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Antioxidant and Anti-inflammatory Activities of Different Solvent Extracts from *Ipomoea pes-caprae* (L.) R. Br. in Lipopolysaccharide Stimulated RAW 264.7 Macrophages

Chantarawan Saengkhae[1,*], Napaporn Uawongyart[2], Sunantha Osiri[2], Klaokwan Srisook[3] and Marut Tangwattanachuleeporn[4]

[1]*Research Unit in Proficient Laboratory for Natural Products, Faculty of Allied Health Sciences, Burapha University, Chonburi 20131, Thailand*
[2]*Faculty of Abhaibhubejhr Thai Traditional Medicine, Burapha University, Chonburi 20131, Thailand*
[3]*Department of Biochemistry and Research Unit of Natural Bioactive Compounds for Healthcare Products Development, Faculty of Science, Burapha University, Chonburi 20131, Thailand*
[4]*Research Unit for Sensor Innovation, Burapha University, Chon Buri 20131, Thailand*

([*]Corresponding author's e-mail: chantars@buu.ac.th)

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Abstract

The oxidative stress and inflammatory environment trigger an unhealthy circle, which can lead to various inflammatory diseases. *Ipomoea pes-caprae*, a traditional medicine mangrove plant, posed many pharmacological activities, including antioxidant, anti-inflammatory and anticancer effects. However, the possible mechanisms involved in *Ipomoea pes-caprae* are still unclear. This study aimed to investigate the antioxidant and anti-inflammatory effects of different solvent extracts from *Ipomoea pes-caprae* on lipopolysaccharide (LPS) stimulated macrophages. Three different solvent gradients were prepared orderly from non-polar to polar: hexane (Hex), supercritical fluid extraction using carbon dioxide plus EtOH as co-solvent (SCO_2) and ethanol (EtOH). All 3 extracts were screened for the cytotoxicity on RAW264.7 cells by MTT assay. The non-toxic doses were investigated for reactive oxygen species (ROS) scavenging by DPPH and DCFH-DA assays and evaluated their anti-inflammatory activities via inhibition against LPS-induced nitric oxide (NO), prostaglandin E_2 (PGE_2), inducible NO synthase (iNOS) and cyclooxygenase-2 (COX-2) mRNA expression. All 3 extracts (25 - 50 µg/mL) exhibited DPPH scavenging and reduced intracellular ROS level in the order of SCO_2 > EtOH > Hex. Further, these extracts suppressed NO and PGE_2 production by regulating iNOS and COX-2 mRNA expression in the order of SCO_2 > Hex > EtOH. Additionally, their inhibitory effects were in a similar pattern as the standard drugs L-NAME and celecoxib. These findings support the traditional use of *Ipomoea pes-caprae* in treating inflammatory diseases due to its attenuation of inflammation in activated macrophage. Also, a wide range of secondary metabolites in unique ecology may be useful as one of the alternative therapies for inflammatory diseases.

Keywords: Antioxidant, Anti-inflammatory, RAW264.7 cells, Lipopolysaccharide, *Ipomoea pes-caprae*

Introduction

Ipomoea pes-caprae (L.) R. Br., (Convolvulaceae) are distributed worldwide in tropical and subtropical coastal beaches. This plant has been used in traditional medicine to treat pain and various inflammation types, including jellyfish sting and dermatitis. Previous research works have confirmed the anti-inflammatory properties of this plant. In this context, the extract of petroleum ether showed considerable anti-inflammatory action when evaluated in different experimental models such as paw-edema and ear-edema model, which were induced by carrageenan and arachidonic acid, respectively. The above-mentioned crude extract contained active compounds such as eugenol, (-)-mellein and 4-vinylguaiacol, which exhibited anti-inflammation via the inhibition of prostaglandin activity [1]. The antinociceptive and anti-inflammatory effect of the hydroethanolic extract of the aerial parts was also reported using the writhing test and carrageenan-induced pleurisy models in mice with lower toxicity [2]. The ethanolic extracts from aerial parts showed anti-inflammatory activities in rats using the cotton pellet-induced granuloma model. The active substances were related to the presence of alkaloids,

glycosides, flavonoids, tannins, and terpenoids [3]. In addition, the methanolic extract exhibited antinociceptive activity against acetic acid-induced abdominal constrictions and against formalin-induced pain in mice, which was equipotent to some analgesic drugs [4]. In a related study, the hydroethanolic extract relieved nociception and inflammation in mice that were injected with cnidarian venom into hind paw [5].

Free radicals in living systems can oxidize biomolecules such as DNA, proteins, lipids resulting in aging, inflammatory diseases, and cancer. Antioxidants are necessary for controlling degenerative diseases caused by reactive oxygen and nitrogen species [6]. The imbalance of antioxidants leads to various inflammatory-associated diseases by motivating the release of inflammatory mediators and cytokines. Chronic inflammation is associated with diseases due to its persistence of inflammation and various immune reactions, which can cause progressive damage. The active inflammatory cells can stimulate signal transduction cascades like inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), and various inflammatory cytokines, which will release more reactive oxygen species [7]. A role in oxidative stress-induced inflammation triggers a vicious circle, which after a long period of time may trigger carcinogenesis, degenerative diseases and metabolic syndromes [8,9]. Therefore, any anti-inflammatory therapy would be most effective under these pathophysiological conditions. Thus, researchers have tried to find medicinal plants or their crude extracts that can perform both antioxidant and anti-inflammatory activities to inhibit various chronic inflammatory-associated diseases.

Another interesting action demonstrated by this plant was its ability to display cytotoxic activity. The hexane-soluble extracts have been demonstrated to have several inhibitory properties on the growth of tumor cell lines such as nasopharyngeal (KB), colon (HCT 15), squamous cell cervical (SQC-1 UISO), and ovarian (OVCAR) carcinomas [10]. The presence of alkaloids, flavonoids, tannins, terpenoids, and glycosides in the crude extract also have anticarcinogenic activities in mice melanoma cancer [11]. In a recent study, it has been shown that the hexane (Hex) and the supercritical fluid extraction by carbon dioxide (SCO_2) induced the KB cell apoptosis without causing excessive damage to normal human peripheral blood mononuclear cells (PBMC) [12]. Interestingly, several studies have demonstrated the significant level of inflammatory cytokines found in cancer microenvironment of various human cancers. However, the literature regarding the antioxidant, anti-inflammatory activities of this plant was not reported in respect of the production of reactive oxygen species (ROS), nitric oxide (NO) and prostaglandin E_2 (PGE_2) in the lipopolysaccharide (LPS) induced RAW 264.7 murine macrophage. In continuation of our research work, we investigated the antioxidant and anti-inflammatory effects of different solvent extracts from *Ipomoea pes-caprae* in LPS-stimulated RAW 264.7 cells as inflammatory model.

Materials and methods

Preparation of plant extract

The aerial parts of *Ipomoea. pes-caprae* (L.) R. Br were collected from Bang Saen Beach in Chonburi Province, Thailand during January-April 2015 and voucher specimen were deposited in the laboratory. After it was washed and oven-dried at 45 °C, hexane (Hex) and ethanol: water (50:50, v/v) (EtOH) were macerated for 7 days and concentrated under vacuum on a rotary evaporator at 45 °C. Supercritical fluid extraction was done using carbon dioxide plus EtOH as co-solvent (SCO_2) operated at 74 bar in combination with temperature at 31 °C as our previous reported [12]. The crude extracts were stored at −80 °C and dissolved in ethanol.

Cell culture and viability assay

RAW 264.7 cell, murine macrophage cell line was obtained from Assoc. Prof. Dr. Klaokwan Srisook. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % fetal bovine serum, 1 % penicillin/streptomycin in a humidified atmosphere of 5 % CO_2, 95 % air at 37 °C. The cell viability was determined by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [12]. Briefly, cells were plated into 96 well plates at a density of 10^5 cells/well and cultured at 37 °C for 24 h. Then, cells were incubated with 1 μg/mL of LPS (from *Escherichia coli* 055:B5, Sigma) with or without various concentrations of test samples for 24 h. Subsequently, a 10 μL of the MTT (5 g/L) was added to each well for 4 h. The culture medium was removed, and the purple formazan crystals were dissolved in 100 μL/well of dimethyl sulfoxide (DMSO) and measured at 570 nm using a microplate reader (Cecil Bioquest 2000 Series). Results were presented as a percentage of the control (100 %) using: Absorbance 570 nm (sample)/Absorbance 570 nm (control)×100.

DPPH radical scavenging assay

The scavenging activity of the stable 1,1-diphenyl-2- picrylhydrazyl (DPPH) free radical was carried out [13]. The test samples with various concentrations in ethanol (20 µL) were mixed with 80 µL of DPPH (0.4 mM) in a 96 well microtiter plate and incubated at room temperature for 30 min. The absorbance at 517 nm was measured with a microplate reader. The percentage inhibition activity was calculated from [A(control) - A(sample)/A(control)] ×100, where A is the absorbance at 517 nm. The inhibition curves were set and IC_{50} values were expressed as concentration to exert half of the scavenging activity.

Measurement of intracellular ROS by DCFH-DA

Intracellular ROS levels were detected using 2',7'-dichlorofluorescein diacetate (DCFH-DA) [14]. RAW 264.7 cells (1×10^5 cells/well) were seeded with culture medium for 24 h and then incubated with the test samples 0 - 50 µg/mL and/or LPS (1 µg/mL) for 24 h. Then, the medium was changed to serum-free DMEM containing 10 µM DCFH-DA and incubated for 30 min in the dark. After washing the cells with PBS for 2 times, the generation of dichlorofluorescein (DCF) resulting from oxidation of DCFH-DA in the presence of ROS was analyzed by flow cytometry using an excitation with a 488-nm argon ion laser (FACSCalibur, BD Biosciences, CA). Each sample were collected at least 10,000 cells.

Measurement of NO

RAW 264.7 cells (1×10^5 cells/well) were seeded into a 96-well plate for 24 h. Subsequently, the cells were treated with test samples (0 - 50 µg/mL) in the presence or absence of LPS (1 µg/mL) for 24 h. The nitrite concentration in the culture medium was measured according to the Griess reaction [15]. The cell culture medium (100 µL) was mixed with the same volume of Griess reagent (equal volumes of 1 % (w/v) sulfanilamide in 5 % (v/v) phosphoric acid and 0.1 % (w/v) naphtylethylenediamine-HCl) and left for 10 min at room temperature. *N*-Nitro-L-arginine methyl ester hydrochloride (L-NAME, 250 µM), a non-selective nitric oxide synthase inhibitor, was used as a positive control. The absorbance was measured at 550 nm and the amount of nitrite in test samples was calculated using a sodium nitrite standard curve.

Measurement of PGE_2

PGE_2 were measured using an immunoenzymatic method according to the manufacturer's instructions. Briefly, RAW 264.7 cells (1×10^5 cells/well) were incubated with test samples in the presence or absence of LPS (1 µg/mL) for 24 h. Celecoxib (20 µg/mL), a selective COX-2, inhibitor, was used as a positive control. The concentration of PGE_2 in the culture medium was determined using a specific enzyme immunoassay kit (Bio-Techne / R & D Systems Inc., U.S.A.) against a PGE_2 standard curve [16].

RNA extraction and real-time RT-PCR

After treatment, total RNA from RAW 264.7 cells were isolated with the RNeasy Plus Mini Kit and RNA (10 ng/µL) reversely transcribed to cDNA with RT2 First Strand Kit according to the manufacturer's procedures (Qiagen company, Hilden, Germany). Reverse transcription- generated cDNA encoding iNOS, COX-2 and glyceraldehyde-3-phosphate dehydrogenase (G3PDH: internal standard) genes were amplified using real-time PCR by Taqman Probes (Applied Biosystems) following the manufacturer's recommendations. Relative quantification of gene expression was compared to housekeeping genes [17].

Statistical analysis

Data were expressed as the mean ± SEM (standard error of mean) from 3 independent experiments. Significant differences among the mean of each group were determined using the one-way analysis of variance (ANOVA) with Dunnett's post hoc test. $P < 0.05$ indicated a statistically significant.

Results and discussion

Extraction

In this study, 500 g of the dried plant was extracted by different solvents: Hexane, CO_2 and ethanol: Water, respectively. The Hex extract was found having the highest yield (2.9 %) (w/w), followed by EtOH (1.9 %) and SCO_2 (1.3 %). In our previous data [12], the phytochemical constituents were determined by gas chromatography-mass spectrometry (GC-MS), and the eugenol was chosen as markers because of its well-known anti-inflammatory and anticancer activities [18,19]. The SCO_2 extract yielded

the highest eugenol (3.19 %), which was higher than Hex (2.2 %) and EtOH (0.5 %). In literature review, *Ipomoea pes-caprae* consisted of various phytochemicals due to dissolve in specific solvents so it contains different active compound in various laboratories. Phenolic compounds such as flavonoids, phenolic acids and anthocyanins are the most secondary metabolites found in this plant [10,20]. Moreover, CO_2 is an attractive solvent because it is non-toxic. Oxygen-free environments and supercritical fluid extraction utilize low temperatures, resulting in less deterioration of the active ingredients in this extract [21]. Being a natural source, *Ipomoea pes-caprae* may contain low side effects and benefit new drug discoveries.

The effects of the 3 different solvent extracts from *Ipomoea pes-caprae* on cell viability

The LPS (1 μg/mL) alone or the combine treatment between LPS plus *Ipomoea pes-caprae* extracts (0 - 50 μg/mL) did not affect the viability of the RAW264.7 cells as compared to control (**Figure 1**). However, EtOH, Hex and SCO_2 extracts showed approximately 15 and 20 % cytotoxicity at concentration 75 - 100 μg/mL. According to these results, a series of non-toxic dose range 0 - 50 μg/mL of extract concentrations were chosen for their antioxidant and anti-inflammatory activities. A recent study by our group demonstrated that the same plant extracts exerted their cytotoxic effects on the human nasopharyngeal (KB) cells [12]. In addition, the compounds isolated from *Ipomoea pes-caprae* have also been reported to possess anticancer properties in many cancer cell lines [10,11]. However, these could be due to different in cell types, medium composition, treatment time and experimental conditions.

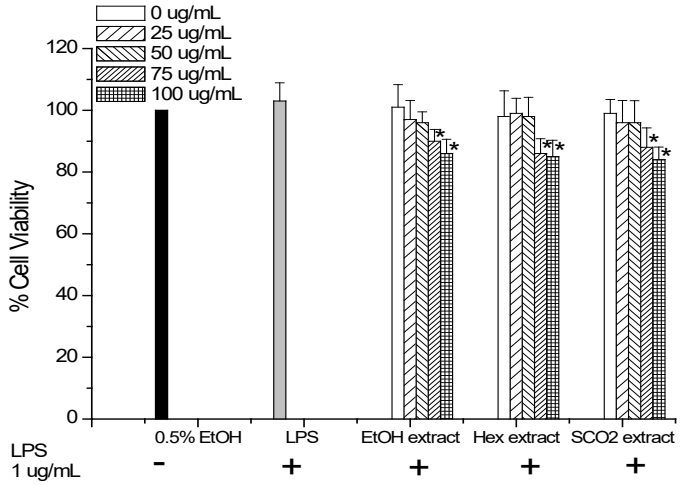

Figure 1 Effects of LPS and/or EtOH, Hex and SCO_2 extracts (0 - 100 μg/mL) for 24 h on the viability of RAW 264.7 cells using MTT assays. Values are the means ± SEM of 3 independent experiments. $^{*}p < 0.05$ indicated significant differences from the control group.

The effects of the 3 different solvent extracts from *Ipomoea pes-caprae* on DPPH scavenging activity

The free radical scavenging activity of the *Ipomoea pes-caprae* extracts was performed, and a concentration-response curve to DPPH assay was obtained in **Figure 2**. The highest scavenging was observed with SCO_2 extract with an IC_{50} value of 9.82 ± 1.17 μg/mL as different from the IC_{50} value of ascorbic acid (4.45 ± 0.66 μg/mL), which is a well-known antioxidant. Hex and EtOH extracts showed scavenging activity with an IC_{50} value of 17.20 ± 2.75 and 25.14 ± 4.27 μg/mL, respectively. The stable DPPH radical contains an odd electron, which can accept an electron donated by an antioxidant compound. Consequently, DPPH test can be quantitatively measured radical scavenging action. In these results, the Hex extract was better than that reported by Banerjee *et al.* [22]. The antioxidant activities of many medicinal plants have a strong correlation with their phenolic compounds because its hydroxyl group donates hydrogen to form stable radical intermediates [23]. Nevertheless, it is also possible to study the *in vitro* antioxidant potential of *Ipomoea pes-caprae* extracts in terms of other experimental models such as measurement of intracellular ROS production.

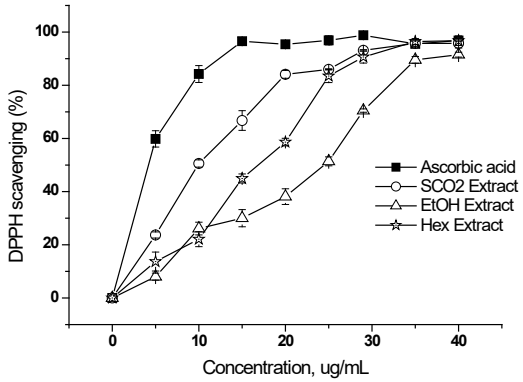

Figure 2 Effects of EtOH, Hex and SCO_2 extracts on DPPH radical scavenging activity. Ascorbic acid was used as the positive control. Data were represented as the means ± SEM of triplicate experiments.

The effects of the 3 different solvent extracts from *Ipomoea pes-caprae* on intracellular ROS

RAW264.7 cells were labeled with the ROS-sensitive fluorescence DCFH-DA that applied to viable cells. The non-fluorescent DCFH-DA dye penetrated the cell membrane and hydrolyzed by esterase to become DCFH [14]. In the presence of ROS, DCFH is oxidized to the fluorescent DCF that can be monitored by flow cytometer. As shown in **Figure 3**, LPS alone was found significantly increased in the ROS level compared to that in the untreated cells. The DCF fluorescent intensity in the LPS group was designated as 100 % and used to calculate the relative expression of DCF fluorescence in other groups. Treatment of 3 different solvents of *Ipomoea pes-caprae* extracts at 25 and 50 μg/mL caused significant reduction of intracellular ROS level ($P < 0.05$). The inhibition percentage of ROS activity at a concentration of 50 μg/mL was in the order of SCO_2 > EtOH > Hex with the percentage inhibition of 56.3 ± 5.68, 47.6 ± 8.24 and 33.0 ± 9.6 %, respectively. LPS produced ROS in macrophages via nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase), resulting in ROS generation as indicated by the increase in DCF fluorescence [24]. Concomitant treatment with *Ipomoea pes-caprae* extracts were found inhibiting the LPS-induced ROS production. These results obtained from a cell-based antioxidant activity assay revealed consistency with the cell free DPPH radical system. Therefore, these results confirmed that *Ipomoea pes-caprae* extracts at non cytotoxic concentration scavenged free radicals in RAW264.7 cells. In addition, the oxidative damage to cellular components may lead to changes in the expressions of inflammatory mediators and cytokines, which are involved in inflammation-related diseases.

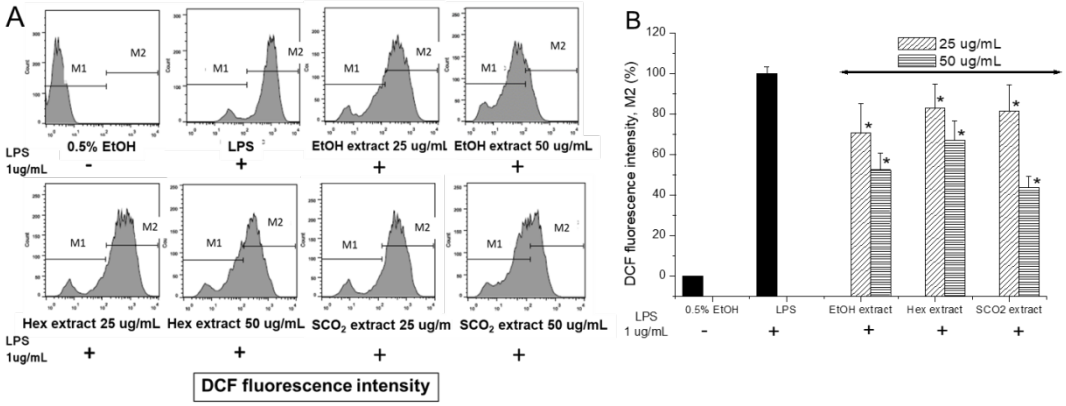

Figure 3 Effects of EtOH, Hex and SCO_2 extracts on LPS induced ROS production in RAW 264.7 cells. The cells were incubated with 0 - 50 μg/mL of extractions in the presence or absence of LPS (1 μg/mL) for 24 h. (A) One representative histogram of flow cytometry at indicated concentrations. (B) Mean fluorescence intensities (M2) ± SEM of triplicate experiments. The LPS group was set to 100 %, and the values of other groups were reliable against it. $^*P < 0.05$ indicated significant differences from the LPS-treated group.

The effects of the 3 different solvent extracts from *Ipomoea pes-caprae* on NO and PGE_2 production

The NO production was measured as nitrite as shown in **Figure 4**. When RAW 264.7 cells were treated with 1 μg/mL of LPS, the NO production increased to 32.4 ± 5.33 μM as compared to 2.22 ± 0.22 μM in the control cells without LPS. All 3 different solvent extracts (25 - 50 μg/mL) significantly reduced LPS-induced NO production ($P < 0.05$). EtOH, Hex and SCO_2 extracts (50 μg/mL) significantly inhibited LPS-induced NO production to 16.4 ± 3.42, 13.34 ± 1.47, 11.1 ± 1.05 μM, respectively with no cytotoxicity to the RAW 264.7 cells. The percentage of NO inhibition was equal to 50, 59 and 65 %, respectively. The PGE_2 production in LPS-induced RAW 264.7 cells were shown in **Figure 5** indicating that LPS treatment (1 μg/mL) increased the amount of PGE_2 formation (1843 ± 93 pg/mL) as compared to untreated controls (180 ± 80 pg/mL). Treatments with EtOH, Hex, and SCO_2 extracts (50 μg/mL) inhibited LPS-induced PGE_2 formation to 1351 ± 96, 1077 ± 121, 812 ± 105 pg/mL, respectively. The percentage of PGE_2 inhibition was equal to 26, 41 and 55 %, respectively. EtOH, Hex, and SCO_2 extracts showed a similar pattern to that of inhibiting NO and PGE_2 production in the LPS induced RAW 264.7 cells, and the relative effectiveness of these extracts was SCO_2 > Hex > EtOH. It has been well established that LPS, outer membrane of Gram-negative bacteria, activated the release of inflammatory mediators through toll like receptor 4 (TLR4) on the cell membrane of the macrophages. The overproduction of inflammatory mediators such as NO, PGE_2, interleukin (IL)-6, IL-1β, and tumour necrosis factor (TNF)-α can damage the host, leading to various chronic inflammatory diseases. Thus, a decrease in the LPS induced expression of inflammatory mediators in macrophages may be useful for preventing the disease in association with inflammation [25].

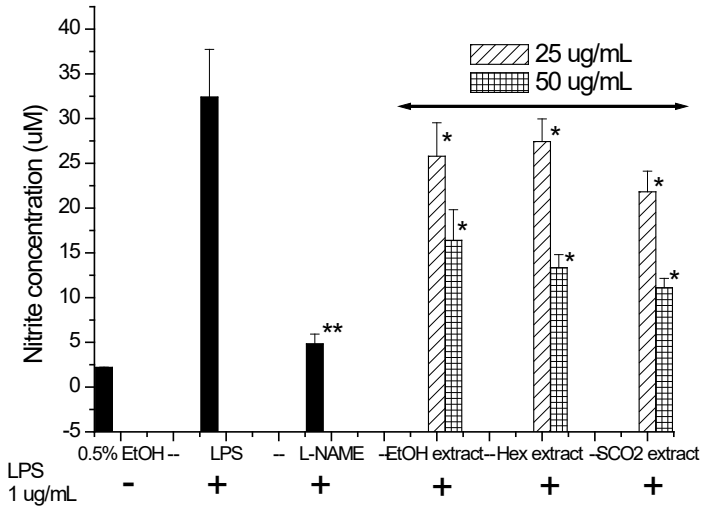

Figure 4 Effects of EtOH, Hex and SCO_2 extracts on LPS induced nitrite production in RAW 264.7 cells. The cells were incubated with 0 - 50 μg/mL of extractions in the presence or absence of LPS (1 μg/mL) for 24 h. L-NAME was used as the positive control. $^{*}P < 0.05$ and $^{**}P < 0.01$ indicated significant differences from the LPS-treated group.

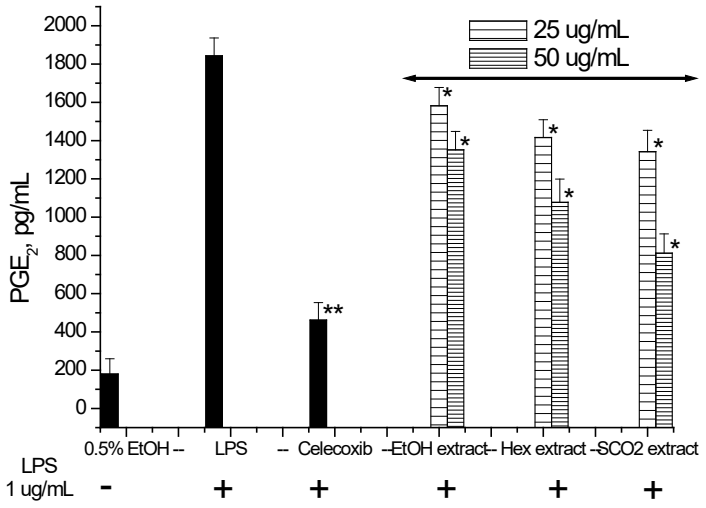

Figure 5 Effects of EtOH, Hex and SCO_2 extracts on LPS induced PGE_2 production in RAW 264.7 cells. The cells were incubated with 0 - 50 μg/mL of extractions in the presence or absence of LPS (1 μg/mL) for 24 h. Celecoxib was used as the positive control. $^{*}P < 0.05$ and $^{**}P < 0.01$ indicated significant differences from the LPS-treated group.

The effects of the 3 different solvent extracts from *Ipomoea pes-caprae* on iNOS and COX-2 mRNA levels

Figure 6 represented the change in mRNA expression for iNOS and COX-2 based on their relative quantification values. LPS at 1 μg/mL induced a significant increase in iNOS and COX-2 mRNA expression as when compared to control cells without LPS. EtOH, Hex, and SCO_2 extracts inhibited iNOS and COX-2 mRNA expression, and the results were consistent with the profile of the inhibition NO and PGE_2 release. These data have also been confirmed at *in vivo* level, demonstrating the reduction of acute inflammation using the writhing test and carrageenan-induced pleurisy models in mice [2] and reduced chronic inflammation using the cotton pellet-induced granuloma model [3]. These results also agreed with Pongprayoon *et al*. [1] reporting that *Ipomoea pes caprae* extract contained active compounds such as eugenol exhibited anti-inflammation via the inhibition of prostaglandin activity. This supposed mechanism is similar to that of L-NAME and celecoxib, the drug used as the positive control, which mediated anti-inflammation via inhibiting NO and PGE_2 productions. In addition, the results from previous studies demonstrated that iNOS has been correlated with the synthesis of PGE_2 in activated macrophages with LPS and resulted in the production of inflammatory diseases [26]. Therefore, targeting the iNOS inhibitor and the COX-2 inhibitor might be beneficial in treating inflammatory-mediated diseases and cancer.

The inhibition of NO and PGE2 production is the main pillars in managing inflammatory and other painful conditions. Overproduction of inflammatory mediators such as NO and PGE2 is highly related to chronic inflammatory disease and will make the inflammatory microenvironment vulnerable to cancer progression. Key molecular cytokines linking inflammation to cancer are NO and PGE2 production in that cytokine signaling could increase microvascular density, leading to an increase of cancer cell proliferation and those by the inhibition of apoptosis [7]. In agreement with our previous studies, Hex and SCO_2 extracts of *Ipomoea pes-caprae* induced apoptosis in KB cells and may be stated as a valid strategy for chemoprevention [12]. A review of the literature showed that the redox status has been closely linked with free radicals, inflammation and cancer. These findings and our previous studies of Ipomoea pes-caprae extracts showed the most promising free radical scavenging and anti-inflammatory and anticancer activities, providing *in vitro* evidence.

The extraction of medicinal plants is an important activity in today's world. Among all the extracts, the relative effectiveness for anti-inflammation was SCO_2 > Hex > EtOH. For general bioactivity screening, the different solvents with different polarities were used in an arrangement from the non-polar to the polar (Hex > SCO_2 > EtOH). In supercritical fluid extraction, carbon dioxide was inherently non-polar solvent, and the addition of ethanol as co-solvents enhanced the solubility of polar molecules;

accordingly, the SCO_2 extract had both polar and non-polar substances. In addition, supercritical fluid extraction has gained popularity for speedy, contamination-free extraction and retrieval of various bio-active compounds [27]. Further, in accordance with our previous results, the chemical compositions of these extracts as compared by gas chromatography showed that Hex and SCO_2 had comparable chemical composition patterns. However, their compositions had more ingredients than those obtained from EtOH extract [12]. In general, the mangrove plants often produce secondary metabolites under stressful high salt conditions. Therefore, it is not surprising that mangrove plants biosynthesis a wide range of secondary metabolites to treat several ailments, including inflammatory-related diseases. Thus, this study provides *in vitro* evidence for the use of Thai traditional plants, *Ipomoea pes-caprae* extracts, as antioxidant, anti-inflammatory activities and confirm the traditional use of this plant toward dermatitis caused by jellyfish stings.

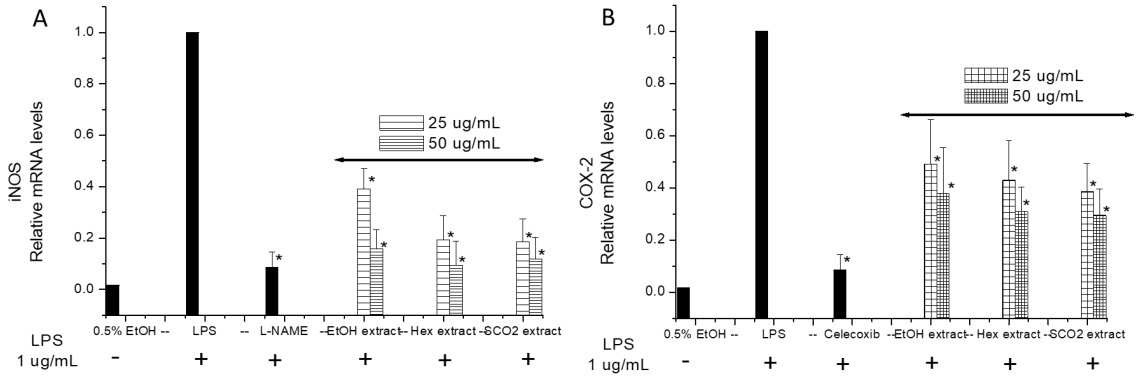

Figure 6 Effects of EtOH, Hex and SCO_2 extracts on LPS induced iNOS (A) and COX-2 (B) mRNA expression in RAW 264.7 cells. The cells were incubated with 0 - 50 μg/mL of extractions in the presence or absence of LPS (1 μg/mL) for 24 h. $^{*}P < 0.05$ indicated significant differences from the LPS-treated group.

Conclusions

EtOH, Hex, and SCO_2 extracts of *Ipomoea pes-caprae* prepared by different extraction methods possessed significant antioxidant and anti-inflammatory activities at non-cytotoxic dose. These effects of *Ipomoea pes-caprae* extracts resulted from the synergistic effect of many phytochemicals compounds in this plant. SCO_2 extract exhibited the strongest activities in scavenged DPPH radical, inhibited radical-mediated cellular oxidation and suppressed NO and PGE_2 production through the regulation of iNOS and COX-2 expression at the mRNA level. Thus, these bioactive compounds contained in *Ipomoea pes-caprae* may function as therapeutic candidates for various inflammatory related diseases.

Acknowledgements

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