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*Original Research Article*

# The Effect of Solvent Extraction on Chemical Composition, Antioxidant, Alpha Glucosidase and Nitric Oxide Inhibitor Activities of Jindamanee, a Thai Traditional Formulation

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ABSTRACT

Jindamanee is a traditional Thai medicine from the Ayutthaya era, historically used to treat various diseases. In this study, the 3 Jindamanee extracts (hexane, $CH_2Cl_2$ and EtOH) were evaluated for antioxidant, alpha glucosidase, and nitric oxide inhibitory activities. The chemical composition was determined by Gas Chromatography Mass Spectrometry (GCMS) and High Performance Liquid Chromatography (HPLC). The result showed that the $CH_2Cl_2$ extract exhibited the highest antioxidant activity, followed by the EtOH and hexane extracts. For alpha glucosidase inhibitory activity, the $CH_2Cl_2$ extract showed an $IC_{50}$ values of 34.21±0.16 µg/mL when compared with the standard drug acarbose ($IC_{50}$ = 194.56±2.32 µg/mL), while EtOH and hexane extracts showed low activity. In addition, the $CH_2Cl_2$ extract showed a higher nitric oxide inhibition in a dose-dependent manner than EtOH and hexane extracts. Moreover, $CH_2Cl_2$ and EtOH extract showed a higher total flavonoid content (TFC) and total phenolic content (TPC), respectively. GC-MS analysis identified isoborneol and endo-borneol as major compounds in all three extracts. HPLC analysis revealed that the quercetin, sinapic acid and hydroxybenzoic acid were the major phenolic compounds in the hexane, $CH_2Cl_2$ and EtOH extracts, respectively. Therefore, this Jindamanee formulation has the potential for the treatment of diabetes and inflammatory diseases.

**Keywords:** Jindamanee, Antioxidant, Alpha glucosidase, Nitric oxide, Chemical composition

## Introduction

Traditional medicine was a medicine based on indigenous knowledge and has significantly influenced modern medicine, which is the importance of preserving medicinal plant-based healing practices.[5] The bioactive compounds were found in medicinal plant, mainly secondary metabolites. Its showed significant health benefits and its have contributed to about one-third of modern medicines. Moreover, bioactive compounds showed fewer side effects and treatment of various chronic diseases.[11] In Thailand, traditional medicine is a primary medical system. The Thai Traditional Medicine was influenced by Ayurveda, Chinese medicine and Buddhism.[19 34] The main precept of treatment is the balancing and harmonization of the four body elements: earth, water, wind, and fire, using herbal medicines, massage, and health maintenance practices.[7] In Thai traditional remedies, there are showed several biological activities such as antipyretic, [24] antibacterial, anti-inflammatory and antioxidant activities.[8]

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However, there are fewer reports on Thai traditional medicine formulations, especially the Jindamanee formulation, which has not been studied for its pharmacological activities and chemical composition. Therefore, Jindamanee formulation was extracted with hexane, $CH_2Cl_2$ and EtOH. The extracts were analyzed for antioxidant, alpha-glucosidase, and nitric oxide inhibitory activities. In addition, the chemical composition was investigated by Gas Chromatography Mass Spectrometry (GCMS) and High Performance Liquid Chromatography (HPLC).

## Materials and Methods

*Chemicals and Reagents*

1,1-diphenyl-2-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman carboxylic acid (Trolox), 2,4,6-tris(2-pyridyl)-S-triazine (TPTZ), and Folin-Ciocalteu reagent were purchased from Sigma-Aldrich (Buchs, Switzerland). 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), Gallic acid, and Quercetin were purchased from Sigma-Aldrich (Wuxi, China). Potassium persulfate ($K_2S_2O_8$), Glacial acetic acid, Ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), and Sodium Carbonate ($Na_2CO_3$) were purchased from Sigma-Aldrich (Hamburg, Germany). Sodium acetate (NaOAc) was purchased from Sigma-Aldrich (Bangalore, India). Hydrochloric acid (HCl) was purchased from Carlo Erba (Milan, Italy). Iron (II) sulfate ($FeSO_4$) was purchased from Thermo Scientific (Mumbai, India). Aluminium chloride ($AlCl_3$) was purchased from QReC™ (New Zealand). Acarbose was purchased from Fujifilm

Wako Pure Chemical corporation (Osaka, Japan). Di-sodium hydrogen phosphate anhydrous ($Na_2HPO_4$), and Sodium dihydrogen phosphate ($NaH_2PO_4$) were purchased RCI Labscan (Bangkok, Thailand). p-nitrophenyl-α-D-glucopyranoside (p-NPG) was purchased from Tokyo Chemical Industry (Telangana, India). Alpha-glucosidase (EC 3.2.1.20, cas: 9001-42-7) was purchased from Megazyme (Wicklow, Ireland). Dimethyl sulfoxide (DMSO) was purchased Merck (Darmstadt, Germany). Gallic acid (GA), protocatechuic acid (PCCA), p-hydroxybenzoic acid (p-HO), chlorogenic acid (ChA), vanillic acid (VA), p-coumaric acid (p-CA), ferulic acid (FA) and sinapic acid (SnA), syringic acid (SyA), rutin (RN) were products of Sigma Aldrich (St. Louis, MO, USA). 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) was purchased from Invitrogen (Eugene, Oregon, USA). Lipopolysaccharides (LPS) from Escherichia coli 0111:B4, sulfanilamide and naphthylethylenediamine dihydrochloride were purchased from Sigma-Aldrich (St. Louis, MO, USA). The n-hexane, dichloromethane ($CH_2Cl_2$), and ethanol (EtOH) were purchased from ACI Labscan (Bangkok, Thailand).

*Herbals Materials*

The medicinal herbs used in Jindamanee formulation were purchased from Chao Krom Poe Co., Ltd., (GPS coordinates: 13°44'32.9"N, 100°30'03.0"E) Bangkok, Thailand. The herbal materials were identified by Asst. Prof. Dr. Supalak Fakkham, an applied Thai traditional medicine. The voucher specimens of herbals materials which are *Nelumbo nucifera* pollen (YS2024-0001), *Kaempferia galanga* rhizome (YS2024-0002), *Myristica fragrans* placenta (YS2024-0003), *Acmella oleracea* flower (YS2024-0004), *Angelica dahurica* root (YS2024-0005), *Atractylodes lancea* root (YS2024-0006), *Crateva religiosa* bark (YS2024-0007), *Cissampelos pareira* (YS2024-0008), Styrax benzoin rubber (YS2024-0009), *Aquilaria crassna* core (YS2024-0010), *Citrus aurantifolia* fruit juice (YS2024-00011) and *Solanum aculeatissimum* fruit juice (YS2024-00012). The voucher specimen was deposited in the Department of Thai Traditional Medicine, Siridhorn Colllege of Public Health.

*Jindamanee extracts Preparation*

Jindamanee formulation was consist of *N. nucifera* pollen (500 g), *K. galanga* rhizome (500 g), *M. fragrans* placenta (500 g), *A. oleracea* flower (500 g), *A. dahurica* root (500 g), *A. lancea* root (500 g), *C. religiosa* bark (500 g), *C. pareira* (500 g), Styrax benzoin rubber (500 g), Sodium borate (500 g), Camphor (500 g), *A. crassna* core (500 g), Honey (1 L), Civet musk (15 g), *C. aurantifolia* fruit juice (1 L) and *S. aculeatissimum* fruit juice (1 L). (Sarikabutra, 1983). The Jindamanee formulation powder 100 g was maceration with 3 solvents which are hexane 300 mL, dichloromethane ($CH_2Cl_2$) 300 mL, and ethanol (EtOH) 300 mL. The extraction method used an ultrasonic cleaner (TRU-SWEEPTM, NY, USA) at 50/60 Hz and 100 watts for 30 minutes (3 replicated). The extracts were filtered with Whatman No.1 filter paper and solvent was reduced by vacuum evaporator (45°C) to obtain hexane extract (5.52%), $CH_2Cl_2$ extract (9.87%) and EtOH extract (31.46%).

*Antioxidant activity*

*1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay*

The different concentrations of extracts (5-1000 µg/mL) were mixed with 200 µM DPPH into 96-well plates in a ratio 1:1. The mixture was incubated at room temperature for 30 min in the dark. After incubation, absorbance was read at 517 nm using a microplate reader (SPECTROstar Nano, Ortenberg, Germany). $IC_{50}$ was determined as the concentration of extract that was capable of causing a 50% inhibition of DPPH and Trolox was used as the positive control. The assay was carried out in triplicate.[7]

*2,2-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assay*

The 28 mM ABTS and 2.45 mM potassium persulfate was prepared in deionized water for 18 hr. before used. The extract 5-1000 µg/mL were mixed with ABTS radical solution into 96-well plates in a ratio 1:1. The mixture was incubated in the dark for 10 minutes. The absorbance was measured at a wavelength of 734 nm using a microplate reader (SPECTROstar Nano, Ortenberg, Germany). $IC_{50}$ was determined as the concentration of extract that was capable of causing a 50% inhibition of ABTS. Trolox was used a positive control. The assay was carried out in triplicate. [30]

*Ferric reducing antioxidant power (FRAP) assay*

The FRAP reagent was prepared by mixing 300 mM acetate buffer pH 3.6, 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3$ solution in ratio 10:1:1. The extract (1000 µg/mL) was mixed with FRAP reagent into 96-well plates. The mixture was incubated in the dark condition for 4 minutes. The absorbance was measured at 595 nm using a microplate reader (SPECTROstar Nano, Ortenberg, Germany). Trolox was used a positive control. The results were expressed in units of mmol $Fe^{2+}$/100 g extract. The assay was carried out in triplicate. [28]

*Alpha glucosidase inhibitory activity*

The extract (25-1000 µg/mL) 50 µL were mixed with α-glucosidase 0.5 U/mL) 15 µL into 96-well plates and 100 mM phosphate buffer (pH 6.8) 20 µL was added, incubated for 10 min at 37°C. After that, 5 mM p-nitrophenyl-α-D-glucopyranoside (p-NPG) 15 µL was added and incubated for 15 min at 37°C. The reaction was stopped by 1 M $Na_2CO_3$ 150 µL. The absorbance was measured at 405 nm using a microplate reader (Ensight, Perkin Elmer Inc., Waltham, MA, USA). $IC_{50}$ was determined as the concentration of samples that was capable of causing a 50% inhibition of α-glucosidase and acarbose was used a positive control. The assay was carried out in triplicate.[25]

*Cell culture*

RAW 264.7 cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS and 1% penicillin-streptomycin. The RAW 264.7 cells were cultured at 37˚C with 5% $CO_2$.

*Cell viability by MTT assay*

RAW 264.7 cells ($2 \times 10^4$ cells/well) were treated with extracts at the various concentrations (31.25-1000 µg/mL) in a 96-well plate and incubated for 24 h. After that, The cells were washed with 1X PBS and 0.5 mg/mL MTT was added and incubated for 3 h. The culture medium was removed, and the cells were dissolved in DMSO. The absorbance was measured at 570 nm using a microplate reader (Ensight, Perkin Elmer Inc., Waltham, MA, USA). The assay was carried out in triplicate.[26]

*Nitric oxide inhibitory assay*

RAW 264.7 cells ($2 \times 10^4$ cells/well) were seeded into 96-well plate. The cells were treated with extracts at the various concentrations (31.25-250 µg/mL) in the presence of 1 µg/mL of lipopolysaccharide (LPS) and incubated for 24 h. After that, the supernatant of cultured medium was collected to determine the NO production. Briefly, the mixture of cell culture medium and of Griess reagent (1% sulfanilamide and 0.1% naphthylethylenediamine dihydrochloride in 2.5% phosphoric acid) was incubated at room temperature for 10 min, and the absorbance was then measured at 550 nm by microplate reader (Ensight, Perkin Elmer Inc., Waltham, MA, USA). The quantity of nitrite was determined from a sodium nitrite standard curve. The assay was carried out in triplicate.[26]

*Total phenolic content*

The 10%Folin-Ciocalteu's reagent was diluted with distilled water before use. The sample (1000 µg/mL) 20 µL was mixture 10% Folin-Ciocalteu's reagent 100 µL into 96-well plates. After 5 min, 7% $Na_2CO_3$ 80 µL was added and incubated for 30 minutes at room temperature in the dark. The absorbance was measured at a wavelength of 760 nm using a microplate reader (SPECTROstar Nano, Ortenberg, Germany). The total phenolic content was shown as gallic acid equivalents (mg GAE/g extract). The assay was carried out in triplicate.[35]

*Total flavonoid content*

The extract (1000 µg/mL) and 2%$AlCl_3$ were mixed into 96-well plate in ratio 1:1 and incubated for 20 minutes. After incubation, the absorbance was measured at a wavelength of 415 nm (SPECTROstar Nano, Ortenberg, Germany). The total flavonoid content was shown as

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quercetin equivalents (mg QE/g extract). The assay was carried out in triplicate.[35]

*Chemical composition analysis by Gas Chromatography Mass Spectrometry (GCMS)*

The GC-MS SHIMADZU QP-2010 instrument (Kyoto, Japan) was used for analyzed the chemical of Jindamanee extracts. The sample 1 µL was injected into Agilent J&W DB-5MS column (30 m × 0.25 mm, 0.25 µm, Santa Clara, CA, USA) with a flow rate was 1 mL/min and helium 5.5 was a carrier gas. The split ratio was set to 1:20. The column temperature was programmed as follows: starting at 40°C (hold time 2 min), with gradual increases in temperature from 40°C to 100°C (5.0°C/min, hold time 5 min), from 100°C to 150°C (5.0°C/min, hold time 10 min), from 150°C to 200°C 5.0°C/min, hold time 10 min), from 200°C to 250°C (5.0°C/min, hold time 5 min), and from 250°C to 320°C 5.0°C/min, hold time 15 min). The peaks were analyzed based on GC retention time and mass spectral identity was by comparison to the NIST17.LIB library.[22]

*Chemical content analysis by High Performance Liquid Chromatography (HPLC)*

The HPLC (Shimadzu Co., Kyoto, Japan) was used for the analyzed the Hydroxybenzoic acid 5 compounds [gallic acid (GA), protocatechuic acid (PCCA), p-hydroxybenzoic acid (p-HO), vanillic acid (VA) and syringic acid (SyA)]. Hydroxycinnamic acid 4 compounds [chlorogenic acid (ChA), p-coumaric acid (p-CA), ferulic acid (FA) and sinapic acid (SnA)] and Flavonoids 2 compounds [rutin (RN) and quercetin (QE)]. The sample 10 µL was injected into C18 column (250 × 4.6 mm, 5 µm, Unisol, Bonna-Agela Technologies, USA) with flow rate at 0.8 mL/min. The mobile phase consists of 1% acetic acid in deionized water (solvent A) and acetonitrile (solvent B) for gradient program from 0 to 5 min, 5% solvent B; from 5-15 min, 9% solvent B; from 15-22 min, 11% solvent B; from 22-38 min, 18% solvent B; from 38-43 min, 23% solvent B; from 43-44 min, 90% solvent B; from 45-70 min, 5% solvent B. The respective UV-diode array detection wavelengths were 280 nm for hydroxybenzoic acids and 320 nm for hydroxycinnamic acids. Phenolics and flavonoids were identified as equivalents of the forementioned standards. The assay was carried out in triplicate.[29]

*Statistical analysis*

The data was presented as mean ± standard deviation. The statistical analysis was done by one-way ANOVA with Tukey HSD to compare the differences between sample groups. The differences were considered to be significant at p<0. 05 using SPSS 23.0 software for Windows. (SPSS Inc., Chicago, IL, USA).

## Results and Discussion

*Antioxidant activity*

The antioxidant potential of the Jindamanee extracts was systematically evaluated using three distinct assays: DPPH, ABTS, and FRAP (Table 1). A statistically significant finding was that the $CH_2Cl_2$ extract exhibited the highest antioxidant activity across all three methods. Specifically, it demonstrated the most potent DPPH radical scavenging capacity ($IC_{50}$ = 175.71±2.27 µg/mL), ABTS ($IC_{50}$ = 53.70±0.18 µg/mL) and FRAP (42.80±3.87 mmol $Fe^{2+}$/100 g extract), the strongest ABTS radical scavenging ($IC_{50}$ = 53.70±0.18 µg/mL), and the highest ferric reducing power FRAP (42.80±3.87 mmol $Fe^{2+}$/100 g extract).

**Table 1:** Antioxidant activity of Jindamanee extract

| Solvents | DPPH; $IC_{50}$ (µg/mL) | ABTS; $IC_{50}$ (µg/mL) | FRAP (mmol $Fe^{2+}$/100 g extract) |
|---|---|---|---|
| Hexane | 348.69±5.79$^b$ | 150.86±4.97$^c$ | 25.81±1.69$^b$ |
| $CH_2Cl_2$ | 175.71±2.27$^b$ | 53.70±0.18$^b$ | 42.80±3.87$^b$ |
| EtOH | 219.44±4.38$^b$ | 55.93±0.81$^b$ | 20.08±1.95$^b$ |
| Trolox | 8.42±0.04$^a$ | 6.47±0.16$^a$ | 795.87±53.95$^a$ |

Note: Letters indicate the significant differences in data between rows in the same columns using one-way ANOVA with Tukey HSD in the same extract ($p < 0.05$).

In contrast, the Hexane and EtOH extracts showed significantly lower antioxidant capacities compared to the $CH_2Cl_2$ extract. While Trolox, served as the standard antioxidant control, it is noteworthy that the Jindamanee extract's ABTS inhibition surpassed that of other Thai traditional medicines, such as the Phikud Benjakot and Phikud Benjathien formulations, which are used to relieve flatulence and fever.[7]

*Alpha glucosidase inhibitory activity*

In the alpha glucosidase inhibitory assay, the $CH_2Cl_2$ extract was identified as the most potent inhibitor, with an $IC_{50}$ value of 34.21±0.16 µg/mL (Table 2). This activity was significantly stronger than that of the EtOH extract ($IC_{50}$ = 49.81±0.23 µg/mL) and the Hexane extract ($IC_{50}$ >200 µg/mL). Notably, the $CH_2Cl_2$ extract demonstrated more potent inhibition than acarbose, the standard antidiabetic drug used as a positive control. This finding is particularly significant, suggesting a strong potential for hyperglycemia management. Furthermore, the potency of the $CH_2Cl_2$ extract ($IC_{50}$ = 34.21 µg/mL) was also superior to other traditional remedies, such as the Krom Luang Chumphon Khet Udomsak remedy ($IC_{50}$ = 46.80 µg/mL), recently studied for similar purposes.[18]

**Table 2:** The α-glucosidase inhibitory activity of Jindamanee extract

| Solvents | α-glucosidase inhibitory activity; $IC_{50}$ (µg/mL) |
|---|---|
| Hexane | >200$^d$ |
| $CH_2Cl_2$ | 34.21±0.16$^a$ |
| EtOH | 49.81±0.23$^b$ |
| Acarbose | 194.56±2.32$^c$ |

Note: Letters indicate the significant differences in data between rows in the same columns using one-way ANOVA with Tukey HSD in the same extract ($p < 0.05$).

*Nitric oxide inhibitory activity*

Nitric oxide (NO) is a critical signaling molecule involved in host defense against pathogens [17] and cytoprotective processes.[9] However, it possesses both pro- and anti-inflammatory properties, and excessive production can cause tissue damage. The modulation of NO synthase (NOS) inhibitors can therefore ameliorate inflammatory responses.[14] In this study, all Jindamanee extracts demonstrated a dose-dependent inhibition of NO release in LPS-stimulated RAW 264.7 cells over the concentration range of 31.25 to 250 µg/mL (Figures 1 and 2). A detailed comparison of the inhibitory percentages reveals that the Hexane extract exhibited the most significant NO inhibitory activity, with inhibition ranging from 25.41% (at 31.25 µg/mL) to 43.38% (at 250 µg/mL). This was followed by the EtOH extract (17.21% to 42.16%). The $CH_2Cl_2$ extract showed the most modest activity in this assay (15.23% to 38.11%). Significantly, all Jindamanee extracts demonstrated superior inhibitory activity compared to other reported Thai traditional medicines, such as the Pok-Kao formulation for osteoarthritis[15] and another polyherbal formulation.[12]

*Total phenolic and total flavonoid content*

The total phenolic (TPC) and total flavonoid (TFC) contents varied significantly based on the extraction solvent (Table 3).

**Table 3:** Total phenolic and total flavonoid content of Jindamanee extract

| Solvents | TPC (mg GAE/g extract) | TFC (mg QE/g extract) |
|---|---|---|
| Hexane | 12.84±1.26$^c$ | 34.08±0.34$^c$ |
| $CH_2Cl_2$ | 36.18±0.16$^b$ | 82.49±1.68$^a$ |
| EtOH | 41.84±1.59$^a$ | 57.50±0.20$^b$ |

Note: Letters indicate the significant differences in data between rows in the same column using one-way ANOVA with Tukey HSD in the same extract ($p<0.05$)

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**Table 4:** Chemical composition of Jindamanee extract by GCMS

| Peak No. | Retention time (min) | MW | Formula | Compound name | %Peak Area | | |
|---|---|---|---|---|---|---|---|
| | | | | | Hexane | $CH_2Cl_2$ | EtOH |
| 1 | 3.03 | 127 | $C_8H_{17}N$ | (S)-(+)-1-Cyclohexylethylamine | 0.04 | | |
| 2 | 8.27 | 136 | $C_{10}H_{16}$ | Sabinene | 0.08 | | |
| 3 | 12.52 | 154 | $C_{10}H_{18}O$ | Isofenchol | 0.45 | 0.33 | 0.19 |
| 4 | 12.65 | 154 | $C_{10}H_{18}O$ | Fenchol | 0.29 | 0.24 | |
| 5 | 13.48 | 152 | $C_{10}H_{16}O$ | Camphor | 0.15 | 0.13 | 0.13 |
| 6 | 13.73 | 154 | $C_{10}H_{18}O$ | Camphene hydrate | 0.12 | | |
| 7 | 13.99 | 154 | $C_{10}H_{18}O$ | Isoborneol | 37.19 | 39.26 | 37.56 |
| 8 | 14.26 | 154 | $C_{10}H_{18}O$ | endo-Borneol | 56.83 | 59.31 | 58.52 |
| 9 | 15.13 | 154 | $C_{10}H_{18}O$ | Isothujol | 0.13 | | |
| 10 | 26.50 | 222 | $C_{15}H_{26}O$ | Agarospirol | 0.11 | | |
| 11 | 26.52 | 204 | $C_{15}H_{24}$ | Alloaromadendrene | | | 0.19 |
| 12 | 26.85 | 222 | $C_{15}H_{26}O$ | β-Selinenol | 0.92 | | 1.73 |
| 13 | 72.51 | 448 | $C_{28}H_{45}ClO_2$ | Cholesteryl chloroformate | 0.11 | | |
| 14 | 74.20 | 3540 | $C_{29}H_{54}O_6$ | Decanoic acid | 0.64 | | |
| 15 | 78.40 | 426 | $C_{30}H_{50}O$ | Lupeol | | 0.31 | 0.91 |
| Total | | | | | 97.06 | 99.58 | 99.23 |

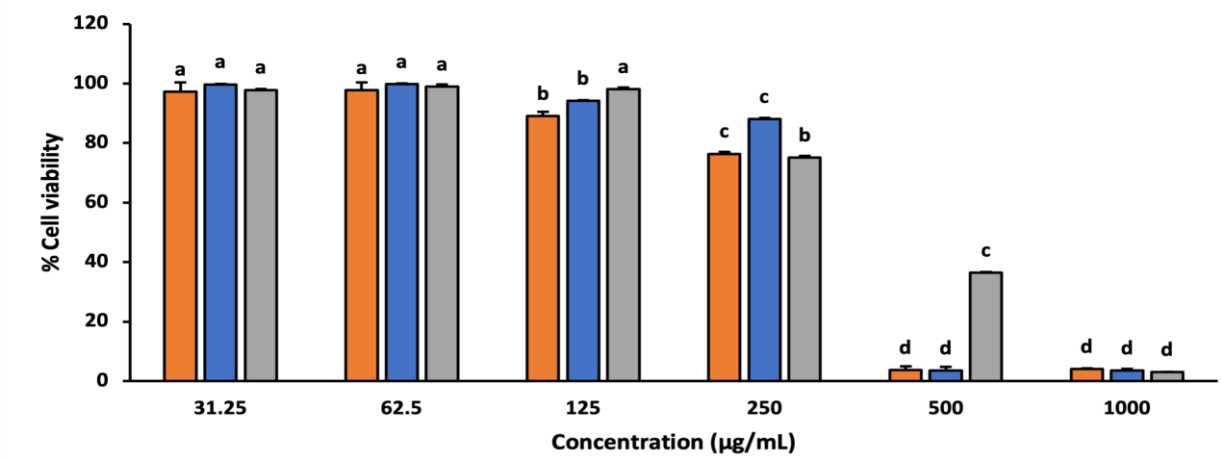


**Figure 1:** The effects of solvent of cell viability on RAW 264.7 cell of Jindamanee hexane extract (■), $CH_2Cl_2$ extract (■) and EtOH extract (■). Letters indicate the significant differences using one-way ANOVA with Tukey HSD in the same extract ($p < 0.05$).

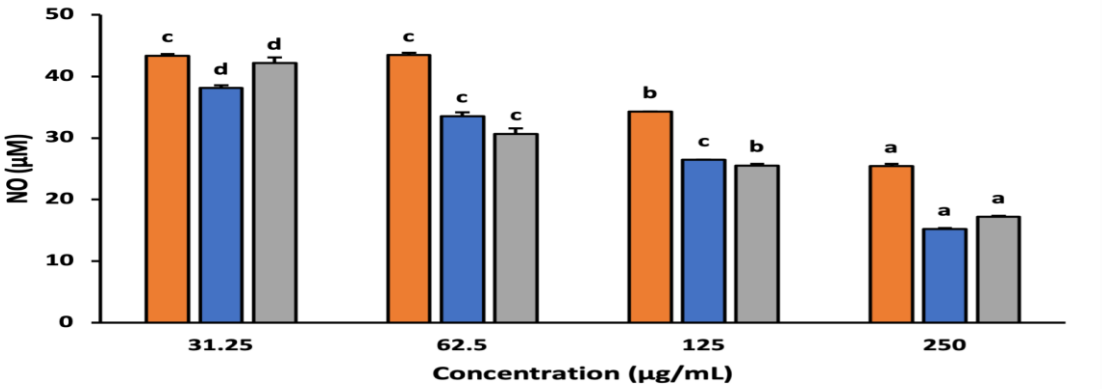


**Figure 2:** The effects of solvent on the production of nitric oxide in LPS-stimulated RAW 264.7 cells of Jindamanee hexane extract (■), $CH_2Cl_2$ extract (■) and EtOH extract (■). Letters indicate the significant differences using one-way ANOVA with Tukey HSD in the same extract ($p < 0.05$).

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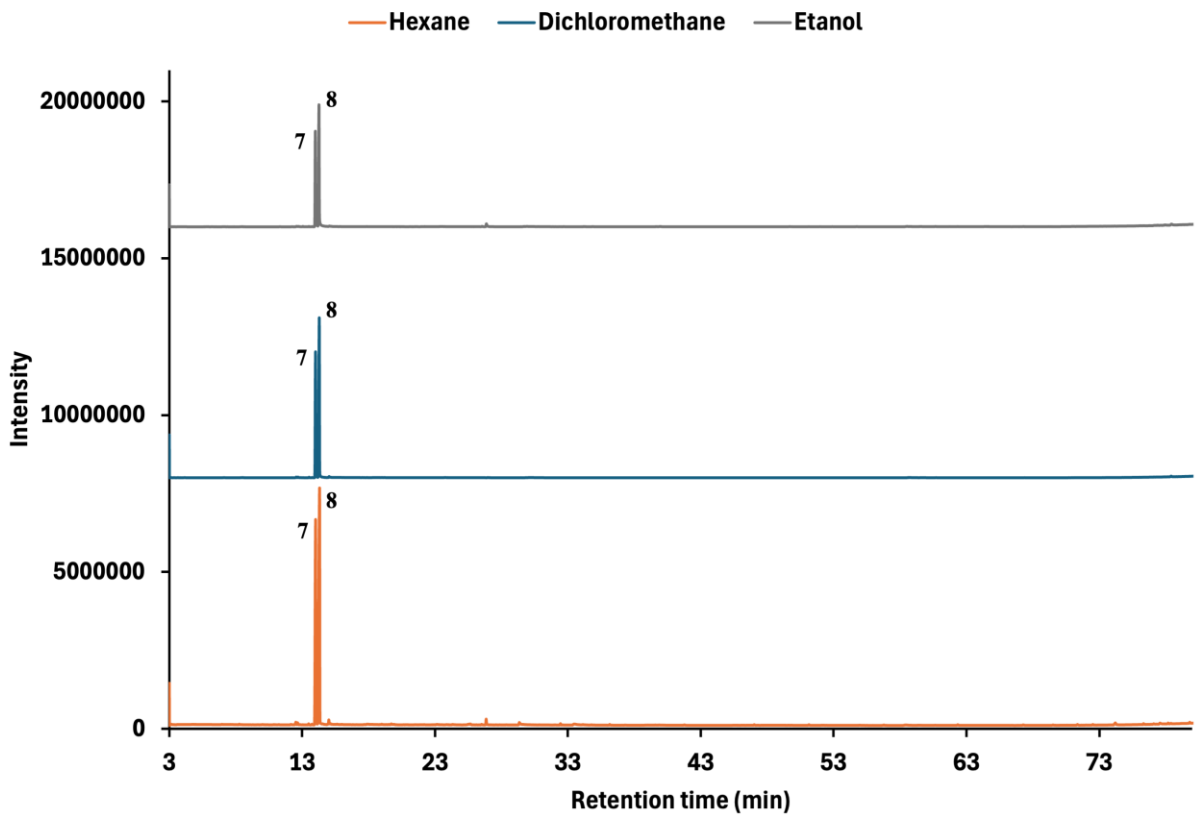


**Figure 3:** The GCMS chromatogram of Jindamanee hexane extract (■), Jindamanee $CH_2Cl_2$ extract (■) and Jindamanee EtOH extract (■).

**Table 5:** HPLC analysis of phenolic and flavonoid in Jindamanee extracts

| Compounds | Chemical contents (µg/g extract) | | |
|---|---|---|---|
| | Hexane | $CH_2Cl_2$ | EtOH |
| Gallic acid | ND | ND | ND |
| Protocatechuic acid | 20.51±0.46$^{c}$ | 151.88±2.49$^{b}$ | 22.91±0.39$^{g}$ |
| Hydroxybenzoic acid | ND | ND | 421.24±6.44$^{a}$ |
| Vanillic acid | ND | ND | 88.05±0.53$^{e}$ |
| Syringic acid | ND | 134.19±1.42$^{c}$ | 267.82±3.42$^{b}$ |
| Chlorogenic acid | ND | ND | ND |
| Coumaric acid | ND | 134.38±0.84$^{c}$ | 68.50±0.23$^{f}$ |
| Ferulic acid | ND | ND | ND |
| Sinapic acid | 114.39±0.20$^{b}$ | 337.34±4.08$^{a}$ | 177.97±2.48$^{c}$ |
| Rutin | ND | ND | ND |
| Quercetin | 115.16±0.28$^{a}$ | 136.93±0.19$^{c}$ | 122.81±3.76$^{d}$ |

Note: ND as not detected. Letters indicate the significant differences in data between rows in the same columns using one-way ANOVA with Tukey HSD in the same extract ($p < 0.05$).

The EtOH extract possessed the highest TPC (41.84±1.59 mg GAE/g extract), followed by the $CH_2Cl_2$ (36.18±0.16 mg GAE/g extract) and Hexane (12.84±1.26 mg GAE/g extract) extracts. Conversely, the $CH_2Cl_2$ extract contained the significantly highest TFC (82.49 mg QE/g extract), far exceeding the levels in the other extracts. This distribution is critical for correlating chemical content with bioactivity. The high TFC in the $CH_2Cl_2$ extract strongly suggests that flavonoids are the primary drivers of its potent antioxidant and alpha-glucosidase inhibitory activities, as flavonoids are well-established anti-inflammatory and antioxidant compounds.[10] Although the EtOH extract had the highest TPC, its biological activities were less potent than the $CH_2Cl_2$ extract. Phenolic compounds are known primary antioxidants[16] and alpha-glucosidase inhibitors.[36] Furthermore, TPC has also been correlated with nitric oxide inhibition,[1] which aligns with the activities observed across all our extracts.

*Chemical composition analysis by GC-MS*

The chemical composition of Jindamanee extract by GC-MS is shown in Table 4 and GCMS chromatogram shown in Figure 3, identifying 13, 6, and 7 compounds in the Hexane, $CH_2Cl_2$, and EtOH extracts, respectively. The most statistically significant finding was the dominance of endo-borneol as the major component in all three extracts, comprising 59.31% of the $CH_2Cl_2$ extract, 58.52% of the EtOH extract, and 56.83% of the Hexane extract. isoborneol was the second most abundant compound. Additionally, β-selinenol (1.73%) was detected in the EtOH extract. This compositional similarity is highly relevant to the observed bioactivities. Borneol is a well-established compound in traditional medicine, known for its anti-inflammatory, analgesic, and antibacterial properties.[3] Isoborneol shares a similar pharmacological profile.[6] This anti-inflammatory potential is particularly noteworthy borneol in *C. camphora* oil significantly reduced xylene-induced swelling.[33] Furthermore, and directly relevant to our findings, borneol has been shown to exhibit moderate alpha-glucosidase inhibition and to significantly inhibit the production of proinflammatory mediators like nitric oxide.[37] The presence of β-selinenol, even at low concentrations, may also contribute to the anti-inflammatory profile.[4]

*Chemical content analysis by HPLC*

HPLC analysis was used to quantify 11 specific phenolic and flavonoid compounds (Table 5). The extracts showed distinct quantitative profiles. The $CH_2Cl_2$ extract was particularly rich in several compounds, showing the highest concentrations of sinapic acid (337.34±4.08 µg/g extract), protocatechuic acid (151.88±2.49 µg/g extract), syringic acid (134.19±1.42 µg/g extract), coumaric acid (134.38±0.84 µg/g extract), and quercetin (136.93±0.19 µg/g extract) compared to the other extracts. In contrast, the EtOH extract contained the highest amount of hydroxybenzoic acid (421.24±6.44 µg/g extract), while the Hexane extract's most abundant quantified compound was quercetin (115.16±0.28 µg/g extract). Gallic acid, chlorogenic acid, ferulic acid, and rutin were not detected in any extract. This chemical disparity correlates strongly with the bioactivity results. The superior $\alpha$-glucosidase and antioxidant activity of the $CH_2Cl_2$ extract aligns with its high concentrations of sinapic acid and quercetin. Sinapic acid is a known potent antioxidant and anti-inflammatory agent,[20] and quercetin are renowned for its broad-spectrum pharmacological activities, including potent antioxidant and anti-inflammatory effects.[32] The high level of hydroxybenzoic acid in the EtOH extract[2] likely contributes to its high TPC and observed anti-inflammatory properties.

## Conclusion

The different solvent extracts of Jindamanee formulation showed that the $CH_2Cl_2$ extract has a potential for α-glucosidase inhibitory and antioxidant activities, which are related to the GCMS analysis showed that isoborneol and endo-borneol are highly abundant in $CH_2Cl_2$, EtOH and hexane extracts. The HPLC analysis showed that quercetin, sinapic acid and hydroxybenzoic acid were a major compound in hexane, $CH_2Cl_2$ and EtOH extracts, respectively. Therefore, the solvent for extraction influenced the biological activity and chemical composition of the Jindamanee formulation, a Thai traditional medicine. Moreover, this formulation demonstrated the potential of antioxidant, anti-inflammatory and α-glucosidase inhibitory activities for the development of a pharmaceutical product.

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

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

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