



GAS CHROMATOGRAPHY-MASS SPECTROMETRY CHEMICAL PROFILING AND ANTIOXIDATION ACTIVITY OF THE THAI HERBAL FORMULA “PIKAD TRISUKKHATI SAMUTHARN” EXTRACT

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ABSTRACT

Pikad Trisukkhati Samutharn (PTS) is a medicinal formula used in Thai traditional medicine for treating severe fever, inflammation, and diarrhea, as well as for general health promotion. This herbal formula was made from the roots of three plants: *Oroxylum indicum* (L.) Kurz, *Sesbania grandiflora* (L.) Pers and *Ficus racemosa* L. The purpose of this study was to investigate the antioxidation activity and chemical profiling of PTS and its herbal components. The antioxidation activity of the extracts was determined using the DPPH free radical scavenging assay, as well as the Folin-Ciocalteu method, to investigate the total phenolic contents. The thiobarbituric acid reactive substance (TBAR) assay was used to test lipid peroxidation activity. The chemical profiling of PTS was demonstrated using gas chromatography–mass spectrometry (GC-MS) analysis. The results revealed that the total phenolic content of the ethanolic extract (79.57 ± 2.33 mg GAE/g extract) was greater than that of the aqueous extract (12.55 ± 2.83 mg GAE/g extract) of the PTS. The DPPH free radical scavenging activity of the PTS ethanolic extract (IC_{50} value 80.69 ± 0.40 mg/mL) was also stronger than that of the PTS aqueous extract (IC_{50} value 650.10 ± 98.20 mg/mL). Lipid peroxidation of the PTS ethanolic extract was moderate with $32.47 \pm 0.71\%$ inhibition. GC-MS revealed the presence of several phenolic compounds contained in the PTS ethanolic extract in which the identified compounds had previously been reported to have several pharmacological activities such as antioxidation, anti-inflammation, and antimicrobial. In conclusion, the antioxidation and chemical constituents of the PTS extract were demonstrated for the first time, and more pharmacological activities of PTS should be explored to confirm its traditional use.

Keywords: antioxidation, *Ficus racemosa*, GC-MS, *Oroxylum indicum*, Pikad Trisukkhati Samutharn, *Sesbania grandiflora*

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Introduction

Free radicals are unpaired independent molecules that are produced from normal physiological metabolism in the body and are received from external substances, such as alcohol, air pollution, tobacco smoke, radioactive agents, and heavy metals. Low and moderate levels of free radicals can be beneficial to the physiological functions of the immune system, but overexpression of free radicals can damage biomolecules such as nucleic acids, proteins, and lipids leading to an increase in oxidative stress involved in pathophysiological diseases such as cancer, cardiovascular diseases, neurodegenerative diseases, and rheumatoid arthritis.¹ Reactive oxygen species (ROS), which participates in the pathogenesis of sepsis and inflammation, is one of the major radical species derived from oxygen. Increased ROS expression in several tissues such as the liver, hypothalamus, and brown adipose tissue during the induction of fever has been observed. Antioxidant molecules scavenge free radical chain reactions by donating an electron to free radicals without destabilizing themselves.^{2,3} Thus, a balance between antioxidant molecules and free radicals is needed.

Phenolic compounds are a large group of secondary metabolites derived from plants that are essential for plant growth and demonstrate multiple properties in humans, particularly antioxidant and anti-inflammatory properties.⁴ The ability of phenolic compounds to eliminate oxidation and prevent oxidative stress is associated with their free radical-scavenging effects.^{5,6}

The utilization of herbal remedies has been a longstanding practice within Thai traditional medicine, serving as a means to address a wide range of illnesses and to enhance overall well-being. Within the framework of Thai traditional medical knowledge, herbal medicines are classified based on taste or properties, resulting in naming conventions that

facilitate ease of remembering. Pikad Trisukkhati Samutharn (PTS), as documented in the authoritative textbook of Thai Traditional Medicine, "Tamra Paetsart Sonkrau Chabub Anurak", is a herbal formula used for the treatment of severe fever, inflammation, and diarrhea, in addition to promoting optimal health.⁷ The PTS formula comprises three herbs that have a bitter taste including the root of *Oroxylum indicum* (L.) Kurz, the root of *Sesbania grandiflora* (L.) Pers, and the root of *Ficus racemosa* L. combined in a ratio of 1:1:1 (w/w). *O. indicum* root contains several flavonoid compounds such as baicalein, baicalein-6-O-glucoside, chysin, oroxylin A, and β -sitosterol and has been reported to have anti-inflammatory, anticancer, antitumor, antiulcer, antidiabetic, and antidyslipidemic properties.^{8,9} The root of *S. grandiflora* contains isoflavonoid compounds including isovestitol, medicarpin, sativa, and betulinic acid, which have been shown to demonstrate antituberculosis properties.¹⁰ *F. racemosa* root, which contains cycloartenol, euphorbol, taraxerone, and tinyatoxin,¹¹ has been reported to show antioxidation effects.¹²

Free radicals and oxidation molecules have been implicated in fever, and PTS has long been employed for the treatment of fever and inflammation. Consequently, there is considerable interest in assessing the biological activities of PTS for its potential anti-inflammatory and antioxidative effects. However, there has been a lack of comprehensive research exploring the pharmacological effects and chemical composition of PTS, thereby hindering the substantiation of its purported beneficial applications in Thai traditional medicine. Consequently, the present study was undertaken to explore the chemical composition using gas chromatography-mass spectrometry (GC-MS) analysis, determine the total phenolic contents, and examine the antioxidative effects of PTS alongside its constituent herbal components.

Materials and methods

Chemicals and standards

Ascorbic acid, DPPH reagent, ferrous sulfate, TBAR reagent, and Trolox were purchased from Sigma Aldrich (St. Louis, MO, USA). Formic acid was of analytical grade and purchased from Merck (Darmstadt, Germany). Gallic acid (purity > 98%) was purchased from the Tokyo Chemical Industry (Tokyo, Japan). Folin-Ciocalteu Reagent was purchased from Loba Chemie (Mumbai, India).

Plant materials

F. racemosa, *O. indicum*, and *S. grandiflora* roots were collected in December 2018 in Phitsanulok Province, Thailand. The plants were authenticated and identified by the Division of Applied Thai Traditional Medicine, Faculty of Public Health, Naresuan University. The voucher specimens No. 05718 for *F. racemosa*, No. 05719 for *O. indicum*, and No. 05720 for *S. grandiflora* were deposited at the PNU Herbarium, Faculty of Science, Naresuan University.

Plant extraction

To obtain the aqueous and ethanolic extracts, all plant materials including *F. racemosa*, *O. indicum*, and *S. grandiflora* roots were separately extracted. PTS was prepared by extracting the formulated of the three herbal plants in a ratio of 1:1:1 (w/w). The plant materials were cut into small pieces, washed with clean water, dried in a hot air oven at 65°C, and then powdered. Aqueous extract was prepared by decoction with water at 90 – 100°C for 45 min with a ratio of dry sample to water of 1:3 w/v. The aqueous extract was then filtrated, frozen at -20°C, and then sublimated in a freeze dryer to concentrate. Ethanolic extract was prepared by maceration with 95% ethanol with a ratio of dry material to solvent of 1:3 w/v for 24 h at room temperature. After filtration, the filtrate was then concentrated under vacuum at 30 - 35°C until dry. The obtained aqueous and ethanolic extracts were stored at -20°C until required for further study.

The yields (%w/w) of the extracts are presented in Table 1.

Determination of total phenolic content

The total phenolic contents of the extracts including the aqueous and ethanolic extracts of PTS and its herbal components were determined using the Folin-Ciocalteu method using gallic acid as the standard.¹³ The stock solution of gallic acid was prepared in 70% methanol to achieve a concentration of 1 mg/mL and then diluted with 70% methanol to obtain concentrations of 150, 120, 90, 60, 30, and 10 µg/mL for the calibration curve. Briefly, 25 µL of 1 mg/mL of sample extract was added to a 96-well microplate followed by 25 µL of Folin-Ciocalteu reagent (3-fold diluted in ultrapure water), and 200 µL of ultrapure water. After incubation for 5 min, 25 µL of 10.6 g/100 mL of Na₂CO₃ was subsequently added, and the solution was then incubated at room temperature (25°C) in the dark for 1 h. The absorbance was measured utilizing a microplate spectrophotometer (BMG Labtech, Germany) at 725 nm with 70% methanol or ultrapure water used as the control. The measurements were performed in triplicate. The total phenolic content of the sample was calculated from the gallic acid calibration curve and expressed as the milligram equivalent of gallic acid per gram of extract (mg GAE/g extract).

DPPH free radical scavenging assay

The DPPH free radical scavenging activity of the aqueous and ethanolic extracts of PTS was assessed by the standard method.¹⁴ The stock solution of the extracts was prepared in 100% ethanol to achieve a concentration of 10 mg/mL, and the dilutions were made with 70% ethanol. Then, 75 µL of diluted solution was added to 96-well plates and mixed with 150 µL of ethanolic solution of DPPH. After incubating for 30 min in the dark at room temperature (25°C), the absorbance was measured at 515 nm using a microplate spectrophotometer (BMG Labtech, Germany). The control sample contained all

the reagents except the extract. The measurements were performed in triplicate. Butylated hydroxytoluene (BHT) was used as a positive control. The percentage of DPPH scavenging activity was calculated using Equation 1, where $A_{\text{(sample)}}$ is the absorbance of the sample extract, $A_{\text{(blank)}}$ is the absorbance of the sample extract without DPPH solution and $A_{\text{(control)}}$ is the absorbance of the control reaction (containing all reagents except the sample extract).

$$\text{DPPH free radical scavenging activity (\%)} = \left\{ \frac{\Delta A_{\text{(control - blank)}} - \Delta A_{\text{(sample - blank)}}}{\Delta A_{\text{(control - blank)}}} \right\} \times 100 \dots \text{Eq. 1}$$

IC_{50} values were determined from the percentage inhibition versus log concentration plot. The extracts that showed the highest DPPH free radical scavenging activity were further examined below for their lipid peroxidation inhibitory activity and analyzed for their chemical composition.

Measurement of lipid peroxidation inhibitory activity

The lipid peroxidation inhibitory activity of the ethanolic extracts of PTS and its herbal components was determined by a thiobarbituric acid reactive substance (TBAR) assay using rat brain homogenate as the lipid source.¹⁵ The stock solution of the extracts was prepared in 70% methanol to achieve a concentration of 1 mg/mL. Briefly, 20 μL of the sample solution was added to a microcentrifuge tube followed by 140 μL of 5.71 mg/mL (total protein) rat brain homogenate; the final concentration of rat brain homogenate was 4 mg/mL. After incubation at 37°C in a hot air oven for 30 min, 40 μL of a mixture of 2 mM ferrous sulfate and 1 mM ascorbic acid was added. The solution was then incubated at 37°C for 1 h to stop the reaction, and 200 μL of TBAR reagent was added and the solution was incubated at 90°C for 1 h. The test solution was then centrifuged at 10,000 rpm for 5 min, and 100 μL of supernatant was extracted and transferred to a 96-well plate. The

absorbance was measured at 535 nm. The measurements were performed in triplicate and Trolox was used as a positive control. The percentage of lipid peroxidation inhibitory activity was calculated using Equation 2.

$$\text{Lipid oxidation inhibitory activity (\%)} = 1 - \left\{ \frac{\Delta A_{\text{(sample - blank)}}}{A_{\text{(control)}}} \right\} \times 100 \dots \text{Eq. 2}$$

GC-MS analysis

Preparation of sample solutions

The sample extracts were weighed, dissolved with methanol at a concentration of 10 mg/mL, and sonicated for 30 min. The solutions were then redissolved in methanol (3 mg/mL) and filtered through nylon syringe filters with a 0.45 μm pore size.

Instruments and chromatographic conditions

GC-MS chemical profiles of the ethanolic extracts of PTS and its herbal components were carried out on a Bruker Scion 436-GC equipped with a single quadrupole mass spectrometer and CP-8410 autosampler. A SCION-5MS column (30 m x 0.25 mm id x 0.24 μm film thickness, 5% phenyl/95% dimethyl polysiloxane as a stationary phase) was used for the analysis. Helium gas (99.999%) was used as the mobile phase. The oven temperature was programmed to heat from 80°C to 250°C at a rate of 5°C/min with a holding time of 4 min, and the final temperature was increased from 250°C to 310°C at a rate of 20°C/min with a holding time of 15 min. The injector temperature was maintained at 250°C, the ion-source temperature was 280°C, the column flow was 1.2 mL/min, the split:splitless ratio was 1:50, the positive electron ionization (EI) mode was at 70 eV, the scanned mass was ranged from 40 to 400 Da, the solvent delay was 0 to 5 min, and the total running time was 56 min. The identification of chemical compounds of PTS and its herbal components was analyzed by comparing the mass spectra with the data from the National Institute Standard and Technology (NIST) library using MS Workstation 8 software.

Results and discussion

Total phenolic contents of PTS and its herbal components

Phenolic compounds have been widely recognized for their potential health benefits due to their antioxidant properties. Therefore, quantifying the total phenolic contents can provide valuable information regarding the potential antioxidative effects of PTS.^{5,6} In this study, we evaluated the total phenolic compound content in PTS and its herbal components. All results are shown in Table 1. The regression equation obtained from the calibration curve of gallic acid was $y = 0.0068x - 0.0171$, $R^2 = 0.9998$. The results showed that the highest total phenolic content among all sample extracts was obtained from the PTS ethanolic extract (PTSE, 79.57 ± 2.33 mg GAE/g extract). This value was approximately seven times higher than that of the PTS aqueous extract (PTSA, 12.55 ± 2.83 mg GAE/g extract). *O. indicum* ethanolic extract (OIE) also showed a high content of phenolic compounds (60.49 ± 1.75 mg GAE/g extract). The aqueous extract of *O. indicum* (OIA) showed the lowest phenolic content (6.62 ± 1.33 mg GAE/g extract). However, the amounts of phenolic compounds contents in *O. indicum*, *S. grandiflora*, and *F. racemosa* were slightly different from those in previous studies, which may be due to the difference in the solvent used for extraction, method of extraction, or geologic.¹⁶⁻¹⁸

The difference in the phenolic content between PTSE and PTSA may be due to the efficiency of the extraction method. Ethanol, methanol, and water are commonly used solvents due to their ability to dissolve a wide range of organic compounds, including phenolics. However, different solvents have varying polarities and extraction capabilities, which can affect the overall phenolic yield. The maceration extraction efficiency is based on the duration of maceration while shaking and higher temperatures can also increase the extraction yield. However, it is important to consider the impact of temperature on the decomposition of phenolic molecules during

extraction. While higher temperatures may enhance extraction yield, excessively high temperatures can lead to degradation of the phenolic compounds.¹⁹ The temperature that was used in the extraction of PTSA may have contributed to a lower phenolic content compared to PTSE. Also, the phenolic contents in PTSE were higher than in each single herbal extract which may be due to the multitudinous phenolic compounds obtained from the mixture of the three herbal components.

The findings of this study provide valuable insights into the most suitable method for extracting phenolic compounds from PTS. The comparison between maceration in ethanol and decoction highlights the effectiveness of maceration in ethanol as a preferred extraction method.

DPPH free radical scavenging activity of PTS and its herbal components

The IC_{50} values of the DPPH free radical scavenging activity of PTS and its herbal components were calculated (Table 1). The results showed that PTSE had an IC_{50} of 80.69 mg/mL, while PTSA had an IC_{50} of 650 mg/mL, and PTSE had a greater DPPH free radical scavenging ability than PTSA. Our results, discussed above, showed that PTSE contains the highest amounts of phenolic compounds.

Lipid peroxidation inhibitory activity of PTS and its herbal components.

Lipid peroxidation is an oxidation process that can cause irreversible damage to cell membranes and is related to some pathological diseases such as Alzheimer's disease, Parkinson's disease, and atherosclerosis.¹ The inhibition of lipid peroxidation of the extracts was expressed as a percentage of lipid peroxidation inhibition at a final concentration of 100 μ g/mL and is presented in Table 2. Trolox, a positive control, eliminated $63.97 \pm 1.73\%$ of lipid peroxidase at 100 μ g/mL. PTSE, OIE, and SGE showed moderate lipid peroxidation inhibitory activity, with lipid peroxidation inhibition of $32.47 \pm 0.71\%$ for PTSE, $30.28 \pm 0.65\%$ for OIE, and $31.44 \pm 1.83\%$ for SGE. In

agreement with a previous study, OIE was able to alleviate myocardial lipid peroxidation levels in doxorubicin-induced cardiac damage rats.²⁰ However, FRE had the lowest inhibition of lipid peroxidation with a percentage of 10.05 ± 1.68%. A previous study found that FRE was potent in inhibiting lipid peroxidation in liposomes containing

egg phosphatidylcholine with an IC₅₀ of 0.61 µg/mL.²¹ The results from the present study differ from that previous study. The difference in the contrast effect may be due to the difference in lipid source. In our study, rat brain homogenate was used as a lipid source, but a previous study used egg phosphatidylcholine as a lipid source.

Table 1 Total phenolic contents and DPPH free radical scavenging activity of PTS extract

Sample	% Yield of an extract	Total phenolic content (mg GAE/g extract)	IC ₅₀ against DPPH scavenging (mg/mL)
Aqueous extract			
PTSA	4.52	12.55 ± 2.83	650.10 ± 98.20
OIA	1.81	6.62 ± 1.33	ND
SGA	1.46	11.80 ± 1.60	ND
FRA	2.26	31.01 ± 2.68	ND
95% Ethanol extract			
PTSE	2.44	79.57 ± 2.33	80.69 ± 0.40
OIE	0.76	60.49 ± 1.75	ND
SGE	1.59	17.57 ± 1.11	ND
FRE	1.51	20.13 ± 2.96	ND
BHT (Positive control)		ND	0.006 ± 0.000

Abbreviations: BHT; butylated hydroxytoluene, FRA; *F. racemosa* aqueous extract, FRE; *F. racemosa* ethanolic extract, OIA; *O. indicum* aqueous extract, OIE; *O. indicum* ethanolic extract, PTSA; *Pikad Trisukkhathi Samutharn* aqueous extract, PTSE; *Pikad Trisukkhathi Samutharn* ethanolic extract, SGA; *S. grandiflora* aqueous extract, SGE; *S. grandiflora* ethanolic extract, ND; Not determined.

Table 2 The percentage of lipid peroxidation inhibition of the PTSE and its herbal components, including *O. indicum* ethanolic extract (OIE), *S. grandiflora* ethanolic extract (SGE), and *F. racemosa* ethanolic extract (FRE), at a final concentration of 100 µg/mL

Sample	% Lipid peroxidation inhibition
PTSE	32.47 ± 0.71
OIE	30.28 ± 0.65
SGE	31.44 ± 1.83
FRE	10.05 ± 1.68
Trolox (Positive control)	
	63.97 ± 1.73

GC-MS chemical profiling of PTSE

The chemical profiling of PTSE was conducted by GC-MS analysis and the GC-MS chromatograms are presented in Figure 1. All data, including the name of the compound, retention time, CAS number, molecular formula, and molecular weight (MW) are shown in Table 3. The chemical profile of PTSE was also compared to the chemical profiles of the three sample herbal plants to identify the source of the compound found in PTSE. The results revealed the presence of 34 compounds identified in PTSE, of which 16 compounds were detected in OIE, 28 compounds were found in SGE, and 23 compounds were present in FRE.

The phytochemical composition using GC-MS analysis of *O. indicum* root extract in various solvents, including water, n-butanol, chloroform, and ethyl acetate was identified in a previous study.²² Nonadecane, eicosane, n-hexadecanoic acid, squalene, oroxilin, and beta-sitosterol have also been detected in *O. indicum* root extracts from previous studies.^{22,23} Also, the compounds identified by GC-MS analysis of *S. grandiflora* leaves²⁴ and *F. racemosa* fruit²⁵ extracts have previously been investigated. However, no study on the GC-MS analysis of *S. grandiflora* and *F. racemosa* root extracts has been reported.

Most of the identified compounds were phenolic compounds, of which nineteen compounds have previously been investigated for their pharmacological activities (Table 4), and several of these compounds have been reported to possess antioxidation effects, including vanillin,^{26,27} 2,4-di-tert-butylphenol,²⁸ hexadecanoic acid,²⁹ homopterocarpin,³⁰ squalene,³¹ beta-sitosterol,³² and beta-amyrin³³ which may correspond to the antioxidation activity of PTSE found in our study. Some compounds in PTSE have antipyretic and anti-inflammatory effects including pentadecane,³⁴ eicosane,³⁴ n-hexadecanoic acid,³⁵ oroxilin A,³⁶ beta-amyrin,³³ beta-amyrone,³⁷ and lupeol,³⁸ which may

be related to the traditional use of PTS for the treatment of severe fever and inflammation.

Conclusion

In conclusion, this study demonstrated the antioxidation activity of PTS extract for the first time, and the results showed that extraction of PTS with ethanol resulted in stronger antioxidation than extraction with water. The results also provide evidence that extraction with ethanol is more appropriate than extraction with water for extracting phenolic compounds from PTS. The stronger antioxidant activity of the PTS ethanolic extract than that of the PTS aqueous extract may be due to the higher phenolic compound content. Ethanolic extracts of PTS, *O. indicum*, and *S. grandiflora* also exhibited moderate inhibitory activity against lipid peroxidation. Therefore, this study suggests that PTS should be prepared by maceration in ethanol to achieve a higher efficiency. The GC-MS chemical profiling was demonstrated, and PTS was found to contain several phenolic compounds in which the identified compounds have been reported to have antioxidation, anti-inflammation, and antimicrobial activities. Some tentatively identified compounds might be responsible for the antioxidant properties of the PTS extracts, and further studies should be investigated. However, the qualification analysis and linear retention index calculation for the tentatively identified compounds were limited in this study, and the major constituents were not identified. Also, GC-MS is limited to analytes with only volatile and thermally labile molecules and is limited to analytes with highly polar compounds owing to their low volatility and thermolability. Therefore, further studies should consider other chromatography techniques, such as liquid chromatography-mass spectrometry (LC-MS), for the phytochemical analysis of PTS. Likewise, further exploration related to the traditional use of PTS should be undertaken.

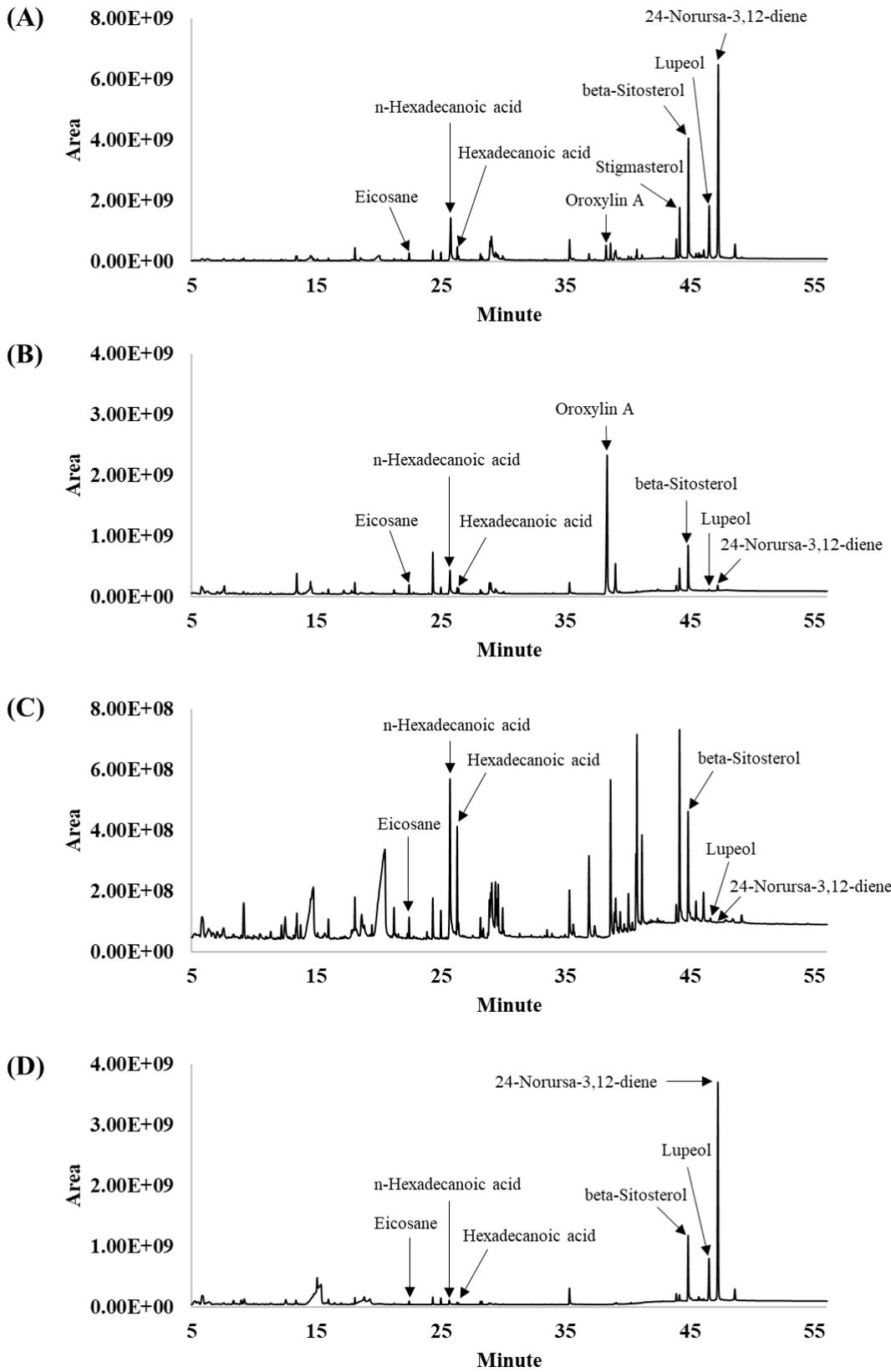


Figure 1 GC-MS chromatograms of (A) PTSE, (B) OIE, (C) SGE, and (D) FRE at a concentration of 10 mg/mL

Table 3 Tentatively identified compounds in the PTSE extract by GC-MS analysis

No	R/T	Name of the compound	CAS no.	Molecular Formula	MW (g/mol)	R match	Also found in		
							OIE	SGE	FRE
1	8.37	Catechol	120-80-9	C ₆ H ₆ O ₂	110	888			✓
2	9.00	Benzofuran, 2,3-dihydro-	496-16-2	C ₈ H ₈ O	120	902			✓
3	9.18	m-Guaiacol	150-19-6	C ₇ H ₈ O ₂	124	943		✓	
4	10.02	Nonanoic acid	112-05-0	C ₉ H ₁₈ O ₂	158	880		✓	✓
5	12.22	Phenol, 2,6-dimethoxy-	91-10-1	C ₈ H ₁₀ O ₃	154	916		✓	
6	13.46	Vanillin	121-33-5	C ₈ H ₈ O ₃	152	963	✓	✓	✓
7	16.00	2,4-Di-tert-butylphenol	96-76-4	C ₁₄ H ₂₂ O	206	951	✓	✓	✓
8	18.13	Nonadecane	629-92-5	C ₁₉ H ₄₀	268	947		✓	
9	22.47	Eicosane	112-95-8	C ₂₀ H ₄₂	282	954	✓	✓	✓
10	24.37	Lidocaine	137-58-6	C ₁₄ H ₂₂ N ₂ O	234	959	✓		✓
11	25.81	n-Hexadecanoic acid	57-10-3	C ₁₆ H ₃₂ O ₂	256	961	✓	✓	✓
12	26.34	Hexadecanoic acid, ethyl ester	628-97-7	C ₁₈ H ₃₆ O ₂	284	883	✓	✓	✓
13	26.44	Tetracosane	646-31-1	C ₂₄ H ₅₀	338	955		✓	✓
14	28.96	9,12-Octadecadienoic acid	60-33-3	C ₂₂ H ₄₄ O ₂	280	904		✓	✓
15	29.98	Eicosanoic acid, ethyl ester	18281-05-5	C ₂₇ H ₅₆	340	837			✓
16	30.07	Heptacosane	593-49-7	C ₂₄ H ₂₆ BrO ₈	380	896		✓	
17	35.65	Homopterocarpin	606-91-7	C ₁₆ H ₁₄ O ₄	284	832		✓	
18	36.90	9-Methoxy-6a,11a-dihydro-6H-benzofuro[3,2-c] chomen-3-ol	607363-34-8	C ₂₀ H ₁₆ O ₇	270	942		✓	
19	38.26	Oroxilin A, diacetate	981-92-0	C ₂₀ H ₁₆ O ₇	368	812	✓		
20	38.62	3-(2,4-dimethoxyphenyl) choman-7-ol	71831-00-0		286	939		✓	✓
21	38.96	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	3443-82-1	C ₁₆ H ₁₆ O ₄	354	883	✓	✓	✓
22	40.06	2H-1-Benzopyran-7-ol, 3,4-dihydro-3-(2-hydroxy-4-methoxyphenyl)-	56701-24-7	C ₃₀ H ₅₀	272	930		✓	
23	40.29	Squalene	111-02-4	C ₁₆ H ₁₂ O ₄	410	953		✓	✓
24	40.65	4H-1-Benzopyran-4-one, 7-hydroxy-3-(4-methoxyphenyl)-	485-72-3	C ₁₇ H ₁₄ O ₅	268	935		✓	
25	40.74	6,4'-Dimethoxy-7-hydroxyisoflavone	550-79-8	C ₂₉ H ₅₀ O ₂	298	802		✓	
26	43.92	Campesterol	474-62-4	C ₂₉ H ₄₈ O	400	903	✓	✓	✓
27	44.17	Stigmasterol	83-48-7	C ₂₉ H ₅₀ O	412	891	✓	✓	✓
28	44.88	beta-Sitosterol	83-46-5	C ₂₉ H ₄₆	414	910	✓	✓	✓
29	45.11	24-Noroleana-3,12-diene	201358-24-9	C ₃₀ H ₅₀ O	394	856	✓	✓	✓
30	45.49	beta-Amyrin	559-70-6	C ₃₀ H ₄₈ O	426	924		✓	
31	45.72	beta-Amyrone	638-97-1	C ₃₂ H ₅₂ O ₂	424	843	✓	✓	✓
32	45.88	9,19-Cyclolanost-24-en-3-ol, acetate, (3 beta.)-	1259-10-5	C ₃₀ H ₅₀ O	468	898	✓		✓
33	46.11	Lupeol	545-47-1	C ₂₉ H ₄₆	426	865	✓	✓	✓
34	47.27	24-Norursa-3,12-diene	201358-25-0	C ₂₉ H ₄₆	394	878	✓	✓	✓

Abbreviations: FRE; *F. racemosa* ethanolic extract, OIE; *O. indicum* ethanolic extract, SGE; *S. grandiflora* ethanolic extract.

Table 4 Bioactivity of tentatively identified compounds in the PTSE

No.	Name of the compound	Nature of compound	Biological activity
1	Catechol	Phenolic compound	Antibacterial, Antifungal ³⁹
2	Benzofuran, 2,3-dihydro-	Phenolic compound	<i>Chlamydia trachomatis</i> inhibition ⁴⁰
3	Nonanoic acid	Saturated fatty acid	Antimicrobial, ⁴¹ Anti-epileptics ⁴²
4	Pentadecane	Alkane compound	Anti-inflammatory, Analgesic, Antipyretic, ³⁴ Antileishmanial activity, ⁴³ Antimicrobial ⁴⁴
5	Vanillin	Aldehyde compound	Antioxidation, ^{26, 27} Antitumor, ⁴⁵ Antifungal, ⁴⁶ Antimicrobial, ⁴⁷
6	2,4-Di-tert-butylphenol	Phenolic compound	Antifungal, ^{28,48} Antioxidation ²⁸
7	Eicosane	Alkane compound	Anti-inflammatory, Analgesic, Antipyretic, ³⁴ Antifungal, ⁴⁹ Antitumor ⁵⁰
8	Lidocaine	Amino amide	Local anesthetic, ⁵¹ Anticancer ⁵²
9	n-Hexadecanoic acid	Fatty acid	Anti-inflammatory ³⁵
10	Hexadecanoic acid, ethyl ester	Ester	Antioxidation and Antibacterial ²⁹
11	Homopterocarpin	Flavonoid compound	Anticancer, ⁵³ Antiulcerogenic, ⁵⁴ Antioxidation, and hepatoprotection ³⁰
12	Oroxylin A	Flavonoid	Anti-inflammatory, Anti-bacterial, Anti-viral, Anticancer ³⁶
13	Campesterol	Phytosterol	Antiangiogenic ⁵⁵
14	Squalene	Triterpene	Antioxidation, Antitumor ³¹
15	Stigmasterol	Phytosterol	Anti-osteoarthritic, ⁵⁶ Anticancer, ^{57, 58} Anti-inflammatory ⁵⁹
16	β -Sitosterol	Phytosterol	Antioxidation, Anticancer, Anti-diabetic, Antimicrobial, Immunomodulatory activities, ³² Antidiabetic ⁵⁹
17	β -Amyrin	Triterpene	Antihyperglycemic, hypolipidemic, ⁶⁰ Anti-inflammatory, ⁶¹ Antioxidation, ³³ Antifungal ⁶²
18	β -Amyrone	Triterpene	Anti-inflammatory ³⁷
19	Lupeol	Triterpene	Anticancer, Antiprotozoal, Chemopreventive, and Anti-inflammatory ³⁸

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