The study of Antioxidant Activity and Chemical Compound by Gas Chromatography - mass spectrometry (GC-MS) of the Pra-Ang-Kab-Pra-Sen Recipe and its herbal components extract

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Abstract

This study evaluates the antioxidant activity, total phenolic content, and phytochemical composition of the Phra Angkhop Phra Sen recipe and its herbal components. The total phenolic content was determined using the Folin-Ciocalteu method, revealing a phenolic content of 59.43 ± 0.003 mg GAE/g for the Phra Angkhop Phra Sen recipe. Among its components, *Cinnamomum verum* exhibited the highest phenolic content (243.59 ± 0.03 mg GAE/g), followed by *Tamarindus indica* and *Zingiber montanum*. Antioxidant activity, assessed through the DPPH radical scavenging assay, showed that *Cinnamomum verum* had the strongest activity (EC50 = 0.01 mg/ml), comparable to the synthetic antioxidant BHT. The Phra Angkhop Phra Sen recipe, *Tamarindus indica*, and *Zingiber montanum* exhibited similar antioxidant potential (EC50 = 0.16 mg/ml), while *Crinum asiaticum* and *Nigella sativa* showed lower activity (EC50 = 0.23 mg/ml and 0.78 mg/ml, respectively). Gas Chromatography-Mass Spectrometry (GC-MS) analysis identified 10 phytochemical compounds in the recipe. D-Fructose, 3-O-methyl was the most abundant (14.50% area), followed by 2,3,4,5-Tetrahydroxypentanal (10.52%) and 4-O-Methylmannose (5.39%).

The findings underscore the significant antioxidant potential of the Phra Angkhop Phra Sen recipe, primarily attributed to its phenolic content, particularly from Cinnamomum verum. This supports its traditional use for managing oxidative stress and inflammation and highlights its potential for developing therapeutic applications in modern medicine.

Keywords: Phra Angkhop Phra Sen recipe, Antioxidant activity, DPPH radical scavenging assay, herbal ingredients

1. Introduction

Free radicals, generated continuously within the human body through processes like respiration, stress, metabolism, and environmental exposures (pollution, sunlight, poor diet), can have detrimental effects. They contribute to oxidative stress, a condition characterized by

cellular and tissue damage (Ames, Shigenaga, & Hagen, 1993). This oxidative stress is implicated in the development of chronic diseases, including cancer, diabetes, heart disease, cardiovascular conditions, and neurodegenerative disorders like Alzheimer's. Free radicals also play a role in muscle and tissue damage, leading to inflammation. Herbal medicine is a rich source of antioxidants, such as phenolic compounds, carotenoids, flavonoids, and various vitamins. These antioxidants play a crucial role in combating oxidative stress by neutralizing free radicals, thereby protecting cells and tissues from damage (Cornish & Garbary, 2010). Research indicates that natural antioxidants, frequently derived from medicinal plants, may present a safer alternative to synthetic antioxidants. Thorough research in herbal medicine is essential. This includes scientific investigations to validate its quality, efficacy, effectiveness, and safety, along with the exploration of its chemical properties, active compounds, and biological activities. Such research is crucial for enhancing the credibility and safety of herbal medicine and fostering its wider acceptance in present society. This research specifically focuses on the Phra Angkab Phra Sen formula, a traditional remedy for pain and muscle aches (Department of Protection and Promotion of Thai Traditional Medicine and Alternative Medicine, Ministry of Public Health, 2012).

The study will analyze its active compounds, assess its antioxidant activity, and determine its total phenolic content. Phenolic compounds are a major group of antioxidants commonly found in medicinal plants (Khophai et al., 2025). The objective is to utilize these findings to develop the Phra Angkab Phra Sen formula into various product forms suitable for practical applications.

1.1 Objective of the Study

1. To investigate the antioxidant activity of herbal ingredients and the Phra Angkab Phra Sen recipe extract using the DPPH radical scavenging assay.
2. To study the total phenolic content of herbal ingredients and the Phra Angkab Phra Sen recipe extract using the Folin-Ciocalteu method.
3. To analyze the chemical composition of herbal ingredients and the Phra Angkab Phra Sen recipe extract using Gas Chromatography-Mass Spectrometry (GC-MS).

2. Methodology

Plant materials

The herbal plants used in the research were the Phra Angkhop Phra Sen recipe, which contains the following herbal ingredients: black cumin (*Nigella sativa* L.) used as seeds, cinnamon (*Cinnamomum verum* J.Presl.) used as bark, Phlai (*Zingiber montanum* (Koenig) Link ex Dietr.) used as rhizomes, Crinum lily (*Crinum asiaticum* L.) used as leaves, and tamarind (*Tamarindus indica* L.) used as leaves.

Chemicals and Reagents

Water was distilled and purified, along with gallic acid, trolox, butylated hydroxytoluene (BHT) (Sigma, Germany), Folin—ciocalteu reagent, Sodium Carbonate (Merck, Germany), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Kandel, Germany). All solvents used were analytical or HPLC grade.

Preparation and extraction of herbal ingredients and the Phra Angkab Phra Sen recipe

The herbal ingredients were dried in a hot-air oven at 50 °C for 24 hours ground to a coarse powder and sieved using a sieve no. 60. A hundred grams of each herbal ingredient was extracted by maceration with 50% ethanol for one week, after filtering, the residue was macerated again using the same procedure. The filtrates of each ingredient obtained from the first and the second time maceration were combined and evaporated until no alcohol was left. The semisolid residue was dried using a freeze-drying machine, and the dry powder was collected and kept in a closed container until use.

The Phra Angkab Phra Sen recipe was composed of 5 species including 20 g of black-cumin (*N. sativa*) used as seeds, 40 g of cinnamon (*C. verum*) used as bark, 80 g of Phlai (*Z. montanum*) used as rhizomes, 160 g of Crinum Lily (*C. asiaticum*) used as leaves, and 360 g of Tamarind (*T. indica*) used as leaves. Its were prepared by mixing equal weight of each ingredient and grinding. The recipe was macerated with 50% ethanol for one week and filtered. The filtrate was kept. The residue was done repeatedly using the same procedure. Two filtrates were combined and evaporated until no alcohol remained in the extract. The semisolid extract was dried using a freeze-drying machine; the dry powder was collected and kept in a closed container for performing biological assay.

Determination of total phenolic content

The content of total phenolic compounds was determined by the modified Folin-Ciocalteu method (Dechayont, Hansakul, & Itharat, 2012), (Rajeshwari & Jyoti, 2013) as the reaction between specific redox reagent (Folin- Ciocalteu reagents) and phenolic substances in the crude extract. If plants consist of phenolic substance, the solution will form a blue complex. In experiment, gallic acid was used as a standard for the calibration curve. In short, 20 µL of extract was added to a 96-well plate and mixed with 100 µL of 10% Folin–Ciocalteu reagent, followed by the addition of 80 µL of a 7.5% Na_2CO_3 solution. After incubation at room temperature for 30 min in the dark with slight shaking, the absorbance at 765 nm was measured on a microplate reader. Gallic acid was used as a standard for the calibration curve. The total phenolic content was expressed as gallic acid equivalent (GAE) milligrams per g of lyophilized extract. All measurements were made in triplicate.

Calculation Formula

$TPC\ (\%\ w/w\ GAE) = PS \times V \times D \times 10^{-6} \times 100/W$

PS = phenolic content of the solution (µg/ml)

V = Total volume of sample (mL)

D = dilution factor

W = sample weight (g)

Determination of Antioxidant Activity Using a DPPH (2,2-Diphenyl-1-picrylhydrazyl) Radical Scavenging Assay

The DPPH radical scavenging activity was measured according to Brand-Williams (Brand-Williams, Cuvelier, & Berset, 1995) with some modifications. DPPH is a stable synthetic free radical with a deep purple color. When DPPH reacts with antioxidants, which are dissolved in ethanol, the purple color of DPPH fades to yellow. This color change indicates the scavenging activity of the antioxidant. For the assay, 100 µL of extract was added to a 96-well plate and mixed with 100 µL of 2.0 mM DPPH. The plate was kept in the dark at room temperature for

15 min. Decreases in absorbance at 517 nm were measured on a microplate reader. Trolox and Butylated hydroxytoluene (BHT) solutions in the concentration range of 6.25–100 µg/mL were used as a standard, and ethanol was used as a control. The extract was tested in a range of concentrations to establish the EC50 (the concentration that reduced the absorbance of DPPH by 50%).

The radical scavenging activity was calculated using the following formula:

$$\% \text{ Inhibition} = [(A0 - A1)/A0] \times 100$$

where A0 is the absorbance of the control and A1 is the absorbance of the sample.

Different sample concentrations were used to obtain antiradical curves for calculating the EC50 values. Antiradical curves were plotted based on concentration on the x-axis and their relative scavenging capacity on the y-axis. The EC50 values were processed using statistical programs (GraphPad Prism® version 5.01 (San Diego, CA)).

Gas chromatography-mass spectroscopic analysis of the Phra Angkhop Phra Sen recipe and its herbal components extract

GC-MS analysis of the Phra Angkhop Phra Sen recipe and its herbal components extract was performed using the equipment Shimadzu GCMS-QP2020 with an HP-5MS 30 m x 0.25 mm ID x 0.25 mm column (non-polar fused silica capillary with a 5% phenyl-methylpolysiloxane stationary phase). The helium gas flow rate into the column was set to 1.0 mL/min and 1 µL of sample was injected at a split ratio of 1:20. The column temperature was programmed at 70 ℃ for 2 min, 5 ℃/min to 200 ℃ for 10 min, 5 ℃/min to 230 ℃ for 10 min, 5 ℃/min to 250 ℃ for 5 min, and 5 ℃/min to 320 ℃ for 20 min. The ion source was 250 ℃ in the Electron Impact Ionization (EI) system, giving the separation results of the extract components as Total Ion Chromatogram (TIC) in the Scan Mode system using the Mass range of 35 to 500 AMU (Atomic Mass Unit) by comparing with the NIST17.lib database (Gomathi et al., 2015).

Data analysis

Results were expressed as the Mean ± Standard Deviation (SD) of three independent experiments for each antioxidant; following the statistical interpretation of the data, EC50 values were expressed as a 95% confidence interval.

Data analysis was performed using the algorithms implemented in six frequently used statistical programs: GraphPad Prism® version 5.01 (San Diego, CA).

3. Results and Discussion

Herbal ingredients and the Phra Angkhop Phra Sen recipe extract

The Phra Angkhop Phra Sen recipe was used to treat Muscle pain, which has been described in Prathomjinda scripture for a long time (Foundation of Thai Traditional Medicine, 2007). This recipe was composed of 5 species including black cumin (*N. sativa*) used as seeds, cinnamon (*C. verum*) used as bark, Phlai (*Z. montanum*) used as rhizomes, Crinum Lily (*C. asiaticum*) used as leaves, and Tamarind (*T. indica*) used as leaves. Each plant was divided into two portions the first portion was used for experimental analysis and another portion was mixed for the Phra Angkhop Phra Sen recipe preparation. All crude drugs were extracted by maceration (50% ethanol), and the percent yields of black cumin, cinnamon, Phlai, Crinum Lily, and tamarind extracts were 24.24, 5.34, 13.52, 21.40, and 20.48% respectively as shown in Table

1. The Phra Angkhop Phra Sen recipe preparation, was prepared by maceration with 50% ethanol for antioxidant activity. The percent yield was 19% as shown in Table 1.

Table 1: Percentage yield of plant extracts

Thai /common name	Scientific name	Part of used	Sample Weight (g)	Crude Extract Weight (g)	% yield (w/w) 50% Ethanol
Thian Dam	*N. sativa*	Seeds	500	121.19	24.24
cinnamon	*C. verum*	Bark	500	26.72	5.34
Phlai	*Z. montanum*	Rhizomes	500	67.58	13.52
Crinum Lily	*C. asiaticum*	Leaves	500	106.98	21.40
Tamarind	*T. indica*	Leaves	500	102.41	20.48
Phra Angkhop Phra Sen recipe	-	-	680	129.21	19

Determination of total phenolic compounds

The total phenolic content of the extracts was determined from the standard curve of gallic acid, with the equation y = 0.0036x + 0.0064 and R =0.9999 (Figure 1). The phenolic content was expressed as gallic acid equivalents (GAE) per gram of dry weight. The Phra Angkhop Phra Sen recipe extract contained the total phenolic compounds at 59.43±0.003 mg GAE/g of dry extract. The maximum content of the total phenolic compounds was found in *C. verum* (243.59±0.03 mg GAE/g of dry extract), followed by *T. indica* (73.59±0.003 mg GAE/g of dry extract), Z. *montanum* 67.85±0.001 mg GAE/g of dry extract), and *C. asiaticum* (50.26±0.01 mg GAE/g of dry extract). The extract of *N. sativa* showed the lowest content of phenolic compound at 38.78±0.004 GAE/g of dry extract as shown in Table 2.

Figure 1: Standard calibration curve of gallic acid for determining phenolic compounds

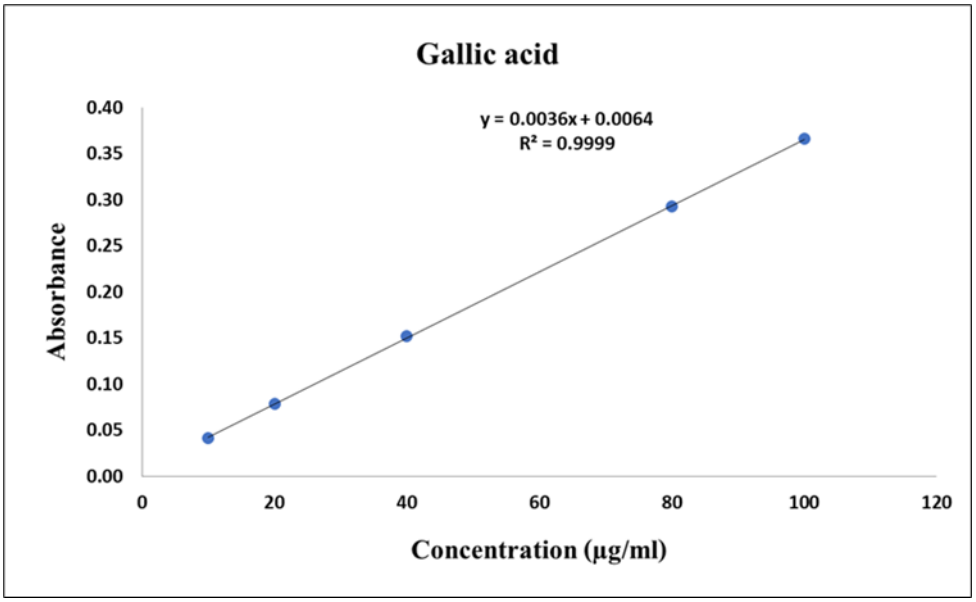

Table 2: The total phenolic compounds in the ethanolic extract of the Phra Angkhop Phra Sen recipe and its herbal components

Thai /common name	Scientific name	Total phenolic contents (mg GAE/g of dry extract)
cinnamon	*C. verum*	243.59±0.03
Tamarind	*T. indica*	73.59±0.003
Phlai	*Z. montanum*	67.85±0.001
Crinum Lily	*C. asiaticum*	50.26±0.01
Thian Dam	*N. sativa*	38.78±0.004
Phra Angkhop Phra Sen recipe	-	59.43±0.003

Express as mean ± SD (n=3)

Determination of Antioxidant Activity Using a DPPH (2,2-Diphenyl-1-picrylhydrazyl) Radical Scavenging Assay

The results of the antioxidant activity test for the extracts of the Phra Angkhop Phra Sen recipe, its herbal components, and the butylated hydroxytoluene (BHT) standard (Table 3) revealed that the *C. verum* extract exhibited the highest antioxidant activity, with an EC50 value of 0.01 mg/ml. This was followed by the Phra Angkhop Phra Sen recipe, *T. indica*, and *Z. montanum*, which demonstrated equally valuable antioxidant activities, each with an EC50 value of 0.16 mg/ml. Meanwhile, *C. asiaticum* and *N. sativa* showed EC50 values of 0.23 mg/ml and 0.78 mg/ml, respectively. The BHT standard also exhibited an EC50 value of 0.01 mg/ml.

Table 3: Antioxidant effect (EC50) in DPPH radicals in the ethanolic extract of the Phra Angkhop Phra Sen recipe and its herbal components

Thai /common name	Scientific name	EC50 (mg/ml)
cinnamon	*C. verum*	0.01
Phra Angkhop Phra Sen recipe		0.16
Tamarind	*T. indica*	0.16
Phlai	*Z. montanum*	0.16
Crinum Lily	*C. asiaticum*	0.23
Thian Dam	*N. sativa*	0.78
BHT		0.01

Gas chromatography-mass spectroscopic analysis of the Phra Angkhop Phra Sen recipe extract

The crude extract of the Phra Angkhop Phra Sen recipe prepared with 50% ethanol solvent revealed the presence of 10 phytochemical compounds, including 2-[2-[2-[2-(2-Butoxyethoxy)ethoxy]ethoxy], .alpha.-Methyl mannofuranoside, 3-O-Methyl-d-glucose,3,4,6-Tri-O-methyl-d-glucose, 4-O-Methylmannose, Galactitol, 2,3,4,5-Tetrahydroxypentanal, D-Fructose, 3-O-methyl-, beta.-Sitosterol acetate, Phenol,2,4-bis(1,1-dimethyl ethyl)-, phosphite. The analysis highlights the diversity of phytochemical compounds present in the Phra Angkhop Phra Sen recipe, with D-Fructose, and 3-O-methyl (with a % area of 14.50) as the most abundant component, as shown in Figure 2 and Table 4.

Figure 2: Chromatographic and Preliminary Phytochemical Analysis of the Extract from the Phra Angkhop Phra Sen Recipe Fermented with 50% Ethanol Solvent Using Gas Chromatography-Mass Spectrometry (GC-MS)

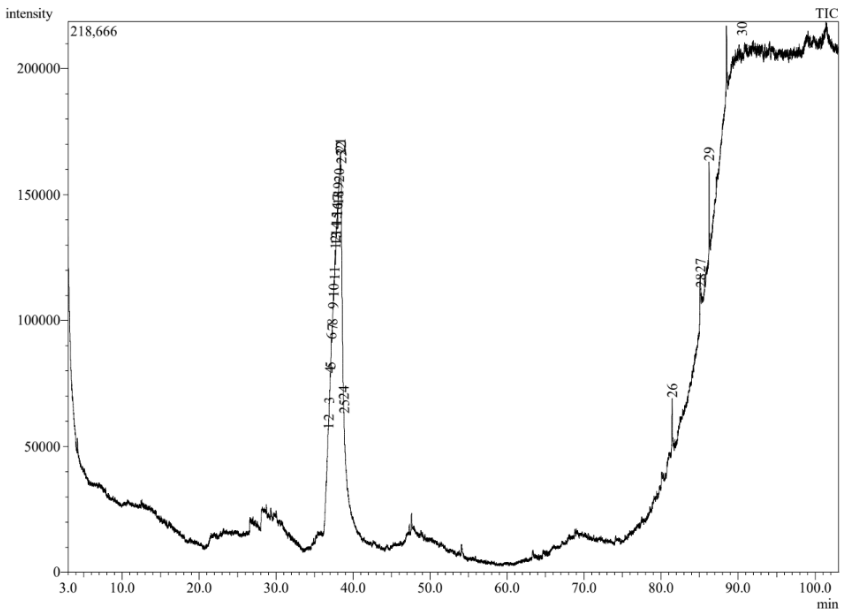

Table 4: Chromatographic and Preliminary Phytochemical Analysis of the Extract from the Phra Angkhop Phra Sen Recipe Fermented with 50% Ethanol Solvent Using Gas Chromatography-Mass Spectrometry (GC-MS)

No.	peak	RT (min)	Name	% area	% match
1.	1	36.785	-	1.00	0
2.	2	36.825	-	0.68	0
3.	3	36.905	-	2.67	0
4.	4	37.035	2-[2-[2-[2-(2-Butoxyethoxy)ethoxy]ethoxy]	1.23	72
5.	5	37.070	-	2.13	0
6.	6	37.200	.alpha.-Methyl mannofuranoside	3.66	76
7.	7	37.265	-	1.23	0
8.	8	37.310	3-O-Methyl-d-glucose	3.58	71
9.	9	37.375	3,4,6-Tri-O-methyl-d-glucose	2.66	71
10.	10	37.430	-	4.41	0
11.	11	37.540	4-O-Methylmannose	5.32	74
12.	12	37.669	4-O-Methylmannose	5.39	78
13.	13	37.780	Galactitol	5.56	75
14.	14	37.810	-	2.05	0
15.	15	37.850	3-O-Methyl-d-glucose	4.25	73
16.	16	37.925	-	2.82	0
17.	17	37.968	.alpha.-Methyl mannofuranoside	3.85	76
18.	18	38.035	-	2.89	0
19.	19	38.070	.alpha.-Methyl mannofuranoside	6.16	76
20.	20	38.170	2,3,4,5-Tetrahydroxypentanal	10.52	75
21.	21	38.345	-	6.59	0
22.	22	38.390	4-O-Methylmannose	3.85	75
23.	23	38.443	D-Frutose, 3-O-methyl-	14.50	72
24.	24	38.765	-	0.72	0
25.	25	38.825	-	1.01	0
26.	26	81.425	beta. -Sitosterol acetate	0.94	82
27.	27	85.075	-	0.64	0
28.	28	85.135	-	0.34	0
29.	29	86.220	Phenol,2,4-bis(1,1-dimethyl ethyl)-, phosphite	1.42	87
30.	30	88.486	-	0.92	0

4. Conclusion

The study aimed to evaluate the antioxidant activity, total phenolic content, and phytochemical composition of the Phra Angkhop Phra Sen recipe and its herbal components using a series of chemical and analytical techniques.

The total phenolic content of the Phra Angkhop Phra Sen recipe was 59.43 ± 0.003 mg GAE/g of dry extract, indicating a moderate presence of phenolic compounds. Among the individual ingredients, *C. verum* (Cinnamon) exhibited the highest phenolic content (243.59 ± 0.03 mg GAE/g of dry extract), followed by *T. indica* (Tamarind) and *Z. montanum* (Phlai), which suggests that cinnamon is the primary contributor to the antioxidant potential of the recipe.

Antioxidant Activity (DPPH Assay) *C. verum* displayed the highest antioxidant activity, with an EC50 value of 0.01 mg/ml, comparable to the synthetic antioxidant BHT. The Phra Angkhop Phra Sen recipe, *T. indica*, and *Z. montanum* showed equal antioxidant efficacy with an EC50 value of 0.16 mg/ml. *C. asiaticum* (Crinum Lily) and *N. sativa* (Thian Dam) demonstrated lower antioxidant activities, with EC50 values of 0.23 mg/ml and 0.78 mg/ml, respectively.

Phytochemical Composition (GC-MS Analysis). The GC-MS analysis identified 10 phytochemical compounds in the Phra Angkhop Phra Sen recipe. The most abundant compound was D-Fructose, 3-O-methyl, with a % area of 14.50, followed by 2,3,4,5-Tetrahydroxypentanal (10.52%) and 4-O-Methylmannose (5.39%). These compounds are known to have antioxidant properties and play roles in cellular protection against oxidative stress.

The findings demonstrate that the Phra Angkhop Phra Sen recipe has notable antioxidant properties, attributed primarily to its phenolic content, particularly from *C. verum* (Cinnamon). The high antioxidant activity of the recipe suggests its potential for therapeutic applications in reducing oxidative stress-related conditions, including inflammation and chronic diseases. Additionally, the GC-MS analysis identified bioactive compounds that contribute to the recipe's overall efficacy. The presence of natural antioxidants such as phenolic compounds and other phytochemicals underscores the value of traditional herbal remedies **(**Cornish & Garbary, 2010).

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