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Original Article

# Bioactive components, antioxidant, and anti-inflammatory activities of the wood of *Albizia myriophylla*

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ABSTRACT

The scientific basis corresponding with the folkloric use of *Albizia myriophylla* Benth., Fabaceae, for the treatment of inflammation-related diseases was established by measuring antioxidant potential using 2,2-diphenyl-1-picrylhydrazyl, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) free radicals, and ferric reducing antioxidant power assays as well as anti-inflammatory effect using nitrite assay and ethyl phenylpropiolate (EPP)-induced rat ear edema model. Both ethanol extract (DPPH, $IC_{50}$ 46.23 μg/ml; ABTS, $IC_{50}$ 57.14 μg/ml; FRAP, 950.14 mM Fe (II)/g) and dichloromethane fraction (DPPH, $IC_{50}$ 29.54 μg/ml; ABTS, $IC_{50}$ 40.36 μg/ml; FRAP, 946.69 mM Fe (II)/g) from *A. myriophylla* demonstrated a promising antioxidant activity. Furthermore, it was found that the ethanol extract of *A. myriophylla* showed significant inhibitory activity against lipopolysaccharide (LPS)-induced nitric oxide production in murine macrophage cells ($IC_{50}$ 13.8 μg/ml). The ethanol extract (15% w/v) also exhibited the maximum percentage inhibition (81–95%) of inflammation in the ear edema model at all assessment times comparable to indomethacin (0.5 mg/ear). Among all isolates **1**–**5** from the active extract of *A. myriophylla*, indenoic acid (**1**) (DPPH, $IC_{50}$ 8.96 μg/ml; ABTS, $IC_{50}$ 10.12 μg/ml) and 8-methoxy-7,3′,4′-trihydroxyflavone (**2**) (DPPH, $IC_{50}$ 5.05 μg/ml; ABTS, $IC_{50}$ 7.89 μg/ml) had potent free radical scavenging effects comparable to those of ascorbic acid (DPPH, $IC_{50}$ 2.12 μg/ml; ABTS, $IC_{50}$ 3.26 μg/ml). Compound **2** also displayed remarkable reducing power in FRAP test (261.81 mg QE/g) and showed a marked inhibition of the cellular nitric oxide production ($IC_{50}$ 27.7 μg/ml). Our results suggest that the anti-inflammatory mechanism of *A. myriophylla* is most probably based on its capacity to suppress nitric oxide production as well as to be free radical scavenger.



## Introduction

The medicinal liana *Albizia myriophylla* Benth., Fabaceae, is widely found in Southeastern Asian countries (Yoshikawa et al., 2002). The various parts of this medicinal plant, especially the wood, have long been used in Thai folk medicine for treating many human diseases (Saralamp et al., 1996). Interestingly, preparations from the wood of *A. myriophylla* have been recorded as folk remedies for tonic and treating inflammation-associated disorders (sore throat, skin infection, toothache, and fever) (MRD, 1998; Chusri et al., 2012). To date, pharmacological evidence associated with antioxidant and anti-inflammatory activities of *A. myriophylla* wood has not earlier been reported. Currently, it is well known that many phytochemicals play important roles in the therapeutic effects of plant based drugs (Charoonratana et al., 2014). Chemical information on the main active ingredients of the herbal drugs would generally serve as a useful parameter to guarantee their efficacy and safety (Zhao et al., 2012; Joycharat et al., 2016). So far, the bioactive phytochemicals responsible for antioxidant and anti-inflammatory activities have not yet been characterized from *A. myriophylla*.

Inflammation is part of a complex defense mechanism of the body to harmful stimuli such as the injurious chemical or biological agents (White, 1999). Free radical damage contributes to either the etiology or the complication of oxidative stress-related

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disorders including inflammatory diseases (Temple, 2000). Anti-inflammatory medicines are among treatments recommended for symptomatic relief of inflammation in multiple diseases such as arthritis, muscle pain, sore throat, toothache, and fever. The long-term administration of available conventional drugs either steroidal or non-steroidal anti-inflammatory chemicals both orally and topically as gels, creams, or sprays might lead to severe side effects. Consequently, natural remedies with very little side effects, proven efficacy and safety are required for as substitutes for chemical therapies (Ong et al., 2007). Medicinal plants that have pharmacological evidences supporting their uses in folk medicines as antioxidant and anti-inflammatory agents may serve as valuable sources of natural medical therapies for inflammatory diseases (Yang et al., 2017).

Therefore, the aim of this study was to examine the potential efficacy correlating with ethnomedical utilization of *A. myriophylla* wood as folk medicines for inflammation-related diseases. The ethanol extract and its various solvent soluble fractions from the wood of *A. myriophylla* were investigated for the antioxidant activity using free radical (DPPH and ABTS) scavenging method and FRAP assay as well as their anti-inflammatory activity using *in vitro* inhibition of cellular NO production and *in vivo* model of EPP-induced ear edema. The isolation and characterization of the major compounds responsible for the antioxidant and anti-inflammatory activities of this medicinal plant were performed as well.

## Materials and methods

### General

The equipments used in this work are as follows: IR, Vertex 70, FTIR (Bruker, Germany); 1D and 2D NMR, FTNMR spectrometer 500 MHz (Varian, Germany); ESIMS, Bruker Daltonics micrOTOF (Bruker Corporation, New Orleans, LA); Absorbance (OD), Microplate reader (Biotek, USA) and Genesys 10 Series (Madison, WI). Silica gel (70–230 mesh, Merck, New York, USA) and Sephadex LH-20 (20–100 mm, Sigma, St. Louis, MO) were used as stationary phases for column chromatography. Precoated sheets of silica gel 60 $F_{254}$ (Merck, New York, USA) were used for thin-layer chromatography (TLC). Fractions and compounds were detected using TLC sprayed with a solution of anisaldehyde-sulfuric acid. DPPH, ABTS, ascorbic acid, antibiotics, and indomethacin were bought from Sigma (St. Louis, USA). Ethyl phenylpropiolate (EPP) was purchased from Sigma–Aldrich (Steinheim, Germany). Thiopental sodium was obtained from Scott-Edil Pharmacia Ltd. (Solad, India). Propylene glycol was from Vidhyasom Co., Ltd. (Bangkok, Thailand). Tween 80 was from P.C. Drug Center Co., Ltd. (Bangkok, Thailand). All laboratory solvents were from commercial sources.

### Plant material

The wood of *Albizia myriophylla* Benth., Fabaceae, was collected in July 2014 and June 2015 in Patalung, Thailand. Identification was done by Dr. Oratai Neamsuvan. The voucher specimens (CB-S22 and NJ-S22) have been deposited at the Faculty of Traditional Thai Medicine, Prince of Songkla University.

### Preparation of the extracts

The ethanol extract and its different solvent soluble fractions were prepared from the dried wood of *A. myriophylla* (4 kg) according to the method mentioned previously (Joycharat et al., 2016). The percentage yield of ethanol extract (3.67%) and its various solvent-soluble fractions (hexane, 0.36%; dichloromethane, 0.16%; *n*-butanol, 0.26%) was documented.

### Isolation and purification

In the present work, the compounds **1**–**5** were isolated using the purification method described previously (Joycharat et al., 2016, 2018). The dichloromethane fraction (19.71 g) was applied to silica gel column using a gradient elution system of $CH_2Cl_2$–acetone (100:0→0:100) and finally washed down with MeOH. The fractions with a similar profile on TLC were then combined and numbered (ID–VID). Fraction IIID (1.64 g) eluted with 30–50% acetone in $CH_2Cl_2$ was further chromatographed on silica gel column using a gradient elution system of $CH_2Cl_2$–ethyl acetate (100:0→0:100) and finally washed down with MeOH. The fractions with a similar TLC pattern were then combined to afford seven subfractions (I–VII). Subfraction III (740 mg) was further purified by repeated gel filtration chromatography (four successive Sephadex LH-20 columns eluted with MeOH), followed by repeated silica gel chromatography ((i) CC gradient $CH_2Cl_2$–MeOH (100:0→0:100) and (ii) CC MeOH–$CHCl_3$ = 10:90) to give compound **1** (5.1 mg). Subfraction V (344 mg) was further purified by three successive Sephadex LH-20 columns eluted with MeOH to give compound **2** (7.8 mg) and compound **3** (7.6 mg). For the isolation of compounds **4** and **5**, the hexane fraction (8.17 g) of *A. myriophylla* was fractionated on silica gel column using a gradient elution system of hexane-acetone (100:0→0:100) and finally washed with MeOH. The fractions were then combined and numbered (IH-XH) in line with their similar TLC profiles. Fraction IIIH was purified on silica gel column eluted with 20% acetone in $CH_2Cl_2$ to afford compound **4** (3.6 mg). Fractions IIH and IVH were recrystallized from acetone to obtain compound **5** (6.4 mg) and a mixture of β-sitosterol and stigmasterol, respectively. Flow chart for the isolation of compounds **1**–**5** from *A. myriophylla* wood can be seen in Fig. 1.

### DPPH radical scavenging assay

The DPPH radical scavenging activity of the extracts and compounds was assessed using the method mentioned by Brand-Williams et al. (1995) with slight modifications. The test samples were prepared in 70% ethanol, and a 20 μl aliquot of each sample (0.24 to 250 μg/ml for extracts and 0.24 to 150 μg/ml for pure compounds) was mixed with 180 μl of DPPH-methanol solution. The reaction mixtures were incubated for 30 min. The absorbance was measured at 515 nm in a microplate reader. Ascorbic acid was used as the reference standard. All tests were carried out in triplicate. Results are performed as the $IC_{50}$ values.

### ABTS radical scavenging assay

For ABTS assay, the procedure followed that described previously with some modifications (Pellegrini et al., 1999). The test samples (0.24 to 250 μg/ml for extracts and 0.24 to 150 μg/ml for pure compounds) were allowed to react with freshly prepared ABTS radical cation ($ABTS^{\bullet+}$) solution for 5 min. Ascorbic acid was used as the reference standard. The absorbance was measured at 750 nm in a microplate reader. All tests were carried out in triplicate. Results are presented as the $IC_{50}$ values.

### Ferric reducing antioxidant power (FRAP) assay

The reducing power of the extracts was assessed using the method described by Butsat and Siriamornpun (2010) with slight modifications. Briefly, the test extracts (each 125 μg/ml; 100 μl) were separately mixed with 900 μl of the fresh FRAP solution in test-tubes and the resulting solution was incubated for 30 min.

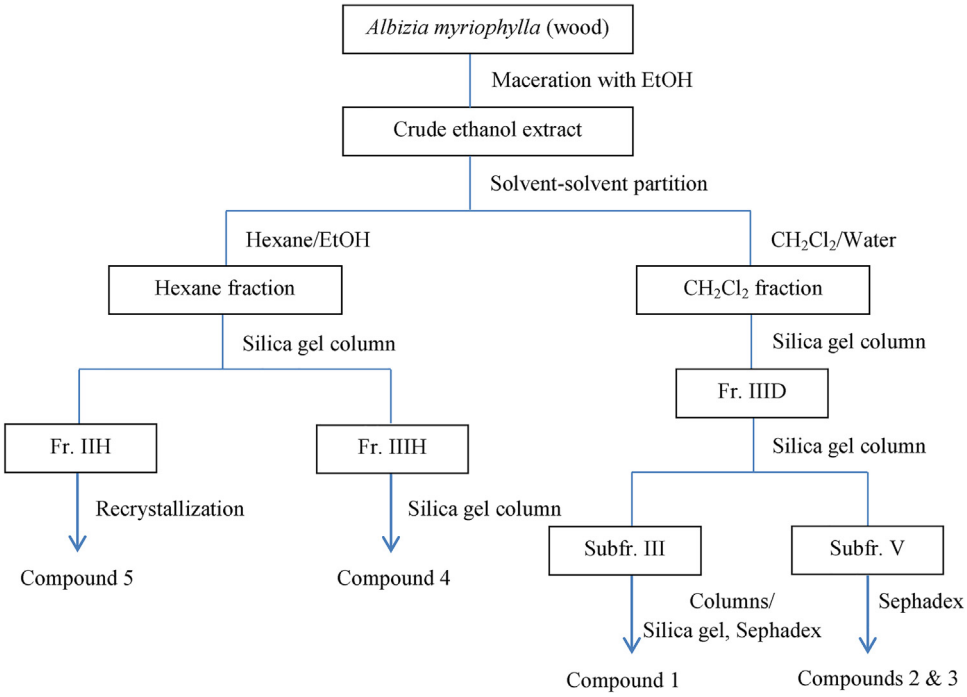



**Fig. 1.** Flow chart for the isolation of compounds **1**–**5** from *A. myriophylla* wood.

The absorbance was measured at 596 nm in a UV-vis spectrophotometer. Ferrous sulphate ($FeSO_4$) was used as the standard for a calibration curve. Results are expressed as mM $FeSO_4$ equivalent per gram of dry extract (mM Fe (II)/g). For FRAP assay of pure compounds, this was carried out as described earlier (Yildirim et al., 2001) with some modifications. The absorbance was determined at 700 nm in a microplate reader. The calibration curve was obtained by plotting the absorbance value against each concentration of standard quercetin. Results are given as mg quercetin equivalent per gram dry mass (mg QE/g DW). All assays were performed in triplicate.

*Cell culture*

The murine macrophages RAW264.7 purchased from the American Type Culture Collection were cultured under previously described condition (Sae-Wong et al., 2011).

*Cell viability assay*

Cell viability was evaluated by the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay as described previously (Sae-Wong et al., 2011). Briefly, RAW264.7 cells were incubated with various concentrations of the test samples in a 96-well plate. After 18 h of incubation, a MTT (10 μl, 5 mg/ml in PBS) reagent was added to each well. The optical density of the purple formazan product was measured with a microplate spectrophotometer by reading the absorbance at 570 nm.

*Nitrite assay*

Assay for NO production in RAW264.7 cells was performed as described previously (Sae-Wong et al., 2011). Briefly, the RAW264.7 cells were grown in a 96-well microplate at a density of $2.5 \times 10^5$ cells/100 μl/well. After 6 h of incubation, the cells were pretreated with various concentrations of samples for 10 min and further stimulated for 18 h with 10 μg/ml of lipopolysaccharide (LPS). The concentration of NO was measured using Griess's reaction. The percentage inhibition was calculated and expressed as mean ± S.E.M. of four determinations. Indomethacin and $N^G$-methyl-L-arginine acetate salt (L-NMMA) were used as reference standards.

*Ethyl phenylpropiolate (EPP)-induced ear edema in rats*

Healthy male Wistar rats (200 ± 50 g) were obtained from the Southern Laboratory Animal Facility, Prince of Songkla University, Thailand. The animals were maintained at controlled room temperature (25 ± 2 °C) and relative humidity 50–55% under a 12:12 h light-dark cycle, with access to water and food *ad libitum*. They were starved of food except water for 16 h prior starting the experiments. Male Wistar rats were randomly divided into five groups which consisted of five animals in each group. The experimental protocols involved the use of animals were approved by the Prince of Songkla University Animal Ethics Committee (MOE 0521.11/062).

Ethyl phenylpropiolate at the dose of 1 mg/ear in 20 μl was dissolved in acetone and topically applied to the inner and outer surfaces of the right ear. The ethanol extract of *A. myriophylla* (AME) was dissolved in a vehicle which composed of propylene glycol, Tween 80, and distilled water (4:1:4). The vehicle, AME (1%, 7.5%, and 15% w/v) or indomethacin (0.5 mg/ear in 20 μl of acetone, positive control) were applied to the right ear 30 min before EPP application. The edema volume was measured using a pocket thickness gauge at before (0) and 15, 30, 60, and 120 min after EPP treatment. The inhibitory effect on the edema formation was compared with that of the control group and percent inhibition was calculated.

*Statistical analysis*

Data are presented as means ± S.E.M. (for anti-inflammatory results) and means ± S.D (for antioxidant results). The Student's *t*-test and one-way ANOVA were used to evaluate the significant differences ($p < 0.05$) between the means.

**Table 1**
Antioxidant activity of the ethanol extract, fractions, and isolated compounds from *Albizia myriophylla*.

| Test samples | Free radical scavenging ($IC_{50}$; μg/ml) | | FRAP | |
|---|---|---|---|---|
| | DPPH• | $ABTS^{•+}$ | (mM Fe (II)/g) | (mg QE/g DW) |
| Ethanol extract | 46.23 ± 0.11 | 57.14 ± 0.13 | 950.14 ± 9.65 | ND |
| Hexane fraction | 142.53 ± 0.54 | 224.15 ± 0.42 | 239.18 ± 6.28 | ND |
| $CH_2Cl_2$ fraction | 29.54 ± 0.16 | 40.36 ± 0.11 | 946.69 ± 1.82 | ND |
| *n*-Butanol fraction | 126.24 ± 0.15 | 183 ± 0.11 | 381.40 ± 8.09 | ND |
| **1** | 8.96 ± 0.02 | 10.12 ± 0.03 | ND | 58.86 ± 0.44 |
| **2** | 5.05 ± 0.01 | 7.89 ± 0.02 | ND | 261.81 ± 0.88 |
| **3** | >150 | >150 | ND | NA |
| Ascorbic acid | 2.12 ± 0.005 | 3.26 ± 0.01 | ND | ND |

Values are means ± SD; $n = 3$.
ND, not done; NA, no activity at the tested concentrations.

## Results

### *Antioxidant activity of the ethanolic extract and fractions from the wood of* Albizia myriophylla

It is known that natural products with antioxidant potential may possess anti-inflammatory effects. Therefore, the evaluation of antioxidant activities of ethanol extract and its fractions from *A. myriophylla* wood was carried out using DPPH, ABTS, and FRAP assays. In order to investigate the antioxidant principles of *A. myriophylla*, the compounds isolated from the most active fraction were then tested in the same antioxidant models as well.

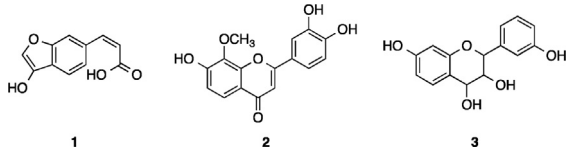
DPPH assay was used to evaluate the radical scavenging potential of ethanol extract from the wood of *A. myriophylla* and its different solvent soluble fractions (hexane, $CH_2Cl_2$, and *n*-BuOH fractions) and the results were compared to that of ascorbic acid. The four test samples showed DPPH radical scavenging activity with $IC_{50}$ values ranging from 29.54 to 142.53 μg/ml (Table 1). The ethanolic wood extract of this plant exhibited potent antioxidant activity against DPPH with $IC_{50}$ of 46.23 ± 0.11 μg/ml. Among the various solvent soluble fractions obtained from the ethanol extract, the dichloromethane fraction showed the best DPPH radical scavenging activity with $IC_{50}$ of 29.54 ± 0.16 μg/ml, followed by the *n*-butanol fraction with $IC_{50}$ of 126.24 ± 0.15 μg/ml, whereas the lowest radical scavenging activity was observed with the hexane fraction. However, the ethanol extract and its dichloromethane soluble fraction demonstrated no statistically significant difference in DPPH radical scavenging activity ($p > 0.05$).

$ABTS^{•+}$ scavenging assay was another method of determining the antioxidant property of ethanol extract and fractions from *A. myriophylla*. The $IC_{50}$ values of the analyzed samples using ABTS method were in the range of 40.36–224.15 μg/ml (Table 1). Among all test samples from *A. myriophylla*, both ethanol extract and its dichloromethane soluble fraction exhibited the best $IC_{50}$ values of 57.14 ± 0.13 and 40.36 ± 0.11 μg/ml, respectively, with no statistically significant difference ($p > 0.05$) in $ABTS^{•+}$ scavenging activity.

Additionally, FRAP assay was also used to investigate the antioxidant potential of ethanol extract and fractions from *A. myriophylla*. The analyzed samples demonstrated antioxidant property with FRAP test in the range of 239.18–950.14 mM Fe (II)/g extract (Table 1). The highest antioxidant potential was found with ethanol extract (950.14 ± 9.65 mM Fe (II)/g extract), followed by the dichloromethane (946.69 ± 1.82 mM Fe (II)/g extract), *n*-butanol (381.40 ± 8.09 mM Fe (II)/g extract), and hexane (239.18 ± 6.28 mM Fe (II)/g extract) fractions, respectively. The ethanol extract and dichloromethane fraction exhibited no statistically significant difference in FRAP test ($p > 0.05$).

### *Antioxidant components from the wood of* Albizia myriophylla

The phytochemical profiles have significant roles in the biological activities of the herbal medicines. In this work, five compounds including indenoic acid (**1**), 8-methoxy-7,3′,4′-trihydroxyflavone (**2**), 3,4,7,3′-tetrahydroxyflavan (**3**), lupeol (**4**), stigmasta-5,22-dien-3-one (**5**) as well as a mixture of β-sitosterol and stigmasterol were characterized from fractions obtained from the active ethanolic wood extract of *A. myriophylla*. From the dichloromethane fraction, compounds **1**–**3** were isolated and their structures were identified by spectroscopic methods, including IR, ESIMS, 1D and 2D NMR as well as through comparing their $^1H$ and $^{13}C$ NMR data with those previously reported (Joycharat et al., 2013, 2018). On the contrary, the triterpene **4** as well as the sterol **5** were found to be the chemical ingredients of the hexane fraction and were identified by comparing their $^1H$ and $^{13}C$ NMR data with those established earlier (Faizi et al., 2001; Malca et al., 2015). The antioxidant activity of compounds **1**–**3** was evaluated using DPPH, ABTS, and FRAP assays. Both **1** (DPPH, $IC_{50}$ 8.96 ± 0.02 μg/ml; ABTS, $IC_{50}$ 10.12 ± 0.03 μg/ml) and **2** (DPPH, 5.05 ± 0.01 μg/ml; ABTS, 7.89 ± 0.02 μg/ml) gave strong radical scavenging properties against DPPH and $ABTS^{•+}$. On the contrary, the flavan **3** was not found to be scavenger of DPPH and $ABTS^{•+}$. The reference standard ascorbic acid had DPPH and $ABTS^{•+}$ scavenging activities with $IC_{50}$ of 2.12 ± 0.005 and 3.26 ± 0.01 μg/ml, respectively. In FRAP assay, compound **2** (261.81 ± 0.88 mg QE/g DW) had significantly stronger reducing power compared with **1** (58.86 ± 0.44 mg QE/g DW), whereas **3** showed no activity at the test concentration.




### In vitro *and* in vivo *anti-inflammatory activity of* Albizia myriophylla

The scientific basic corresponding with the uses of *A. myriophylla* for the treatment of inflammation-related diseases in folk medicines was established by measuring *in vitro* anti-inflammatory activity using lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages model. With regard to the significant antioxidant effects of the most active extract and compound from *A. myriophylla*, the ethanol extract and flavone **2** were therefore selected for determining the inhibitory activity toward LPS-induced NO production in RAW264.7 cells. The ethanol extract and flavone **2** revealed the potential to inhibit the cellular NO production in a dose-dependent manner without cellular toxicity (Table 2). The NO release in LPS-treated RAW264.7 cells was

**Table 2**
Inhibition of cellular NO production of ethanol extract and **2** from *Albizia myriophylla*.

| Samples | Inhibition (%) at various concentrations (μg/ml) | | | | | | $IC_{50}$ (μg/ml) |
|---|---|---|---|---|---|---|---|
| | 0 | 1 | 3 | 10 | 30 | 100 | |
| **2** | 0.0 ± 0.4 | 15.6 ± 2.2 | 24.8 ± 2.3 | 26.3 ± 3.6 | 51.7 ± 3.0 | 80.1 ± 0.2 | 27.7 |
| Ethanol extract | 0.0 ± 0.4 | 25.8 ± 3.3 | 39.8 ± 1.4 | 45.6 ± 1.6 | 61.2 ± 1.2 | 83.5 ± 0.4 | 13.8 |
| Indomethacin | 0.0 ± 0.4 | 22.2 ± 0.6 | 39.4 ± 2.6 | 52.3 ± 1.0 | 62.1 ± 1.2 | 86.5 ± 0.2 | 8.5 |
| L-NMMA | 0.0 ± 0.4 | 8.0 ± 0.8 | 41.0 ± 1.5 | 53.2 ± 0.6 | 61.5 ± 1.0 | 97.9 ± 0.2 | 7.8 |

Percentage inhibition values are means ± S.E.M.; $n = 4$.

**Table 3**
Effects of an ethanol extract from *Albizia myriophylla* (AME) and indomethacin on EPP-induced ear edema in rats.

| Group | Edema thickness (μm) | | | | Edema inhibition (%) | | | |
|---|---|---|---|---|---|---|---|---|
| | 15 min | 30 min | 60 min | 120 min | 15 min | 30 min | 60 min | 120 min |
| Control | 74 ± 9 | 72 ± 14 | 74 ± 6 | 72 ± 11 | – | – | – | – |
| Indomethacin | −2 ± 2[a] | 10 ± 7[a] | 4 ± 2[a] | 4 ± 2[a] | 103 | 86 | 95 | 94 |
| 1% AME | 30 ± 17[a] | 55 ± 9 | 67 ± 5 | 28 ± 18[a] | 59 | 24 | 9 | 62 |
| 7.5% AME | 26 ± 10[a] | 70 ± 8 | 62 ± 4 | 48 ± 11 | 65 | 3 | 16 | 33 |
| 15% AME | 6 ± 5[a] | 14 ± 12[a] | 4 ± 4[a] | 4 ± 4[a] | 92 | 81 | 95 | 94 |

Values are means ± S.E.M; $n = 5$.
[a] Significant difference as compared with control ($p < 0.05$). –, no activity.

strongly suppressed by the ethanol extract and flavone **2** from *A. myriophylla* with $IC_{50}$ values of 13.8 and 27.7 μg/ml, respectively. L-NMMA and indomethacin used as positive controls had $IC_{50}$ values of 7.8 and 8.5 μg/ml, respectively. Consequently, the various concentrations (1%, 7.5%, and 15%) of ethanol extract from *A. myriophylla* (AME) were investigated topically in rat ear edema induced by EPP after 15, 30, 60, and 120 min. The results showed that the ear edema thickness of the rats in the control group was maintained for 2 h of assessment (Table 3). 1% AME significantly inhibited the ear edema formation at 15 and 120 min after EPP application. 7.5% AME exhibited an inhibitory effect on the ear edema formation induced by EPP at 15 min. Furthermore, AME (15%) and the reference drug indomethacin (0.5 mg/ear) showed significant inhibitory activity on the ear edema formation at all assessment times.

## Discussion

Traditional medicine has been found to be valuable source for more effective and safer alternative therapeutic agents of future drugs (Leonti and Casu, 2013). The medicinal plants used in folk medicine always play major role in primary health care of both rural and urban populations of several countries including Thailand (Johnson, 1999; Neamsuvan et al., 2015). Although, herbal medicines have been used for long to maintain good health and to cure diseases in many countries, they have not been officially recognized world-wide because qualitative and quantitative data on their safety and efficiency would generally not sufficient to meet the standard criteria for drugs. A quality control of herbal drugs has always been a key issue for the development of traditional medicines (Charoonratana et al., 2014). In general, herbal products contain complex mixtures of secondary metabolites, therefore selection of suitable marker compounds responsible for the pharmacological activities is currently an important approach to their quality control (Shi et al., 2014). Several previous reports have shown many plants species used in traditional medicine for the management of inflammation showing antioxidant and anti-inflammatory properties (Qiao et al., 2007; Babu et al., 2009). In order to obtain scientific evidence of the health benefits relevant to ethnomedical claims of *A. myriophylla*, the plant was investigated for its anti-inflammatory and antioxidant activities.

Antioxidants have been documented to prevent the oxidative stress of free radicals associated with the pathogenesis of a number of chronic diseases, including diabetes and inflammation-associated disorders (Evans et al., 2002). Recently, the capacity of antioxidants *in vitro* has been widely investigated by various methods under different conditions. The DPPH and ABTS radical scavenging assays as well as FRAP model are among the frequently used methods to evaluate the antioxidant potential of various natural products, especially from plant origin, because of their good repeatability, simple and inexpensive technique as well as the relatively short time required for investigation (Thaipong et al., 2006). In the present study, these three analysis methods were carried out to determine and confirm the antioxidant activity of *A. myriophylla*. The results showed that antioxidant potential of each test extract except for that of dichloromethane and ethanol was significantly different from all others ($p < 0.05$). The effect of the test extracts on DPPH and ABTS radicals scavenging is suggested to be due to their hydrogen-donating potential, whereas the ability to reduce ferric to ferrous iron in FRAP reagent is commonly associated with the capacity of test samples to donate an electron to free radicals and to convert them into more stable molecules. The ethanolic extract partitioned against dichloromethane was the most active fraction exhibiting the best activity in all antioxidant assays in this study. For these reasons, the antioxidant assay of the isolates **1**–**3** from dichloromethane fraction was carried out. The results clearly indicated that 8-methoxy-7,3′,4′-trihydroxyflavone (**2**) exhibited potent antioxidant activity against DPPH and ABTS radicals, comparable with that of ascorbic acid and it also showed strong antioxidant capacity in FRAP assay. The antioxidant activity of **1**–**3** was documented in this work for the first time. The 4-oxo group, a double bond between C-2 and C-3, and a 3′,4′-catechol group in B-ring were suggested to be important features for the remarkable radical scavenging of **2** (Zhang et al., 2015). On the contrary, flavan **3**, due to the presence of only one hydroxyl in B-ring and the lack of conjugation between the 2,3-double bond and the 4-oxo group, showed no antioxidant activity (Zhang et al., 2015). Available reports tend to demonstrate that flavonoids are responsible for a wide range of pharmacological activities (Zhang et al., 2015). The health-promoting efficacy of flavonoids may be associated with their high antioxidant activity both *in vitro* and *in vivo* systems (Awaad et al., 2012; Zhang et al., 2015). Regarding to the overall antioxidant property of **2**, it could be considered as the major active principle responsible for the antioxidant activity of *A. myriophylla*. Additionally, studies demonstrated that the use

of a drug in combination to an antioxidant from natural sources was superior when compared to individual use (Chegaev et al., 2013). Therefore, the efficacy of the combination of flavone **2** from *A. myriophylla* and standard drugs such as N-acetylcysteine (NAC) and doxorubicin in the prevention and the treatment of oxidative stress-related disorders would be interesting for further investigation.

Inflammation is a complex biological response process of living tissue to injury or infection (White, 1999). Nitric oxide (NO) is a water soluble free radical produced normally in macrophages and endothelial cells during inflammation reaction (White, 1999). The overproduction of NO can cause tissue damage and the pathogenesis of inflammation-related diseases (White, 1999). Therefore, suppressing the overproduction of NO represents among the beneficial strategies to manage inflammatory condition. Several studies have suggested that the NO production inhibitory activity contributed to the anti-inflammatory property of many medicinal plants (Sae-Wong et al., 2011; Rédei et al., 2018). Inflammation in EPP model involves the release of inflammatory mediators which are capable of promoting vasodilation and increasing vascular permeability as well as producing edema characteristic. Inhibition of this response is a likely indication of anti-inflammatory activity of test substances. Many studies have demonstrated that the members of *Albizia* used in traditional medicines for the treatment of inflammatory conditions had anti-inflammatory effects in various *in vivo* models (Qiao et al., 2007; Babu et al., 2009). An ethanol is traditionally used for certain preparations such as a tincture, applied either orally or topically, in folk medicines. Interestingly, since the application of medicinal plants in the form of ethanol extract is very convenient owing to its simple and high yielding preparation, it would be practical for primary health care of all individuals, especially those far away from the health services. The present study demonstrated that the ethanolic wood extract of *A. myriophylla* had anti-inflammatory potential both *in vitro* and *in vivo*, through inhibiting the NO production in LPS-stimulated RAW264.7 macrophages model and reducing skin swelling in rat ear edema model. A number of studies have revealed the synergistic effects of the combination bioactive components in plant extracts using *in vivo* models of inflammation (Mo et al., 2013). Accordingly, in this work, the synergistic effect of a mixture of phytochemicals in *A. myriophylla* extract may contribute to the ethnomedical capacity of this medicinal plant for treating inflammation-associated disorders. Among the isolated compounds from *A. myriophylla*, lupeol and β-sitosterol were known to contribute to the anti-inflammatory activity both *in vitro* and *in vivo* assays (Awaad et al., 2012; Rédei et al., 2018). Regarding the most potent antioxidant activity of the flavone **2** in this study, we therefore evaluated its anti-inflammatory property in a cell line based *in vitro* system. Our data showed that the anti-inflammatory capacity of this flavone isolated from *A. myriophylla* was associated with the inhibition of cellular NO production. Therefore, we suggest that **2** could be partially rational for a therapeutic potential of *A. myriophylla* in the management of inflammatory condition. Many studies have previously shown that the anti-inflammatory activity of flavonoids was related with the inhibition of the generation of excessive inflammatory mediators including NO (Sae-Wong et al., 2011; Rédei et al., 2018). According to the previously observed information on the structure-activity relationships for anti-inflammatory activity (Peluso et al., 2010), the molecular planarity, lipophilicity, hydroxylation and methoxylation patterns of flavonoid structure have been proposed to be structural aspects provided anti-inflammatory effects of this chemical class. Taken together, the double bond between C-2 and C-3, the B-ring catechol moiety as well as the *O*-methylation in A-ring of the flavone **2** were predicted to be associated with its anti-inflammatory activity.

Quantitative analysis of marker compounds, especially those relevant to the claimed therapeutic effects, would serve as an additional approach for ensuring the quality, efficacy, safety, and reproducibility of the herbal drugs (Charoonratana et al., 2014). Previous phytochemical studies of *A. myriophylla* wood resulted in the identification of different classes of compounds, including flavonoids, phenolic acids, triterpenes, lignan glycosides, and iminosugars (Yoshikawa et al., 2002; Joycharat et al., 2013). The metabolites **4** and **5** of phytosterol family were commonly found in plants and reported to exhibit a spectrum of pharmacological activities (Adcox et al., 2001; Siddique and Saleem, 2011). On the contrary, compounds **1** and **3** were relatively rare in other plants, while **2** was previously reported to be the component of few other plants (Lazari et al., 1998). Previously, indenoic acid (**1**), 8-methoxy-7,3′,4′-trihydroxyflavone (**2**), and 3,4,7,3′-tetrahydroxyflavan (**3**) have been reported from the wood of *A. myriophylla* (Joycharat et al., 2013; 2016). Regarding to the amount of the isolates **1**–**3** in *A. myriophylla*, our result indicated that compound **2** was the most abundant component, followed by **3** and **1**, respectively, similar to previous studies (Joycharat et al., 2013; 2016). Based on these results, compounds **1**–**3** may be classified as the characteristic substances of *A. myriophylla* wood.

## Conclusion

The evidence presented in this work highlights the potent anti-inflammatory and antioxidant activities of *A. myriophylla* wood, correlating with the ethnomedical claims of this plant species. The mechanisms responsible for the anti-inflammatory activity of the ethanolic wood extract of *A. myriophylla* appear to be related with its capacity to suppress NO production as well as to be free radical scavenger. Furthermore, we report herein, for the first time, the antioxidant efficacy of indenoic acid (**1**) and 8-methoxy-7,3′,4′-trihydroxyflavone (**2**) as well as the marked anti-inflammatory activity of **2**. Regarding to the overall antioxidant and anti-inflammatory properties of **2**, it could be considered as the principal bioactive compound responsible for the therapeutic and health-promoting potential of *A. myriophylla* and might probably be used as the bioactive marker compound for the quality control of this plant species for its future development as a natural therapeutic agent for the treatment of inflammation-associated disorders.

## Ethical disclosures

**Protection of human and animal subjects.** The authors declare that no experiments were performed on humans for this study. The experimental protocol involved the use of animals was in accordance with all institutional and national guidelines for the care and use of laboratory animals.

**Confidentiality of data.** The authors declare that no patient data appear in this article.

**Right to privacy and informed consent.** The authors declare that no patient data appear in this article.

## Authors' contributions

NJ collected the plant material, isolated and purified compounds, analyzed the NMR data, and prepared the final manuscript. NB prepared extracts and performed antioxidant activity. NK and CTY performed anti-inflammatory assay and participated in writing manuscript. SPV contributed analysis tools. All authors read and approved the final manuscript.

## Conflicts of interest

The authors declare no conflicts of interest.

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