the Kasai Scripture.

Scope of the Research

1. Variable Scope

This study focused on the herbal compress formulation described in the Kasai Scripture of Thai Traditional Medicine. The formulation consists of nine medicinal plants in equal proportions. The independent variable was the extraction method, comprising a 95% ethanolic extract prepared by maceration and an aqueous extract prepared by decoction for 20 minutes. The dependent variables were preliminary phytochemical constituents, total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activities (DPPH and ABTS radical scavenging assays) of the ethanolic and aqueous extracts.

2. Time Scope

This study was conducted from April to June 2026 at the laboratory of Thai Traditional Medicine, College of Muay Thai Studies and Thai Traditional Medicine, Muban Chombueng Rajabhat University, Ratchaburi Province, Thailand.

Literature Review

The medicinal plants included in the Kasai Scripture herbal compress formulation are rich sources of bioactive compounds, particularly phenolic compounds, flavonoids, terpenoids, and alkaloids, which contribute to antioxidant activity by scavenging free radicals and reducing oxidative stress. The phytochemical composition and antioxidant capacity of these plants are influenced by factors such as plant species, plant part, extraction method, and solvent polarity (Leng et al., 2017; Kongkwamcharoen et al., 2021; Sookying et al., 2022).

Previous studies have shown that ethanolic and methanolic extracts of several constituent herbs, including *Tamarindus indica L.*, *Acacia concinna (Willd.) DC.*, *Zingiber montanum (J.Koenig) Link ex A.Dietr.*, *Zingiber zerumbet (L.) Roscoe ex Sm.*, and *Zingiber officinale Roscoe*, generally contain higher total phenolic content (TPC) and total flavonoid content (TFC), resulting in stronger antioxidant activities as determined by DPPH and ABTS assays (Ghasemzadeh et al., 2010; Leng et al., 2017; Rohmah, 2022; Thiraphatthanavong et al., 2023; Rinthong et al., 2024). Although fewer studies have investigated *Crinum asiaticum* and *Ricinus communis*, these species also contain phenolic compounds and other phytochemicals with potential antioxidant activity (Ahmed & Iqbal, 2018; Kongkwamcharoen et al., 2021).

Despite the reported antioxidant properties of the individual herbs, scientific evidence regarding the phytochemical profile and antioxidant activity of the complete Kasai Scripture herbal compress formulation remains limited. Therefore, this study aimed to determine the total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activities using DPPH and ABTS assays to provide scientific evidence for the phytochemical characterization, quality standardization, and future development of this traditional herbal formulation.

Research Methodology

1. Materials

The dried medicinal plants used in the herbal compress formulation were purchased from Chao Krom Poe Co., Ltd., Bangkok, Thailand, except for the fresh leaves of *Crinum asiaticum* L. and *Drypetes roxburghii* (Wall.) Hurus., which were collected in April 2026 from Nakhon Ratchasima and Ratchaburi, Thailand, respectively. All plant materials were authenticated by Asst. Prof. Dr. Supalak Fakkham, an expert in Applied Thai Traditional Medicine. Voucher specimens were prepared for the following medicinal plants: *Ricinus communis* L. leaves (AS2026-001), *Tamarindus indica* L. leaves (AS2026-002), *Acacia concinna* (Willd.) DC. leaves (AS2026-003), *Crinum asiaticum* L. leaves (AS2026-004), *Drypetes roxburghii* (Wall.) Hurus. leaves (AS2026-005), *Zingiber montanum* (J.Koenig) Link ex A.Dietr. rhizomes (AS2026-006), *Zingiber zerumbet* (L.) Roscoe ex Sm. rhizomes (AS2026-007), *Zingiber officinale* Roscoe rhizomes (AS2026-008), and *Sesamum indicum* L. seeds (AS2026-009). The voucher specimens were deposited at the College of Muay Thai Studies and Thai Traditional Medicine, Muban Chombueng Rajabhat University, Ratchaburi, Thailand. The freshly collected leaves of *Crinum asiaticum* L. and *Drypetes roxburghii* (Wall.) Hurus. were washed, oven-dried at 50°C for 48 h, ground, passed through a No. 16 sieve, and stored in airtight containers at room temperature until extraction.

2. Preparation of herbal extracts

Equal amounts of each powdered medicinal plant (100 g each) were thoroughly mixed to prepare the polyherbal formulation, which was extracted using two different methods.

2.1 Ethanolic extraction: The polyherbal formulation was extracted with 95% ethanol (1:5, w/v) by maceration at room temperature for 7 days with daily shaking. The extract was filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator at 45°C. The concentrated extract was further dried to constant weight in a hot-air oven at 45°C to ensure complete removal of residual solvent. The extraction yield of the ethanolic extract was 9.58% (w/w).

2.2 Aqueous extraction: The polyherbal formulation was extracted with distilled water (1:5, w/v) by decoction for 20 min. The extract was filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator at 45°C.

The concentrated extract was further dried to constant weight in a hot-air oven at 45°C to ensure complete removal of residual moisture. The extraction yield of the aqueous extract was 8.14% (w/w).

The ethanolic and aqueous crude extracts were transferred to amber glass containers, protected from light, and stored at 4°C for no longer than 1 month before phytochemical and antioxidant analyses.

3. Preliminary Phytochemical Screening

Preliminary phytochemical screening of the ethanolic and aqueous extracts was performed using qualitative color and precipitation reactions, as modified by Koodkaew et al. (2018). Five classes of phytochemicals, including tannins, alkaloids, saponins, terpenoids, and cardiac glycosides, were evaluated.

3.1 Tannin Test: Crude extract (0.20 g) was mixed with 5 mL of distilled water and boiled for 2 minutes. After filtration, three drops of 1% (w/v) ferric chloride ($FeCl_3$) solution were added. The appearance of a greenish-black or bluish-black coloration indicated the presence of tannins.

3.2 Alkaloid Test: Crude extract (0.20 g) was mixed with 15 mL of 2% (v/v) sulfuric acid (H_2SO_4) and heated for 2–3 min. After filtration, Dragendorff's reagent was added. The formation of an orange-red precipitate indicated the presence of alkaloids.

3.3 Saponin Test: Crude extract (0.20 g) was boiled with 5 mL of distilled water and filtered. Two drops of olive oil were added, followed by vigorous shaking. Persistent formation indicated the presence of saponins.

3.4 Terpenoid Test: A crude extract (0.20 g) was extracted three times with 3 mL of petroleum ether for 3 minutes each. The combined extract was then mixed with 2 mL of chloroform until homogeneous. Concentrated sulfuric acid (H_2SO_4) was carefully added, and the mixture was observed for changes. The appearance of a distinct interface indicated the presence of terpenoids.

3.5 Cardiac Glycoside Test: Crude extract (0.20 g) was extracted three times with 3 mL of petroleum ether for 3 minutes each. Methanol (3 drops), acetic acid (3 drops), and sulfuric acid (3 drops) were subsequently added. The appearance of a blue or bluish-green color indicated the presence of cardiac glycosides.

4. Determination of Total Phenolic Content (TPC)

Total phenolic content was determined using the Folin–Ciocalteu colorimetric assay, as described by Kaisoon et al. (2011). Briefly, 125 µL of diluted Folin–Ciocalteu reagent was mixed with 15 µL of sample extract (2.5 µg/mL). After incubation at room temperature in the dark for 5 min, 125 µL of sodium carbonate solution (200 mg/mL) was added. The mixture was further incubated in the dark for 90 min, then absorbance was measured at 725 nm using a microplate reader. Gallic acid (0–100 µg/mL) was used to construct the calibration curve. The results were expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract). All measurements were performed in triplicate.

5. Determination of Total Flavonoid Content (TFC)

Total flavonoid content was determined using the aluminum chloride colorimetric method described by Kim et al. (2003). Briefly, 20 µL of sample extract (2.5 µg/mL) was mixed with 6 µL of 5% sodium nitrite solution and incubated for 5 min. Subsequently, 6 µL of 10% aluminium chloride solution and 40 µL of 1 M sodium hydroxide were added. The absorbance was measured at 510 nm using a microplate reader. Catechin (0–100 µg/mL) was

used to construct the calibration curve. Results were expressed as milligrams of catechin equivalents per gram of extract (mg CE/g extract). All assays were conducted in triplicate.

6. Antioxidant Activity Assay

6.1 DPPH Radical Scavenging Assay

The antioxidant activity was evaluated using the DPPH radical scavenging assay, as described by Samarakoon et al. (2011). The ethanolic crude extract was dissolved in DMSO, and the aqueous crude extract was dissolved in distilled water. Each crude extract was prepared as a series of two-fold serial dilutions to yield 12 concentration levels, ranging from 2.5 to 0.001 mg/mL, for subsequent biological assays. Twenty microliters of each sample solution were mixed with 180 µL of 80 µM DPPH solution prepared in methanol. Absorbance was measured at 517 nm using a microplate reader. Trolox was used as the positive control. The percentage inhibition was calculated using the following equation: % inhibition = $[Abs_{Control} - (Abs_{Sample} - Abs_{Blank})]/ Abs_{Control} \times 100$. The IC_{50} value was determined from the dose–response curve and represents the concentration required to inhibit 50% of the DPPH radicals. All experiments were performed in triplicate.

6.2 ABTS Radical Scavenging Assay

The ABTS radical scavenging activity was determined according to Re et al. (1999). The ethanolic crude extract was dissolved in DMSO, and the aqueous crude extract was dissolved in distilled water. Each crude extract was prepared as a series of two-fold serial dilutions to yield 12 concentration levels, ranging from 2.5 to 0.001 mg/mL, for subsequent biological assays. A volume of 2.5 µL of each sample solution was mixed with 250 µL of $ABTS^{\bullet+}$ working solution. Absorbance was measured at 734 nm using a microplate reader. Trolox was used as the reference antioxidant. The percentage inhibition was calculated using the following equation: % inhibition = $[Abs_{Control} - (Abs_{Sample} - Abs_{Blank})]/ Abs_{Control} \times 100$. The IC_{50} value was determined from the dose–response curve and represents the concentration required to inhibit 50% of the ABTS radicals. All experiments were performed in triplicate.

7. Statistical Analysis

All assays were performed in technical triplicate using extracts prepared from a single extraction batch, and data are presented as the mean ± standard deviation (SD). Statistical analyses were performed using IBM SPSS Statistics version 32. Comparisons between the ethanolic and aqueous extracts for total phenolic content (TPC) and total flavonoid content (TFC) were conducted using an independent-samples t-test. Differences in antioxidant activity (DPPH and ABTS IC_{50} values) among the ethanolic extract, aqueous extract, and the reference standard (Trolox) were analyzed using one-way ANOVA, followed by Tukey's HSD. Statistical significance was set at $p < 0.05$.

Research Results

1. Preliminary Phytochemical Screening

The preliminary phytochemical screening of the ethanolic and aqueous extracts showed positive results for tannins, saponins, and terpenoids. In contrast, alkaloids and cardiac glycosides were present only in ethanolic extracts and were not detected in aqueous extracts.

2. Determination of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

The extraction yields, total phenolic content (TPC), and total flavonoid content (TFC) of the herbal compress formulation described in the Kasai Scripture are presented in Table 1. The ethanolic extraction yielded 86.25 g (9.58% w/w), whereas the aqueous

extraction yielded 73.27 g (8.14% w/w). The total phenolic content was calculated from the gallic acid standard curve ($y = 339.89x + 0.0268$; $R^2 = 0.9987$) to obtain TPC values of 41.06 ± 0.96 mg GAE/g extract and 29.83 ± 0.22 mg GAE/g extract for the ethanolic and aqueous extracts, respectively ($p = 0.003$). The total flavonoid content was calculated from the catechin standard curve ($y = 50.567x + 0.0013$; $R^2 = 0.9875$) to obtain TFC values of 20.28 ± 0.41 mg CE/g extract and 14.32 ± 0.49 mg CE/g extract for the ethanolic and aqueous extracts, respectively ($p < 0.001$). The ethanolic extract exhibited significantly higher TPC and TFC than the aqueous extract.

Table 1: TPC and TFC of the herbal compress formulation in the Kasai Scripture

Formulation extract	Weight of extract (g)	Yield (%w/w)	TPC* (mg GAE/g extract)	TFC* (mg CE/g extract)
Ethanolic extract	86.25	9.58	41.06 ± 0.96[a]	20.28 ± 0.41[a]
Aqueous extract	73.27	8.14	29.83 ± 0.22[b]	14.32 ± 0.49[b]
p-value	-	-	0.003	<0.001

Remark * Data are presented as mean ± SD; each experiment was performed in triplicate.
[a,b]Means bearing different superscript letters in the same column are significantly different ($p < 0.05$)

3. Antioxidant Activity Assay

The antioxidant activities of the herbal compress extracts were evaluated using the DPPH and ABTS radical scavenging assays (Table 2). In the DPPH assay, the aqueous extract exhibited significantly stronger radical scavenging activity than the ethanolic extract, with IC_{50} values of 0.83 ± 0.09 and 2.15 ± 0.20 mg/mL, respectively ($p < 0.05$). Conversely, in the ABTS assay, the ethanolic extract demonstrated significantly greater antioxidant activity than the aqueous extract, with IC_{50} values of 2.80 ± 0.28 and 4.33 ± 0.22 mg/mL, respectively ($p < 0.05$). However, both extracts were less active than Trolox in both assays.

Table 2: DPPH and ABTS radical scavenging capacities of the herbal compress formulation in the Kasai Scripture

Formulation extract	Antioxidant capacities*	
	DPPH radical scavenging activity	ABTS radical scavenging activity
Ethanolic extract	2.15 ± 0.20[c]	2.80 ± 0.28[b]
Aqueous extract	0.83 ± 0.09[b]	4.33 ± 0.22[c]
Trolox	0.02 ± 0.00[a]	0.35 ± 0.00[a]

Remark * Data are presented as IC_{50} (mg/mL) ± SD; each experiment was performed in triplicate.
[a,b,c] Means bearing different superscript letters in the same column are significantly different ($p < 0.05$)

Discussion

The present study demonstrated that the ethanolic extract of the Kasai Scripture herbal compress formulation contained significantly higher total phenolic content (TPC) and total flavonoid content (TFC) than the aqueous extract ($p < 0.05$), suggesting that ethanol more efficiently extracted phenolic and flavonoid compounds because of its intermediate polarity (Dai & Mumper, 2010). Similar observations have been reported for *T. indica*, *A. concinna*, *Z. montanum*, *Z. zerumbet*, and *Z. officinale*, in which ethanolic extracts generally exhibited higher phytochemical contents and stronger antioxidant activities than aqueous extracts (Leng et al., 2017; Ghasemzadeh et al., 2010; Rohmah, 2022; Thiraphatthanavong et al., 2023; Rinthong et al., 2024). Consistent with the higher TPC and TFC, the ethanolic extract showed significantly greater ABTS radical-scavenging activity, in agreement with previous reports demonstrating positive associations between phenolic compounds and ABTS antioxidant activity (Sookying et al., 2022; Hanif Ali et al., 2022). In contrast, the aqueous extract exhibited significantly stronger DPPH radical-scavenging activity despite its lower TPC and TFC. This was an observed finding in the present study and may reflect the presence of water-soluble antioxidant constituents with high DPPH-scavenging activity; however, this explanation remains hypothetical and requires confirmation by phytochemical characterization. The discrepancy between the DPPH and ABTS assays may be explained by differences in their reaction mechanisms and solvent compatibility. The ABTS assay primarily reflects electron transfer reactions, whereas the DPPH assay mainly measures hydrogen atom transfer, although both assays may involve mixed mechanisms. Furthermore, ABTS can be applied in both aqueous and organic media, whereas DPPH is generally performed in organic solvents, potentially contributing to differences in antioxidant responses among extracts (Prior et al., 2005; Re et al., 1999; Apak et al., 2016). Moreover, crude herbal extracts contain complex mixtures of phytochemicals that may interact synergistically or antagonistically; therefore, antioxidant activity cannot always be explained solely by the total phenolic or flavonoid contents (Apak et al., 2016). The TPC and TFC assays also have inherent limitations. The Folin–Ciocalteu assay is not specific for phenolic compounds because it also reacts with other reducing substances, while the aluminium chloride colorimetric assay primarily detects flavonoids capable of forming complexes with aluminium ions and may not accurately quantify all flavonoid subclasses (Everette et al., 2010; Chang et al., 2002). Therefore, the TPC and TFC values reported in this study should be regarded as estimates rather than absolute quantitative measurements. Overall, the antioxidant activity observed in the Kasai Scripture herbal compress formulation is likely attributable to the combined effects of multiple phytochemical groups rather than a single class of compounds. Further studies employing chromatographic techniques (e.g., GC-MS, HPLC or LC–MS) together with complementary antioxidant assays are warranted to identify the bioactive constituents responsible for the observed activities and to clarify their mechanisms of action.

The present study demonstrated that both the ethanolic and aqueous extracts of the Kasai Scripture herbal compress formulation contained phenolic and flavonoid compounds and exhibited in vitro antioxidant activity in the DPPH and ABTS assays. The ethanolic extract contained significantly higher total phenolic content (TPC) and total flavonoid content (TFC) than the aqueous extract and showed greater ABTS radical-scavenging activity, indicating that the extraction solvent influenced both phytochemical recovery and antioxidant activity. However, these findings are limited to phytochemical characterization and in vitro antioxidant assays and should not be interpreted as evidence of clinical efficacy, safety, or therapeutic effectiveness.

Recommendations

1. Recommendations for the application of research results

The findings of this study provide preliminary scientific evidence on the phytochemical composition and in vitro antioxidant activity of the herbal compress formulation described in the Kasai Scripture. These findings may serve as a basis for the standardization and quality control of Thai herbal compress formulations and support future phytochemical and pharmacological investigations.

2. Recommendations for future research

Further studies should identify the major bioactive compounds responsible for the antioxidant activity using chromatographic techniques (e.g., HPLC and GC–MS), investigate the antioxidant mechanisms using additional assays, and evaluate the formulation's anti-inflammatory activity, safety, stability, and clinical efficacy to support its therapeutic application.

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