# In Vitro and in Silico Anti-Inflammatory Activity of Phenoliccompounds Isolated from Dillenia Indica L.

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## Abstract

Four known compounds (1-4) were isolated from the fruit of *Dillenia indica* L., a medicinal plant belonging to the Dilleniaceae family. The structures were characterized by spectroscopic methods and direct comparison of their physical properties with those reported in the literature. There was three flavone, including kaempferide (1), Dillenetin (2), and quercetin (3), together with gallic acid (4). Compounds 1-4 exhibited significant anti-inflammatory activity by preventing bovine serum albumin's denaturation with the $IC_{50}$ of 2.18, 5.07, 0.51, and 4.66 mM, respectively. Moreover, a molecular docking study of the most potent compound was performed to find the inhibitors' binding mode into the binding site of bovine serum albumin.

**Keywords:** Inflammation, Anti-denaturation, Dillenia indica, Molecular docking

## Introduction

Significant non-specific defense response to tissue injuries caused by a pathogen or wound is inflammation characterized by warmth, redness, pain, and swelling (Chen et al., 2017). Furthermore, protein denaturation that leads to antigen formation may cause inflammation. Also, multiple human illnesses are related to inflammation (Elisha, Dzoyem, McGaw, Botha, & Eloff, 2016). The non-steroidal anti-inflammatory medicines (NSAIDs) are the therapeutic drug generally used to manage inflammatory conditions that have possible side effects, including indigestion, stomach ulcers, allergic reaction, and reappearance of symptoms following discontinuation (Jackson & Hawkey, 2000). Several non-steroidal anti-inflammatory drugs such as diclofenac sodium prevent denaturation of bovine serum albumin (BSA) at

pathological pH (Hasan, 2019). Thus, compounds capable of preventing protein denaturation could be of potential value in treating inflammatory.

Dillenia indica L. (Dilleniaceae) is a medicinal plant used in Southeast Asia, and it is called Ma-tad in Thai. The pulp from the fruit gives a sour taste and is used in Thai Mon cuisine in curries, jams, and jellies. The crude extract from leaves of D. indica was investigated for bioactive metabolites, and flavonoids were isolated and identified. Bioactive compounds from D. indica were found to possess xanthine oxidase inhibitory activity (Khammee, Rattanapittayapron, Rangjaroen, Jaratrungtawee, & Kuno, 2019). However, other activities, such as anti-denaturation activities, have not been reported. Therefore, the present study's main objective was to investigate the anti-inflammatory potential of D. indica by testing its in vitro and in silico anti-denaturation activities.

## Materials and Methods

### General Experimental Procedures

H NMR and C NMR spectra were recorded on a Bruker AVANCE III 500 NMR. High-resolution electrospray ionization-time of flight (HRESI-TOF) mass spectra were recorded on a Bruker MicroTOF mass spectrometer. UV spectra were recorded using a PerkinElmer VICTOR Nivo. IR spectra were obtained from a Bruker TENSOR II FTIR by using the attenuated total reflectance (ATR) technique. Column chromatography was performed on silica gel 60 (Merck Code No. 7734).

#### Plant

Fruits of D. indica were collected from Nonthaburi province (13.906289, 100.483087), Thailand, in August 2019.

#### Extraction and Isolation

The fresh fruits of D. indica (3.5 kg) were ground and soaked in acetone (3.5 L x 3). The acetone extract was evaporated to dryness to provide a crude extract. The 41.4 g of acetone extract was chromatographed on a quick silica gel column (10 x 4.6 cm, 150 g) eluted by a gradient of n-hexane-acetone (0-100%, stepwise) to provide ten fractions (D1 to D10). Fraction D3 (2.67 g) was further chromatographed on a silica gel column (4 x 63 cm) eluted with a gradient solvent from n-hexane-acetone (100:0) to 100% acetone to give seven fractions (D3.1

to D3.7). Fraction D3.4 was filtered and washed with cold hexane to give a yellow solid of 1 (1.05 g, kaempferide (1), 2.53 %).

Fraction D4 (475.3 mg g) was separated by silica gel column chromatography (CC) (10% acetone-n-hexanes) to give subfraction D4.1–D7. Subfraction D4.4 (209.8 mg), after repeated silica gel CC (20% acetone-n-hexane) and recrystallization from CH2Cl2-MeOH (1:9), provided yellow needles of compound 2 (55.7 mg, dillenetin (2), 0.13%). Fraction 6 (2.13 g), after silica gel CC (15% acetone-n-hexane), yielded subfraction D6.1–D 6.9. Fraction D 6.5 (2.13 g) gave 3 (935.7 mg, quercetin (3), 2.66%) as yellow needles, after recrystallization (MeOH–CH2Cl2). Fraction D8 (935.4 mg) was purified by CC eluted with a gradient system of acetone – n-hexane and MeOH to give ten fractions, D8.1-D8.5. Fraction D8.4 (0.231 g) was purified by recrystallization with EtOH to give colorless crystals of 4 (85.4 mg, gallic acid (4), 0.2%).

**Kaempferide (1):**yellow solid; mp 226 - 228 °C [mp 224–226°C (Nguyen, Shi, Luan, & Wang, 2015)]; $R_f$ 0.5(40% acetone - hexane); $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$) : 247 (3.02), 260 (3.00), 298 (2.99), 362 (2.93), 395 (2.91); ATR-FTIR $cm^{-1}$: 3463 (OH), 1651(conjugated C=O), 1614 (aromatic C=C), 1293 (C-O); $^1$H )400 MHz, acetone-$d_6$ ($\delta$ 12.04 (1H, s, 5-OH, H-5), 8.22 (1H, d, *J* = 8 Hz, H-6′), 8.22 (1H, d, *J* = 8 Hz, H-2′), 7.11 (1H, d, *J* = 8 Hz, H-3′), 7.11 (1H, d, *J* = 8 Hz, H-5′), 6.54 (1H, d, *J* = 1.6 Hz, H-8), 6.27 (1H, d, *J* = 1.6 Hz, H-6), 3.90 (3H, s, 4′-$OCH_3$); $^{13}$C NMR )125 MHz, acetone-$d_6$ ( $\delta$ 177.5 (C-4), 165.0 (C-7), 162.1 (C-5), 157.9 (C-4′), 151.6 (C-8a), 146,6 (C-2), 136.9 (C-3), 130.3 (C-2′), 130.3 (C-6′), 124.4 (C-1′), 114.9 (C-3′), 114.9 (C-5′), 104.2 (C-4a), 99.1 (C-6), 94.0 (C-8), 55.8 (4′-$OCH_3$); HR-ESI-TOFMS: (+ve): m/z 301.0712 (calcd. for $C_{16}H_{12}O_6$+H: 301.0706).

**Dillenetin (2):** yellow needles; mp 292 - 294 °C [mp 291-292°C (Le Mai DeSilva & Bahorun, 2009)]; $R_f$ 0.42 (30% acetone - hexane); $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$) : 249 (3.12), 299 (3.11), 359 (3.06), 393 (3.06); ATR-FTIR $cm^{-1}$: 3430 (OH), 1649 (conjugated C=O), 1251 (C-O); $^1$H NMR )400 MHz, DMSO-$d_6$( $\delta$ 12.42 (1H, s, 5-OH, H-5), 7.79 (1H, dd, *J* = 6.8, 1.2 Hz, H-6′), 7.74 (1H, s, H-2′), 7.13 (1H, d, *J* = 6.8 Hz, H-5′), 6.49 (1H, d, *J* = 1.2 Hz, H-8), 6.19 (1H, d, *J* = 1.2 Hz, H-6), 3.84 (3H, s, 3′-$OCH_3$), 3.84 (3H, s, 4′-$OCH_3$); $^{13}$C NMR )125 MHz, DMSO-$d_6$( $\delta$ 175.9 (C-4), 164.0 (C-7), 160.7 (C-5), 156.2 (C-8a), 148.3 (C-3′), 146.1 (C-2), 146.1 (C-4′), 136.2 (C-3), 123.3 (C-1′), 121.4 (C-6′), 111.5 (C-5′), 110.8 (C-2′), 103.0 (C-4a), 98.2 (C-6), 93.6 (C-8), 55.6 (4′-$OCH_3$), 55.6 (3′-$OCH_3$); HR-ESI-TOFMS: (+ve): m/z 331.0812 (calcd. for $C_{17}H_{14}O_7$+H: 331.0812).

**Quercetin (3):** yellow needles mp 310 - 312 °C [mp 313-314°C (Le Mai DeSilva & Bahorun, 2009)]; $R_f$ 0.25 (40% acetone - hexane); $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 247 (3.08), 260 (3.08), 297 (3.07),

359 (3.03), 394 (3.02); ATR-FTIR $cm^{-1}$: 3381 (OH), 1660 (conjugated C=O), 1600 (aromatic C=C), 1194 (C-O); $^{1}H$ (400 MHz, acetone-$d_6$( $\delta$ 12.16 (1H, s, 5-OH), 7.82 (1H, dd, *J* = 2 Hz, H-2′), 7.69 (1H, dd, *J* = 6.8, 2 Hz, H-6′), 7.00 (1H, d, *J* = 6.8 Hz, H-5′), 6.52 (1H, d, *J* = 0.8 Hz, H-8), 6.26 (1H, d, *J* = 0.8 Hz, H-6); $^{13}C$-NMR (125 MHz, acetone-$d_6$( $\delta$ 176.6 (C-4), 167.6 (C-7), 165.0 (C-5), 157.8 (C-8a), 146.9 (C-2), 146.9 (C-4′), 145.8 (C-3′), 136.8 (C-3), 121.52 (C-6′), 121.5 (C-1′), 116.2 (C-5′), 115.7 (C-2′), 104.1 (C-4a), 99.2 (C-6), 94.5 (C-8); HR-ESI-TOFMS: (+ve): m/z 303.0503 (calcd. for $C_{15}H_{10}O_7$+H: 303.0499).

**Gallic acid (4):** White solid; mp 246-248°C [mp 248-250°C (Fernandes & Salgado, 2016)]; C $R_f$ 0.43 (10% ($CH_2Cl_2$- MeOH); ATR-FTIR $cm^{-1}$: 3351(OH), 1688 (conjugated C=O); $^{1}H$-NMR (400 MHz, DMSO-$d_6$( : $\delta$ 9.15 (1H, s, -COOH), 6.90 (2H, s, H-2,6), 3.33 (3H, br s, 3, 4, 5-OH); HR-ESI-TOFMS: (-ve): m/z 169.0169 (calcd. for $C_7H_6O_5$ -H: 169.0142).

## Inhibition of BSA Denaturation

The anti-inflammatory activity of the isolated compounds was determined using thef Elisha and co-workers method (Elisha et al., 2016) with minor modifications. All tested compounds were dissolved in DMSO to obtain stock solutions (2000 µg/mL). The reaction mixture consisted of the 100 µL tested compounds (final concentration 1.25–1000 µg/mL) and 100 µL of 5 % aqueous bovine serum albumin (BSA); pH was adjusted by adding a glacial acetic acid. The samples were incubated at 37 °C for 20 min and then heated to 70 °C for 10 min. The mixture was allowed to cool for 10 min, after which turbidity was measured at 416 nm by PerkinElmer VICTOR Nivo™ Multimode Plate Reader. The blank contained the sample and distilled water. Distilled water was used as a negative control. The positive control was diclofenac sodium (final concentration of 1.25–500 µg/mL). The percentage of inhibition was calculated using the following formula

$$\text{The percentage of inhibition (\%)} = 100 \times \frac{(\text{Abs}_{\text{Sample}} - \text{Abs}_{\text{Blank}})}{\text{Abs}_{\text{control}}} - 1$$

Whereas: Abs is the absorbance. The $IC_{50}$ was calculated from a graph of inhibition against the different concentrations. The experiment was carried out in triplicate.

## Molecular Docking Simulation

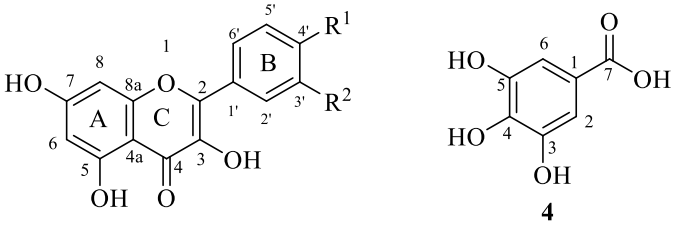
The three-dimensional coordinates of BSA were obtained from the Protein Data Bank (PDB IDs: 4JK4). The 4JK4 represents the X-ray crystal structure of bovine serum albumin co-

crystallized with 3,5-diiodosalicylic acid (Sekula, Zielinski, & Bujacz, 2013). Molecular docking was performed with the AutoDock 4.2 software. Ligands were constructed using ChemSketch and developed using HF/321G by Gaussian 03 molecular software package. Docking was carried out based on a standard protocol using the Lamarckian Genetic Algorithm (Morris et al., 2009). The one hundred independent docking runs were performed for each ligand. Further, the interaction of the ligand with protein models was evaluated using the Discovery Studio.

## Results and Discussion

### Chemical constituents

Phytochemical investigation of the constituents from acetone extract of the fruits of *D. indica* resulted in the isolation of kaempferide (**1**), dillenetin (**2**), quercetin (**3**), and gallic acid (**4**). Compounds **1**–**4** were characterized by analyses of the UV, IR, Mass, $^{1}$H-NMR, $^{13}$C-NMR, HMQC, and HMBC spectra and comparison of the data with those previously reported in the literature (Nguyen, Shi, Luan, & Wang, 2015, Le Mai DeSilva & Bahorun, 2009, Fernandes & Salgado, 2016). Their structures are shown in Figure 1.




**1**: $R^1 = OCH_3$, $R^2 = H$
**2**: $R^1$, $R^2 = OCH_3$
**3**: $R^1$, $R^2 = OH$

**Figure 1** Chemical structures of compounds **1–4.**

**Table 1** Anti-BSA denaturation of compounds isolated from D. indica

| Compounds | $IC_{50}$ (uM) | Compounds | $IC_{50}$ (uM) |
|---|---|---|---|
| kaempferide (**1**) | 2.18 | gallic acid (**4**) | 4.66 |
| dillenetin (**2**) | 5.07 | Sodium diclofenac | 1.90 |
| quercetin (**3**) | 0.51 | (standard drug) | |

### In vitro denaturation of BSA

As shown in Table 1, The inhibition of BSA protein denaturation of **1**-**4** was evaluated. Compound **3** showed maximum inhibition in BSA denaturation with an $IC_{50}$ value of 0.51 uM

($IC_{50}$ value of Diclofenac sodium is 1.9 uM). Kaempferide (**1**) and dillenetin (**2**) demonstrated inhibition activity at 2.18 and 5.07 uM, respectively. In the case of gallic acid (**4**), prevention in protein denaturation with an $IC_{50}$ value of 4.66 uM was shown. This study's result is consistent with several studies that show the interaction with polyphenolic compounds improved the thermal stability of proteins (Moualek, Iratni Aiche, Mestar Guechaoui, Lahcene, & Houali, 2016). Therefore, from the observations of Table 1, compound **3** was selected based on inhibition of BSA protein denaturation activity of the compounds for further molecular docking study.

**Table 2** Molecular docking experiments binding of interactions of the quercetin (**3**) with BSA 4JK4

| BSA Residual | Type of interaction (Amino-ligand) | Distance (Å) |
|---|---|---|
| LEU197 | H-bond | 3.433 |
| ARG198 | H-bond | 3.154 |
| SER201 | H-bond | 2.938 |
| ALA209 | O···H-C | 3.442 |
| LEU210 | C···H-O | 3.115 |
| TRP213 | C···C | 3.143 |
| SER343 | C-H···O | 3.524 |
| LEU346 | C-H···O | 3.564 |
| SER453 | H-bond | 2.813 |
| LEU456 | C-H···O | 2.897 |
| ASN457 | H-bond | 3.556 |
| LEU480 | H-bond | 3.989 |
| VAL481 | H-bond | 2.933 |
| ARG483 | C···C | 2.952 |
| ARG484 | H-bond | 3.294 |

**In silico molecular docking analysis**

Quercetin (**3**), the highest active compound in isolated compounds from biological activities, was selected to analyze the crucial interactions in the BSA binding site**.** The value of the total interaction energies of compound **3** is -8.58 kcal/mol. Our molecular docking

studies revealed that compound **3** formed eight hydrogen bond interaction between hydroxyl groups of the ligand with LEU197, ARG198, SER201, SER453, ASN457, LEU480, VAL481, and ARG484. Besides, the five C–H···O interaction between quercetin (**3**) and ALA209, LEU210, SER343, LEU346, and LEU456 residues were observed. Moreover, two hydrophobic interactions between a ligand's carbon atoms with the carbon atom of TRP213 and ARG483 residues were found**.** Based on the activities and docking studies, compounds **3** were identified as promising anti-inflammatory lead molecules. The docking analysis results were described in Tables 2, and the docking figure was shown in Figure 2.

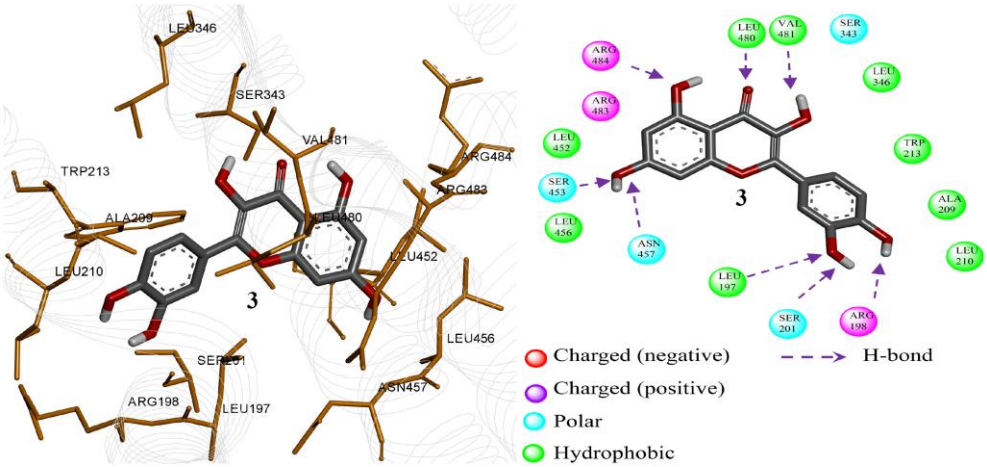



**Figure 2** The 3D interaction poses of quercetin (**3**) in the binding pocket of 4JK4

## conclusion

In this study, we isolated four bioactive compounds, namely, kaempferide (1), dillenetin (2), quercetin (3), and gallic acid (4), from the fruit of D. indica. These compounds were evaluated for their BSA denaturation activity. In vitro studies have demonstrated that quercetin (3) possesses the highest activity with an IC50 value of 0.51 mM, three-fold higher than Sodium diclofenac (standard drug, IC50 = 1.90 mM). Molecular docking simulation results showed that quercetin (3) created efficient interactions with amino acid residues surrounding the BSA binding site. The preliminary result obtained from this study will lead to discovering a new therapeutic agent for the treatment of inflammatory.

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