**Marantodes pumilum** (Blume) Kuntze Inhibited Secretion of Lipopolysaccharide- and Monosodium Urate Crystal-stimulated Cytokines and Plasma Prostaglandin E₂

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**ABSTRACT**

Background: *Marantodes pumilum* is traditionally used for dysentery, gonorrhea, and sickness in the bones. Previous studies revealed its antibacterial and xanthine oxidase inhibitory activities. **Objective:** To evaluate the inhibitory effects of three *M. pumilum* varieties on the secretion of lipopolysaccharide (LPS)- and monosodium urate crystal (MSU)-induced cytokines and plasma prostaglandin E₂ (PGE₂) in vitro. **Materials and Methods:** The leaves and roots of *M. pumilum* var. alata (MPL), *M. pumilum* var. pumila (MPP), and *M. pumilum* var. lanceolata (MLP) were successively extracted with dichloromethane (DCM), methanol, and water. Human peripheral blood mononuclear cells and ELISA technique were used for the cytokine assay, whereas human plasma and radioimmunoassay technique were used in the PGE₂ assay. Flavonoids content was determined using a reversed-phase high-performance liquid chromatography. **Results:** DCM extract of MPL roots showed the highest inhibition of LPS-stimulated cytokine secretion with IC₅₀ values of 29.87, 76.2, 5.84, 25.33, and 5.40 µg/mL for interleukin (IL)-1α, IL-1β, IL-6, IL-8, and tumor necrosis factor (TNF)-α, respectively, while that of plasma PGE₂ secretion was given by DCM extract of MPP roots (IC₅₀ 31.10 µg/mL). Similarly, the DCM extract of MPL roots demonstrated the highest inhibition against MSU-stimulated IL-1α, IL-1β, IL-6, IL-8, TNF-α, and PGE₂ secretion with IC₅₀ values of 11.2, 8.92, 12.29, 49.51, 9.60, and 31.58 µg/mL, respectively. Apigenin in DCM extracts of MPL (0.061 mg/g) and MPP (0.064 mg/g) roots could be responsible for the strong inhibitory activity against IL-1β, IL-6, TNF-α, and PGE₂. **Conclusion:** The results suggested that DCM extracts of MPL and MPP roots are potential anti-inflammatory agents by inhibiting the secretion of LPS- and MSU-stimulated pro-inflammatory cytokines and PGE₂.

**Key words:** Lipopolysaccharide, *Marantodes pumilum*, monosodium urate crystals, pro-inflammatory cytokines, prostaglandin E₂

**SUMMARY**

- Amongst 18 tested extracts, DCM extracts of MPL and MPP roots remarkably inhibited LPS- and MSU-stimulated pro-inflammatory cytokines and PGE₂ secretion
- Phytochemical analysis was performed for the active extracts using RP-HPLC system
- The presence of flavonoids particularly apigenin could be responsible for the anti-inflammatory activity.

**Abbreviations used:** BSA: Bovine serum albumin, COX-2: Cyclooxygenase-2, CPM: Count per minute, DAMP: Danger-associated molecular pattern, DCM: Dichloromethane, DMSO: Dimethyl sulfoxide, ELISA: Enzyme-linked immunosorbent assay, FBS: Fetal bovine serum, H₂O₂: Water, HEPES: 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid, HMC-1: Human mast cell-1, HMG1: High-mobility group box 1, ICAM: Intercellular adhesion molecule, IFN: Interferon, IgG: Immunoglobulin G, IKK: IκB kinase, IL: Interleukin, iNOS: Inducible nitric oxide synthase, LPS: Lipopolysaccharide, MeOH: Methanol, MPA: *Marantodes pumilum* var. alata, MPL: *Marantodes pumilum* var. lanceolata, MPP: *Marantodes pumilum* var. pumila, MSU: Monosodium urate, MT: Methylthiazole tetrazolium, NFκB: Nuclear factor-kappa B, NLR: NOD-like receptor, NLRP3: NLR family pyrin domain containing protein 3, NO: Nitric oxide, NOD: Nucleotide-binding oligomerization domain, NOSAI: Nonsteroidal anti-inflammatory drug, PAMP: Pathogen-associated molecular pattern, PBMC: Peripheral blood mononuclear cell, PBS: Phosphate buffered saline, PGE₂: Prostaglandin E₂, PMACI: Phorbol-12-myristate 13-acetate and calcium ionosphere A23187, PRR: Pathogen recognition receptor, RTFE: Polytetrafluoroethylene, RIA: Radioimmunoassay, RIG: Retinoic acid-inducible gene I, RLR: RIG-I like receptor, RP-HPLC: Reversed-phase high-performance liquid chromatography, RPMI-1640: Roswell Park Memorial Institute-1640, TR: Toll-like receptor, TNF: Tumor necrosis factor, VCAM: Vascular cell adhesion molecule.

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nucleotide-binding oligomerization domain-like receptors (NLRs), and retinoic acid-inducible gene I-like receptors, which differentiate pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs) from normal host protein. PAMPs include various bacterial cell wall components such as lipopolysaccharide (LPS), lipopeptides, peptidoglycans, and teichoic acid that initiate the infectious pathogen-induced inflammation. LPS is well known as the best studied model on innate immunity in order of its role as the prototypic activator of those response. On the other hand, DAMPs include protein DAMPs such as high-mobility group box 1 (HMG1) and hyaluronic fragments, and nonprotein DAMPs such as uric acid. High level of uric acid (>7 mg/dL) leads to crystallization of monosodium urate that causes gouty inflammation. During inflammatory response, a variety of soluble factors are involved in leukocyte recruitment; for example, some derivatives of arachidonic acid including prostaglandins, leukotrienes, and lipoxins. The main therapeutic treatment for inflammation is by administration of steroids and nonsteroid anti-inflammatory drugs that may cause adverse effects such as gastrointestinal toxicity, renal toxicity, or gastrointestinal bleeding. Thus, there is a need to identify alternative anti-inflammatory agents from natural resources that can be used for the treatment of inflammatory disorders.

Marantodes pumilum (Blume) kunze (Primulaceae) is previously named as Labisia pumila (Blume) Fern.-Vill or locally known as Kacip Fatimah in Malaysia. It is a popular herb that has been used by many generations of the Malay women as a traditional medicine for reproductive-related conditions, including to induce and facilitate childbirth and to help women regain strength after giving birth. The preparations have also been used for flatulence, dysentery, dysmenorrhea, gonorrhea, and sickness in the bones. Three varieties of M. pumilum are mainly found in Malaysia, namely, M. pumilum (Blume) Kunze var. alata (Scheff.). Mez. var. pumila, and var. lanceolata (Scheff). Mez. and these can be differentiated based on the characteristics of petiole and leaf-shape, microscopic anatomy, as well as phytochemically. The popularity of traditional herbal products containing M. pumilum has tempted many researchers to investigate the phytochemistry and pharmacological actions of this plant.

Several phytochemical compounds have been characterized, including phenolics (e.g., gallic acid, caffeic acid, pyrogallol, benzoic acid, cinnamic acid, and methyl gallate); flavonoids (e.g., quercetin, myricetin, kaempferol, naringin, and rutin); flavanols (e.g., catechin and epigallocatechin); and isoflavonoids (e.g., daidzein and genistin). Strong antioxidant compounds such as β-carotene and ascorbic acid were also found in M. pumilum. Triterpenoid saponins, alkaloid compounds, and benzooquinone derivatives were also isolated. These compounds have been found to be responsible for many biological activities such as antibacterial, antioxidant, anti-inflammatory, and anticancer. Previous scientific studies have also reported various activities of M. pumilum extracts including antioxidant, antibacterial, antifungal, anticarcinogenic, xanthine oxidase inhibition, and anti-inflammatory.

Karimi et al. reported that M. pumilum inhibited nitric oxide (NO) release in RAW 264.7 cells induced with LPS and interferon-γ in addition, M. pumilum has been used traditionally for bacterial infection such as dysentery and gonorrhea that could initiate inflammatory response. The plant has also been used for bone sickness including gout. However, to the best of our knowledge, the effect of M. pumilum on bacterial and gouty inflammation in pro-inflammatory cytokines and prostaglandin E2 (PGE2) secretion remained to be investigated. The rationale of using LPS and monosodium urate crystals (MSU) to induce inflammation was to mimic the bacterial infection and gouty inflammation pathways, respectively. Thus, this study was aimed to determine the effect of extracts of M. pumilum varieties on the inhibition of LPS- and MSU-stimulated cytokines and plasma PGE2 secretion in vitro. The work will provide some insights for the development of a natural anti-inflammatory agent and an evidence-based herbal supplement.

**MATERIALS AND METHODS**

**Materials**

All chemicals and organic solvents used in this work were of analytical grade. Solvents were purchased from Merck (Darmstadt, Hesse, Germany). High-performance liquid chromatography (HPLC)-grade methanol (MeOH) and acetonitrile were obtained from Merck (Darmstadt, Hesse, Germany) while HPLC-grade orthophosphoric acid was obtained from Fisher Scientific (Loughborough, Leicestershire, UK). HPLC-grade gallic acid (99%), caffeic acid (98%), myricetin (96%), quercetin (98%), apigenin (95%), and kaempferol (97%) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Dexamethasone was supplied from Hospital Canselor Tuanku Muhirz of Universiti Kebangsaan Malaysia. Lymphoprep was obtained from Axis-Shield PoC AS (Oslo, Norway), while unlabeled PGE2, anti-PGE2, LPS from Salmonella abortus equii, uric acid, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, thiazolyl blue (methyl thiazol tetrazolium [MTT]), 20 mM 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), fetal bovine serum (FBS), penicillin-streptomycin solution, Roswell Park Memorial Institute (RPMI)-1640 medium containing L-glutamine, dextran from Leuconostoc mesenteroides, and indomethacin were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Phosphate buffered saline (PBS) was purchased from MP Biomedicals (USA) while bovine serum albumin (BSA) and activated charcoal were purchased from Merck (Darmstadt, Hesse, Germany). IL-8 ELISA kits were purchased from Abnova, Germany, while all of the other kits were purchased from Cayman, USA. Radiolabeled PGE2 (125I-[H]-PGE2, 50 μCi) and liquid scintillation cocktail were purchased from Perkin Elmer (Massachusetts, USA). The leaves and roots of three varieties of M. pumilum were collected from Hutan Gunung Bujang Melaka, Kampar, Perak, Malaysia. The plants were authenticated by Emeritus Professor Dato’ Dr. Abdul Latiff Mohamad. Voucher specimens of M. pumilum var. alata (herbarium number: UKMB 30006/SIM 2622), M. pumilum var. pumila (MPR, UKMB 30007/SIM s.n.), and M. pumilum var. lanceolata (MPL, UKMB 30008/SIM s.n.) were deposited in the herbarium of Universiti Kebangsaan Malaysia.

**Preparation of plant extracts**

The leaves and roots of plant materials were separated, air-dried, and ground. Each dried powder was individually extracted with dichloromethane (DCM) and MeOH by sequential exhaustive maceration. The final residue was then extracted under reflux with distilled water (H2O) for 2 h at 80°C. The organic filtrate was collected and concentrated under reduced vacuum pressure, while the H2O extract was freeze-dried. The percentage yield of extract was calculated with respect to its air-dried powder [Table 1]. The extract was dissolved in dimethyl sulfoxide (DMSO; the final concentration of DMSO was not exceeded 0.5% in medium).

**Study subjects**

Human blood was obtained from healthy volunteers (n = 3, ≥18 years old) who fulfilled the inclusion criteria of nonsmoker, fasted overnight, and had not taken any medicines or supplements. The experimental protocol for the cytokines and PGE, assays was approved by the Human Ethical Committee of Universiti Kebangsaan Malaysia with an approval number of UKM 1.5.3.5/244/NF-040-2011 and UKM.
Table 1: Percentage yield of extracts

| Specimens | Plant parts | Percentage yield (% w/w)* |
|-----------|-------------|---------------------------|
|           | DCM | MeOH | H₂O |
| MPA       | Roots   | 0.85 | 8.08 | 6.00 |
|           | Leaves | 1.80 | 2.23 | 2.74 |
| MPP       | Roots   | 1.59 | 4.63 | 2.58 |
|           | Leaves | 11.38 | 1.09 | 4.56 |
| MPL       | Roots   | 3.16 | 7.21 | 5.16 |
|           | Leaves | 3.30 | 8.70 | 1.03 |

*Percentage yield was calculated based on dry weight. DCM: Dichloromethane; MeOH: Methanol; H₂O: Water; MPA: Marantodes pumilum var. alata; MPP: Marantodes var. pumila; MPL: Marantodes var. lanceolata

1.5.3.5/244/NF-016-2013, respectively. The protocol was in accordance with the principles outlined in the Declaration of Helsinki.[24]

Cytokine assay

**Cell preparation and viability test**

Cell preparation procedure was based on the method of Boyum with slight modification.[24] Briefly, fresh heparinized venous blood from healthy volunteers was diluted in 1:1 ratio with RPMI-1640 medium containing L-glutamine. Peripheral blood mononuclear cells (PBMCs) were separated from the blood using Lymphoprep gradient centrifugation at 600 g for 20 min.[26] The cells were washed twice with RPMI-1640 medium, resuspended in a complete RPMI-1640 medium buffered with HEPES to pH 7.4, and supplemented with 100 U/mL penicillin, 100 µg/mL streptomycin, and 10% FBS (hereafter referred to as a complete medium). The cells were counted using a hemocytometer under a light microscope and then adjusted up to 5 x 10⁶ cells/mL. Cell viability was determined by MTT assay as described by Mosmann with slight modification by using DMSO to dissolve the formazan crystals.[27,28] The PBMCs (100 µL) were incubated at 37°C with 5% CO₂ for 3 h with 100 µL of extracts (50 and 100 µg/mL) or dexamethasone (0.5 and 5 µg/mL) or complete medium with 0.5% DMSO as a negative control. The concentration of extracts used in MTT assays was based on the previous study by Karimi et al.,[25] who performed NO assay on RAW 266.7 cells. After 27 h, 20 µL of MTT (5 mg/mL in sterile PBS) was added to each well and incubated again for 4 h. The supernatant was carefully discarded. The formazan blue crystals formed by cells were dissolved in 100 µL of DMSO (100%) and the absorbance was measured at a wavelength of 570 nm using a microplate reader (Multiskan Go, Thermo Fisher Scientific, USA).

**Determination of cytokine level**

PBMCs (100 µL) were preincubated at 37°C with 5% CO₂ for 3 h with 100 µL of either extracts as test samples or dexamethasone as a positive control with final concentrations being 50 and 5 µg/mL, respectively, while 0.5% DMSO in complete medium was used as a negative control. After 3 h, 20 µL of LPS (1 µg/mL) was added to induce cytokine release from cells and the mixture was incubated based on the type of cytokines, i.e., 12 h for tumor necrosis factor (TNF)-α and interleukin (IL)-1β, 20 h for IL-6, and 24 h for IL-1α and IL-8. Meanwhile, for MSU-induced inflammation, cells were incubated with 20 µL of MSU crystal suspension (200 µg/mL) for 24 h for all cytokines.[29-31] After incubation, cells were centrifuged at 300 x g and 4°C for 10 min to obtain cell-free supernatants and the resultants were stored at -80°C until analysis. The concentration of cytokines was tested using appropriate ELISA kits for human cytokines according to manufacturer’s instruction. The cytokine level was measured using a microplate reader (BioTek, USA). Limit of detection of these kits were 2.0 pg/mL for IL-8, 7.8 pg/mL for IL-6, and 3.9 pg/mL for all the others. The cytokine secretion levels were compared with the negative control that was considered as 100% cytokine secretion. The percentage inhibition (% I) was calculated using equation (1):

\[
\% I = \left(1 - \frac{[\text{Concentration of cytokine or PGE}_2 \text{ in sample or positive control}]}{[\text{Concentration of cytokine or PGE}_2 \text{ in negative control}]} \right) \times 100 \%
\]

The IC₅₀ values were determined for the active extracts in five concentrations ranging from 3.125 to 50 µg/mL.

**Prostaglandin E₂ assay**

**Plasma preparation**

Fresh heparinized blood from healthy volunteers were incubated at 37°C containing 5% CO₂ for 24 h with LPS or MSU after preincubation for 30 min in the presence or absence of extracts (50 µg/mL) or indomethacin (10 µg/mL) as a positive control or 0.5% DMSO in radioimmunoassay (RIA) buffer (PBS [0.01 M; pH 7.4] containing BSA [0.1%] and sodium azide [0.1%]) as a negative control. After incubation, the blood was centrifuged at 3000 x g for 15 min at 4°C and the plasma supernatant was removed carefully and immediately used for the assay.[32]

**Radioimmunoassay of plasma prostaglandin E₂ secretion**

The assay was carried out according to the modified method of Saadawi et al. and Patrignani et al.[32,33] A reaction mixture containing 100 µL of plasma, 100 µL of anti-PGE₂ (diluted with ratio of 1:50,000), and 100 µL of [³⁵S]-PGE₂ (0.1 µCi/mL) was incubated at 4°C for 24 h. After incubation, 200 µL of dextran-coated charcoal was added to the mixture and incubated again for 10 min at 4°C. After centrifugation at 3000 x g for 15 min at 4°C and 300 µL of supernatant was added to 3 mL liquid scintillation cocktail in Pico Pro Vial” (Perkin Elmer, Massachusetts, USA). The radioactivity was measured using a liquid scintillation analyzer (Packard Tri-Carb, Models B3110TR, Hamburg, Germany). The average count per min value of standards and samples was calculated by subtracting the value of antibody-antigen binding in the samples (B) with nonspecific binding (NC; [³⁵S]-PGE₂ and RIA buffer) together with the total binding between antibody and antigen (Bo; [³⁵S]-PGE₂, anti-PGE₂, and RIA buffer). The normalized percentage bound (%B/Bo) was then calculated using equation (2).

\[
\% \frac{B}{Bo} = \left( \frac{B - NC}{Bo - NC} \right) \times 100 \%
\]

The %B/Bo values were plotted using semi-logarithmic graph against the corresponding concentration of standard PGE₂, in picogram. The %B/Bo values of serial dilutions of standard PGE₂ with concentrations ranging from 2.45 to 400 pg/0.1 mL were used to obtain a standard curve plot. The concentration of PGE₂ (pg/0.1 mL) in each sample was determined by interpolating the %B/Bo values using standard curve. Percentage inhibition was calculated by using equation (1). The IC₅₀ values were determined for the active extracts and indomethacin at five concentrations ranging from 3.125 to 50 µg/mL and from 0.625 to 10 µg/mL, respectively.

**High-performance liquid chromatography analysis**

Phytochemical analysis was performed for the most active extracts, i.e., DCM root extracts of MPL and MPP, using an RP-HPLC based on the method described by Karimi et al. to quantify the amount of quercetin and apigenin.[13] The extracts solution (100 mg/mL) and a mixture of reference standard solution containing gallic acid, caffeic acid, myricetin, quercetin, apigenin, and kaempferol (1 mg/mL) were
prepared in HPLC-grade MeOH, sonicated for 5 min, and filtered through 0.45 µm Millipore Millex PTFE membrane (Maidstone, Kent, UK) before analysis using the following validated HPLC conditions: XBridge C-18 column (250 mm × 4.6 mm, 5 µm; Waters, Dublin, Ireland), photodiode array detector (Waters 2998 with Waters Prep Degasser; Waters, Dublin, Ireland) at a wavelength of 330 nm, flow rate of 0.6 mL/min, mobile phase consisted of acetonitrile (A) and acidified H₂O with 0.1% orthophosphoric acid (B) and eluted by a linear gradient of 15%–85% A and 85%–15% B (0–55 min), and isocratic composition of 85% A and 15% B (55–60 min). The column was equilibrated for 20 min before the next injection. Injection volume of each solution was 10 µL.

Statistical analysis
All of the data were analyzed using GraphPad Prism 5 software (GraphPad Software, Inc., La Jolla, CA, USA). Each experiment was carried out in duplicate determinations from three independent experiments (n = 3) and presented as mean ± standard error of mean. Data were analyzed using one-way analysis of variance and post hoc Tukey's test for multiple comparisons and P ≤ 0.05 was considered statistically significant. The IC₅₀ values were determined using GraphPad Prism 5 software.

RESULTS
Cell viability of human peripheral blood mononuclear cells
The concentration of 50 µg/mL of all M. pumilum extracts and 5 µg/mL of dexamethasone gave >90% cell viability [Figure 1]. Thus, the respective concentrations were used as the highest concentration in this experiment.

Effect of Marantodes pumilum extracts on cytokine secretion in lipopolysaccharide-stimulated human peripheral blood mononuclear cells
Of 18 extracts tested, three extracts were strongly active against the cytokine assay [Table 2]. DCM extract of MPL roots had the highest inhibitory activity for five cytokine secretions (IL-1α, IL-1β, IL-6, IL-8, and TNF-α) with values of 99.20, 99.91, 99.65, 51.65, and 100.04%, respectively, followed by MPP roots that inhibited secretion of IL-1α (88.87%), IL-6 (99.68%), and TNF-α (70.29%), while MeOH extract of MPL leaves inhibited only TNF-α (51.15%). The extracts showed inhibition of cytokine secretion in a concentration-dependent manner. Interestingly, DCM extract of MPL roots gave significantly higher (P ≤ 0.05) inhibitory activity against IL-1α, IL-1β, IL-6, and TNF-α compared to dexamethasone, while that of IL-8 was comparable to dexamethasone (P > 0.05). Moreover, DCM extract of MPP roots also had significantly higher (P ≤ 0.05) inhibitory activity against IL-1α and IL-6 and comparable to dexamethasone (P > 0.05) against TNF-α secretion.

As presented in Table 3, DCM extract of MPL roots was found to have the strongest inhibitory activity with IC₅₀ values of 29.87, 7.62, 5.84, 25.33, and 5.40 µg/mL for IL-1α, IL-1β, IL-6, IL-8, and TNF-α, respectively, followed by DCM extract of MPL roots but only for IL-1α (15.86 µg/mL), IL-6 (5.87 µg/mL), and TNF-α (19.92 µg/mL). All active extracts had significantly lower activity compared to dexamethasone (IC₅₀ values of 0.90, 0.03, 0.89, 1.94, and 0.08 µg/mL for IL-1α, IL-1β, IL-6, IL-8, and TNF-α, respectively; P ≤ 0.001).

Effect of Marantodes pumilum extracts on plasma prostaglandin E₂ secretion in lipopolysaccharide -stimulated human whole blood
At the concentration of 50 µg/mL, the DCM extract of MPP roots showed the highest inhibition (72.78%) compared to indomethacin (90.56%) [Table 4]. The results also showed that the DCM extracts of MPL roots, MPL leaves, and MPP leaves, as well as MeOH extract of MPL leaves were active with inhibitory values of 65.79, 63.76, 53.19, and 50.07%, respectively. The IC₅₀ determination revealed that the DCM extract of MPP roots inhibited the secretion of plasma PGE₂ in a concentration-dependent manner with an IC₅₀ value of 31.1 µg/mL followed by DCM extracts of MPL roots (33.66 µg/mL), MPL leaves (37.27 µg/mL), and MPP leaves (48.79 µg/mL) and MeOH extract of MPL leaves (49.81 µg/mL). All extracts had significantly higher IC₅₀ values than indomethacin (IC₅₀ = 0.52 µg/mL; P ≤ 0.001).

Effect of Marantodes pumilum extracts on cytokine secretion in monosodium urate crystals-stimulated human peripheral blood mononuclear cells
In this study, two DCM extracts of MPP and MPL roots (50 µg/mL) actively inhibited cytokine secretion [Table 5]. DCM extract of MPL roots significantly inhibited the secretion of IL-1α (94.96%), IL-1β (99.91%), IL-6 (97.04%), and TNF-α(97.65%) compared to dexamethasone (P ≤ 0.05); however, inhibition of IL-8 (51.74%) was comparable to dexamethasone at

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**Figure 1:** Viability of peripheral blood mononuclear cells after 27 h of exposure to extracts of *Marantodes pumilum*, dexamethasone, and 0.5% of dimethyl sulfoxide. Data are presented as mean ± standard error of mean (n = 3)
concentration of 5 µg/mL (P > 0.05). The activity was followed by MPP roots that inhibited the secretion of IL-1α (73.96%), IL-6 (80.91%), and TNF-α (64.74%) and was statistically comparable (P > 0.05) for IL-1α and TNF-α and significantly higher (P ≤ 0.05) for IL-6 than dexamethasone. Determination of IC₅₀ value of active extracts demonstrated that inhibitory activity was in a concentration-dependent manner. DCM extract of MPP roots strongly inhibited IL-1α, IL-1β, IL-6, and TNF-α with IC₅₀ values of 11.2, 8.92, 12.29, and 9.60 µg/mL, respectively. However, as presented in Table 6, all extracts possessed significantly lower IC₅₀ values compared to dexamethasone (IC₅₀ values of 0.02, 0.71, 0.46, 0.68, and 0.15 µg/mL for IL-1α, IL-1β, IL-6, IL-8, and TNF-α, respectively; P ≤ 0.001).

Effect of Marantodes pumilum extracts on plasma prostaglandin E₂ secretion in monosodium urate crystals-stimulated human whole blood

Only six extracts (50 µg/mL) were found to actively inhibited plasma PGE₂ secretion [Table 7]. DCM extract of MPP roots had the highest inhibitory activity with a value of 72.87%, followed by DCM extracts of MPP roots (66.08%) and MPL leaves (65.72%) and MeOH extracts of MPP roots (54.45%), MPL leaves (52.08%), and MPP leaves (50.28%). However, all active extracts gave significantly lower activity (P ≤ 0.05) compared to indomethacin (10 µg/mL), with an inhibition value of 97.45%. The lowest IC₅₀ value was obtained for DCM extract of MPL roots (31.58 µg/mL), followed by DCM extract of MPP roots (33.01 µg/mL) and DCM extract of MPL leaves (35.26 µg/mL). All extracts showed a concentration-dependent inhibition; however, the activity was significantly lower (P ≤ 0.001) compared to indomethacin (IC₅₀ = 0.35 µg/mL).

High-performance liquid chromatography analysis

Of 18 extracts tested for bioassays, only DCM extracts of MPP and MPL roots showed higher inhibitory activities for five cytokines and PGE₂ secretion against LPS and MSU stimulation compared to the other extracts. From this study, the DCM extract of MPP roots was found to contain quercetin (1.27 mg/g) and apigenin (0.064 mg/g). However, only apigenin (0.051 mg/g) was detected in the DCM extract of MPL roots [Figure 2].

DISCUSSION

The viability of human PBMCs was assessed by MTT assay based on the conversion of MTT to purple-colored formazan by mitochondrial activity. All extracts showed a concentration-dependent inhibition; however, the activity was significantly lower (P ≤ 0.001) compared to indomethacin (IC₅₀ = 0.35 µg/mL).

Table 2: Percentage of inhibitory activities of Marantodes pumilum extracts on cytokine secretion in lipopolysaccharide-stimulated human peripheral blood mononuclear cells

| Specimen | Plant part | Extract | IL-1α | IL-1β | IL-6 | IL-8 | TNF-α | Percentage of inhibition (%±SEM) |
|----------|------------|---------|-------|-------|------|------|-------|----------------------------------|
| MPA      | Roots      | DCM     | ND    | ND    | 2.15±0.48 | 8.06±0.26 | 1.19±0.69 |
|          |            | MeOH    | ND    | 2.40±0.18 | 4.75±0.53 | 12.72±0.27 | 19.95±0.55 |
|          |            | H₂O     | ND    | 0.61±0.10 | 5.78±0.44 | 21.04±0.32 | ND |
|          | Leaves     | DCM     | ND    | 2.41±0.52 | 6.81±0.42 | 12.27±0.29 | 3.53±1.49 |
|          |            | MeOH    | ND    | 1.38±0.82 | 2.21±0.38 | 19.21±0.20 | 10.92±0.73 |
|          |            | H₂O     | ND    | 0.77±0.15 | 5.16±0.54 | 10.44±0.20 | 8.07±1.90 |
| MPP      | Roots      | DCM     | 88.87±0.04 | 34.80±1.22 | 99.68±0.00 | 42.18±0.17 | 70.29±0.06 |
|          |            | MeOH    | ND    | 7.25±0.10 | 23.12±0.64 | 6.22±0.02 | 49.90±0.12 |
|          |            | H₂O     | ND    | 4.00±0.12 | 7.48±0.37 | 0.13±0.30 | ND |
|          | Leaves     | DCM     | ND    | 3.15±0.62 | 5.06±0.68 | ND | 0.34±1.07 |
|          |            | MeOH    | ND    | 6.41±1.22 | 8.60±0.66 | 7.41±0.23 | 48.08±1.54 |
|          |            | H₂O     | ND    | 1.71±0.72 | 7.13±0.40 | ND | 2.78±2.04 |
| MPL      | Roots      | DCM     | 99.20±0.01 | 99.76±0.02 | 99.65±0.00 | 51.65±0.25 | 100.04±0.00 |
|          |            | MeOH    | 14.30±0.32 | 16.39±0.22 | 12.32±0.40 | ND | 39.95±0.39 |
|          |            | H₂O     | ND    | 4.03±0.52 | 10.25±0.31 | ND | 4.17±1.59 |
|          | Leaves     | DCM     | ND    | 4.70±0.42 | 10.69±0.27 | ND | 16.57±1.63 |
|          |            | MeOH    | 28.30±1.79 | 3.58±0.42 | 7.24±0.70 | 4.68±0.24 | 51.15±1.27 |
|          |            | H₂O     | ND    | 2.89±0.92 | 9.50±1.40 | ND | 1.89±0.69 |
| Dexamethasone | ND | 69.68±0.51 | 85.00±2.40 | 65.32±3.37 | 60.68±0.68 | 83.48±1.04 |

Data are presented as mean±SEM (n=3). Data were analysed by using one-way ANOVA followed by post hoc Tukey. Concentration of extracts was 50 µg/mL, while dexamethasone was 5 µg/mL. Percentage inhibition >2.5% was significant at P≤0.05 when compared with negative control. °P<0.05 was considered not significantly different compared to dexamethasone; †P≤0.05. Percentage inhibition was significantly higher than dexamethasone. SEM: Standard error of mean; DCM: Dichloromethane; MeOH: Methanol; H₂O: Water; MPA: Marantodes pumilum var. alata; MPP: Marantodes var. pumila; MPL: Marantodes var. lanceolata; TNF: Tumor necrosis factor; IL: Interleukin; ANOVA: Analysis of variance; ND: Inhibition was not detected.

Table 3: The IC₅₀ values (µg/mL) of Marantodes pumilum extracts on cytokine secretion in lipopolysaccharide-stimulated human peripheral blood mononuclear cells

| Specimen | Plant part | Extract | IL-1α | IL-1β | IL-6 | IL-8 | TNF-α | IC₅₀ values (µg/mL)±SEM |
|----------|------------|---------|-------|-------|------|------|-------|----------------------|
| MPP      | Roots      | DCM     | 15.86±0.88 | 7.62±0.37 | 5.87±0.61 | 5.84±0.25 | 19.92±1.44 |
|          |            | MeOH    | 29.87±1.49 | 15.87±0.37 | 25.33±0.57 | 5.40±0.32 | 42.24±2.97 |
| MPL      | Roots      | DCM     | 0.92±0.16 | 0.03±0.02 | 0.89±0.08 | 1.94±1.38 | 0.08±0.00 |
|          | Leaves     | MeOH    | 0.03±0.16 | 0.03±0.02 | 0.89±0.08 | 1.94±1.38 | 0.08±0.00 |
Table 4: Percentage of inhibition of *Marantodes pumilum* extracts and the IC₅₀ values of active extracts on plasma prostaglandin E₂ secretion in lipopolysaccharide-stimulated human whole blood

| Specimen | Plant part | Extract | Percentage of inhibition (%) | IC₅₀ (µg/mL) |
|----------|------------|---------|-------------------------------|--------------|
| MPA      | Roots      | DCM     | 45.61±0.70                   | -            |
|          |            | MeOH    | 46.27±0.58                   | -            |
|          |            | H₂O     | 43.99±0.46                   | -            |
|          | Leaves     | DCM     | 40.88±0.35                   | 42.28±0.49   |
|          |            | MeOH    | 42.34±0.78                   | -            |
|          |            | H₂O     | 31.24±0.36                   | -            |
| MPP      | Roots      | DCM     | 72.78±0.31                   | 31.10±0.23   |
|          |            | MeOH    | 43.24±0.78                   | -            |
|          |            | H₂O     | 36.81±0.41                   | -            |
|          | Leaves     | DCM     | 53.19±0.60                   | 48.79±0.23   |
|          |            | MeOH    | 45.41±1.30                   | -            |
|          |            | H₂O     | 21.15±0.44                   | -            |
| MPL      | Roots      | DCM     | 65.79±0.97                   | 33.66±0.26   |
|          |            | MeOH    | 37.94±0.53                   | -            |
|          |            | H₂O     | 32.48±0.88                   | -            |
|          | Leaves     | DCM     | 63.76±0.72                   | 37.27±0.25   |
|          |            | MeOH    | 50.07±0.05                   | 49.81±0.18   |
|          |            | H₂O     | 45.97±0.78                   | -            |
| Indomethacin |        |         | 90.56±0.27                   | 0.52±0.01    |
| Negative control | |         | 0                              | -            |

Data are presented as mean±SEM (n=3). Data were analysed by using one-way ANOVA followed by post hoc Tukey. Concentration of extracts was 50 µg/mL; while indomethacin was 10 µg/mL. 

Table 5: Percentage of inhibitory activities of *Marantodes pumilum* extracts on cytokine secretion in monosodium urate crystals-stimulated human peripheral blood mononuclear cells

| Specimen | Plant part | Extract | IL-1α Percentage of inhibition (%)±SEM | IL-1β Percentage of inhibition (%)±SEM | IL-6 Percentage of inhibition (%)±SEM | IL-8 Percentage of inhibition (%)±SEM | TNF-α Percentage of inhibition (%)±SEM |
|----------|------------|---------|----------------------------------------|----------------------------------------|----------------------------------------|----------------------------------------|----------------------------------------|
| MPA      | Roots      | DCM     | 23.86±0.06                             | ND                                     | 13.74±0.52                             | 9.89±0.37                              | 6.78±0.21                             |
|          |            | MeOH    | 24.66±0.36                             | 2.40±0.18                              | 13.04±1.24                             | 4.70±0.15                              | 2.40±0.18                             |
|          |            | H₂O     | ND                                     | 0.62±1.03                              | 17.84±0.84                             | 13.34±2.24                             | 7.13±0.04                             |
|          | Leaves     | DCM     | 28.26±2.16                             | 2.38±0.52                              | 14.91±0.18                             | 14.99±1.28                             | 3.76±0.33                             |
|          |            | MeOH    | 21.96±0.36                             | 1.35±0.83                              | 14.40±0.12                             | 13.10±0.76                             | 0.55±0.07                             |
| MPP      | Roots      | DCM     | 73.96±0.36                             | 44.1±1.18                              | 80.91±1.07                             | 40.10±1.58                             | 64.74±0.93                             |
|          |            | MeOH    | 41.56±1.96                             | 5.82±0.41                              | 9.00±0.74                              | 6.95±0.25                              | 40.4±2.31                             |
|          |            | H₂O     | 8.49±0.66                              | 3.05±0.85                              | 7.91±0.68                              | ND                                     | 2.61±0.20                             |
|          | Leaves     | DCM     | 46.46±0.26                             | 1.88±0.74                              | 10.05±0.48                             | 3.80±1.68                              | 3.79±0.02                             |
|          |            | MeOH    | ND                                     | 6.89±1.72                              | 9.55±0.05                              | 2.76±0.64                              | 4.39±0.07                             |
|          |            | H₂O     | ND                                     | 1.24±0.23                              | 9.92±0.92                              | 0.99±3.72                              | 1.0±0.58                              |
| MPL      | Roots      | DCM     | 94.96±0.96                             | 99.91±0.01b                            | 97.04±0.47a                            | 51.74±0.99b                            | 97.65±0.02b                            |
|          |            | MeOH    | 25.86±1.06                             | 6.94±0.34                              | 10.71±0.11                             | ND                                     | 37.15±0.55                            |
|          |            | H₂O     | 14.76±0.06                             | 5.08±0.23                              | 6.58±1.71                              | 2.37±2.07                             | 2.42±1.74                            |
|          | Leaves     | DCM     | 47.46±0.28                             | 4.23±0.01                              | 41.06±1.26                             | 7.33±1.50                             | 30.84±0.45                            |
|          |            | MeOH    | 20.36±5.36                             | 3.11±0.02                              | 10.26±0.02                             | 0.99±1.58                             | 35.16±0.33                            |
|          |            | H₂O     | ND                                     | 1.02±0.04                              | 1.97±0.64                              | ND                                     | 2.64±0.07                            |
| Dexamethasone |      |         | 87.45±0.83                             | 54.99±2.98                             | 66.63±2.72                             | 60.46±2.11                             | 67.48±2.36                            |
| Negative control | |         | 0                                      | 0                                      | 0                                      | 0                                      | 0                                      |

Data are presented as mean±SEM (n=3). Data were analyzed using one-way ANOVA followed by post hoc Tukey. Concentration of extracts was 50 µg/mL, while dexamethasone was 5 µg/mL. Percentage inhibition >2.5% was significant at P≤0.05 when compared with negative control. SEM: Standard error of mean; DCM: Dichloromethane; MeOH: Methanol; H₂O: Water; MPA: *Marantodes pumilum* var. alata; MPP: *Marantodes var. pumila*; MPL: *Marantodes var. lanceolata*; ANOVA: Analysis of variance; ND: Inhibition was not detected.
Table 6: The IC₅₀ values (µg/mL) of Marantodes pumilum extracts on cytokine secretion in monosodium urate crystals-stimulated human peripheral blood mononuclear cells

| Specimen | Plant part | Extract | IL-1α | IL-1β | IL-6 | TNF-α | IC₅₀ values (µg/mL)±SEM |
|----------|------------|---------|-------|-------|------|-------|-------------------------|
| MPP      | Roots      | DCM     | 19.83±0.13 | -     | 22.81±0.72 | -     | 31.67±0.93 |
| MPL      | Roots      | DCM     | 11.2±0.47 | 8.92±0.21 | 12.29±0.30 | 49.51±1.87 | 9.60±0.15 |
| Dexamethasone |          |         | 0.02±0.002 | 0.71±0.24 | 0.46±0.03 | 0.68±0.14 | 0.15±0.01 |

Data are presented as mean±SEM (n=3). Data were analyzed using one-way ANOVA followed by post hoc Tukey. - Not determined as none of tested concentration exceeded 50% inhibition. All IC₅₀ values of extracts were statistically different compared to dexamethasone (P≤0.001). SEM: Standard error of mean; DCM: Dichloromethane; MPP: Marantodes var. pumila; MPL: Marantodes var. lanceolata; TNF: Tumor necrosis factor; IL: Interleukin; ANOVA: Analysis of variance.

Table 7: Percentage of inhibition of Marantodes pumilum extracts and the IC₅₀ values of active extracts on plasma prostaglandin E₂ secretion in monosodium urate crystals-stimulated human whole blood

| Specimen | Plant part | Extract | Percentage of inhibition (%) | IC₅₀ (µg/mL) |
|----------|------------|---------|------------------------------|-------------|
| MPP      | Roots      | DCM     | 50.33±5.53 | 49.33±5.53 |
| MPL      | Roots      | DCM     | 72.87±0.44 | 72.87±0.44 |
| M. pumila |            |         |                |              |
| M. lanceolata |            |         |                |              |

Data are presented as mean±SEM (n=3). Data were analyzed using one-way ANOVA followed by post hoc Tukey. - Not determined as none of tested concentration exceeded 50% inhibition. All IC₅₀ values of extracts were statistically different compared to indomethacin (P≤0.001). SEM: Standard error of mean; DCM: Dichloromethane; MeOH: Methanol; H₂O: Water; MPA: Marantodes var. alata; MPP: Marantodes var. pumila; MPL: Marantodes var. lanceolata; ANOVA: Analysis of variance; ND: Inhibition was not detected.

MSU can activate NLRP3 inflammasome in gout condition. The activation of NLRP3 converts pro-caspase 1 into caspase-1, which then catalyzes the cleavage of pro-IL-1β into IL-1β. Moreover, MSU also leads to activation of nuclear factor-kappa B (NF-kB) which upregulates the secretion of inflammatory mediators.[2-4] NF-kB is a major transcription factor that plays an important role in gene regulations in inflammation responses by controlling the expression of genes encoding the pro-inflammatory cytokines (e.g., IL-1, IL-6, IL-8, and TNF-α), adhesion molecules (e.g., intercellular adhesion molecule, vascular cell adhesion molecule, and E-selectin), inducible enzymes (e.g., cyclooxygenase-2 [COX-2] and inducible nitric oxide synthase [iNOS]), growth factors, certain acute phase proteins, and immune receptors.[22] Cell activation with LPS and MSU regulates cytoplasmic levels of NF-kB by forming a complex with its inhibitors, the IκBs (IκB-α and IκB-β), that are phosphorylated and degraded via IκB kinases. Therefore, the inhibition of this regulatory enzyme is considered as an important point in inflammatory response in terms of inhibition of pro-inflammatory cytokine secretion.[41] Moreover, activation of NF-kB also mediates the expression of rapid response genes in the inflammatory response to injury, including iNOS and COX-2.

PGE₂ is an eicosanoid that is biosynthesized from arachidonic acid precursor by the action of COX-2 enzyme and PGE₂ synthase in endothelial cells. PGE₂ plays a crucial role as a pro-inflammatory mediator in inflammation. Therefore, an inhibitor of the PGE₂ secretion may be effective as a therapeutic agent for inflammation.[19] The stimulation of pro-inflammatory stimuli such as LPS or MSU has been known to be responsible in COX-2 expression in various cells, leading to excessive production of PGE₂.[42,43] Thus, the inhibition of COX-2 expression, known to be regulated by NF-kB, would be expected to result in the inhibition of PGE₂ secretion and potentially cause anti-inflammatory action.[41,44]

From this study, only DCM extracts of MPL and MPP roots gave strong inhibitory activities in both LPS- and/or MSU-induced inflammation of IL-1α, IL-1β, IL-6, IL-8, TNF-α, and PGE₂ secretion. The inhibitory activities of DCM extract of MPL roots (50 µg/mL) were even higher or comparable to dexamethasone (5 µg/mL). However, the IC₅₀ values were significantly lower than dexamethasone. It is suggested that this extract would be highly potential as an anti-inflammatory. Interestingly, previous study by Mamat et al.[22] reported that M. pumilum possessed xanthine oxidase inhibitory activity that is related to gouty inflammation. Thus, it strengthens the postulation that this plant might be potentially useful for antigout treatment that can reduce uric acid level as well as inflammatory response.

When only two varieties of MPP and MPL were biologically active, it suggested that the active components and amounts affecting the anti-inflammatory activity were different in all species and plant parts. The presence of phenolics and flavonoids as reported by previous studies might be responsible for the activity.[14,45] Flavonoids are ubiquitous phenolic compounds that have been recognized as potential anti-inflammatory, antioxidant, and anticancer agents. It was reported that flavonoids inhibit the activity and gene expression of various pro-inflammatory mediators as well as up- or down-regulate the transcription factors in inflammatory pathway.[45] Previous studies reported that flavonoids such as quercetin, kaempferol, and apigenin exhibited the inhibition of NF-kB signaling.[46] Quercetin was found to inhibit LPS-stimulated iκB phosphorylation in PBMCs and significantly inhibit TNF-α production and gene expression in concentration-dependent manner. Moreover, quercetin was reported to attenuate the MSU-induced inflammation in rats by decreasing the recruitment of leukocytes, cytokines, and chemokines levels in rats.[46,47] Quercetin and kaempferol also showed potential inhibitory activity via gene expression and secretion of IL-1β or IL-6 in phorbol-12-myristate 13-acetate and calcium ionophore A23187-stimulated human mast cell-1, while myricetin inhibited IL-6 and TNF-α, but not IL-1β and IL-8.[46] Park et al.[46] revealed that quercetin and kaempferol scavenged...
reactivity of NO and inhibited iNOS in LPS-stimulated RAW264.7 cells, as well as decreased iNOS and COX-2 protein level in activated Chang liver cells. Several flavonoids including quercetin, galangin, apigenin, and naringin have been reported to decrease PGE$_2$ release in the macrophage cell line J774A.1. Apigenin was reported to inhibit the production of pro-inflammatory cytokines such as IL-1$\beta$, IL-6, and TNF-$\alpha$ in LPS-stimulated PBMCs. The molecular mechanism involves in anti-inflammatory activity of apigenin is suggested to be the inhibition of iNOS, COX-2, IL-1$\beta$, IL-6, and TNF-$\alpha$ gene expression and amelioration of p38-MAPK, JNK, and ERK phosphorylation. In short, flavonoids may inhibit pro-inflammatory cytokine secretion and COX-2 expression, as well as PGE$_2$ secretion. The HPLC analysis in this study substantiated the fact that strong inhibition of DCM extract of MPL roots against IL-1$\alpha$, IL-1$\beta$, IL-6, IL-8, TNF-$\alpha$, and PGE$_2$ could be mainly due to the presence of apigenin as previously reported.

CONCLUSION

This investigation demonstrated that extracts of _M. pumilum_, especially DCM extract of MPL and MFP roots, possessed inhibitory activity of IL-1$\alpha$, IL-1$\beta$, IL-6, IL-8, TNF-$\alpha$, and PGE$_2$ in LPS- and MSU-stimulated inflammation. The presence of flavonoids such as apigenin might contribute to the activity. The findings suggested that _M. pumilum_ has an anti-inflammatory potential against microbial infection and gouty inflammation due to its ability to inhibit inflammatory mediators stimulated by LPS and MSU, respectively. To the best of our knowledge, this is the first report of inhibitory activity of three varieties of _M. pumilum_ in LPS- and MSU-induced cytokines and PGE$_2$ secretion. The results may provide useful data for further investigation of pharmacological activity of _M. pumilum_, especially for antioxidant activity.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Ward PA. Acute and chronic inflammation. In: Serhan CN, Ward PA, Gilroy DW, editors. Fundamentals of Inflammation. New York: Cambridge University Press; 2010. p. 175-85.
2. Aderem A, Ulevitch RJ. Toll-like receptors in the induction of the innate immune response. Nature 2000;406:782-7.
3. Lakshmi R, Jayawardhana KK. The role of toll like receptors in innate immunity. World J Pharm Res 2015;4:687-84.
4. Murphy HS. Inflammation. In: Rubin E, Reiser HM, editors. Essential of Rubin’s Pathology. 6th ed. Philadelphia: Wolter Kluwer Health/Lippincott Williams & Wilkins; 2013. p. 25-34.
5. Baticani M. Nonsteroidal anti-inflammatory drugs: Cardiovascular, cerebrovascular and renal effects. Arq Bras Cardiol 2010;94:556-63.
6. Theplantlist.org. The Plant List Version 1.1; 2013. Available from: http://www.theplantlist.org. [Cited on 2016 Apr 14].
7. Burkhill IH. A Dictionary of the Economic Products of the Malay Peninsula. London: Crown Agents; 1935.
8. Stone BC. Notes on the genus Labisia Lindl. (Myrsinaceae). Malay Nat J 1988;42:43-51.
9. Sunarmo B. Revision of the genus Labisia (Myrsinaceae). Blumea 2005;50:579-96.
10. Aladdin NA, Jamal JA, Talib N, Hamsani NA, Rahman MR, Sabandar CA, et al. Comparative study of three _Marantodes pumilum_ varieties by microscopy, spectroscopy and chromatography. Braz J Pharmacognosy 2016;26:1-14.
11. Chua LS, Latiff NA, Lee SY, Lee CT, Sarmidi MR, Aziz RA. Flavonoids and phenolic acids from Labisia pumila (Kacip Fatimah). Food Chem 2011;127:1186-92.
12. Karimi E, Jaafar HZ, Ahmad S. Phytochemical analysis and antimicrobial activities of methanolic extracts of leaf, stem and root from different varieties of Labisia pumila Benth. Molecules 2011;16:4438-50.
13. Hisham DM, Lip JM, Noh JM, Normah A, Nabiah MF. Identification and isolation of methyl gallate as a polar chemical marker for Labisia pumila Benth. J Trop Agric Food Sci 2011;39:279-84.
14. Karimi E, Jaafar HZ. HPLC and GC-MS determination of bioactive compounds in microwave obtained extracts of three varieties of Labisia pumila Benth. Molecules 2011;16:6791-805.
15. Avula B, Wang YH, Ali Z, Smillie TJ, Khan IA. Quantitative determination of triperpene saponins and alkannin-phenolics from Labisia pumila using an LC-UV/ELSD method and confirmation by LC-ESI-TOF. Planta Med 2011;77:1742-8.
16. Abdullah N, Chermahini SH, Chua LS, Sarmidi MR. Labisia pumila: A review on its traditional, phytotoxicological, and biological uses. World Appl Sci J 2013;27:1297-306.
17. Jaafar HZ, Karimi E, Ibrahim MH, Ghazanmzadeh A. Phytochemical screening and antioxidant activity assessment of the leaf stem and root of _Labisia pumila_. Aust J Crop Sci 2013;7:276-80.
18. Pfie AH, Othman F, Zakaria ZA. Anticarcinogenic activity of Labisia pumila against 1,2-dimethylbenzene (a) racemate (DMBA)/croton oil-induced mouse skin carcinogenesis. Afr J Pharm Pharmacol 2011;5:823-32.
19. Mamat N, Jamal JA, Jantan I, Husain K. Xanthine oxidase inhibitory and DPPH radical scavenging activities of some Primulaceae species. Sains Malaysia 2014;43:1827-33.
Karimi E, Jafarzadeh HA, Alhadad S. Anti-inflammatory and cytotoxic activities of three varieties of Labisia pumila benth: From microwave obtained extracts. BMC Complement Altern Med 2013;13:20.

World Medical Association. Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. World Medical Association; 2008. Available from: http://www.wma.net/en/30publications/10policies/b3/index.html. [Cited on 2017 Jan 10].

Bögum A. Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1 g. Scand J Clin Lab Invest Suppl 1968;97:77-89.

Shield A. Purification of mononuclear cells, monocytes and polymorphonuclear leukocytes. Axis Shield Mini Rev 2011;4:1-15.

Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods 1983;65:55-63.

Riss TL, Moravec RA, Niles AL, Benink HA, Worzella TJ, Minor L. Cell viability assays. In: Sittampalam GS, Gal-Edd N, Arik M, Auld D, Austin C, Bejcek B, et al., editors. Assay Guidance Manual. Bethesda (MD): Eli Lilly & Company and the National Center for Advancing Translational Sciences; 2004. Available from: https://www.ncbi.nlm.nih.gov/books/NBK53196/. [Cited on 2017 Jan 10].

Salim E, Kumolosasi E, Jantani I. Inhibitory effect of selected medicinal plants on the release of pro-inflammatory cytokines in lipopolysaccharide-stimulated human peripheral blood mononuclear cells. J Nat Med 2014;68:647-53.

Eletheriadis T, Pissas G, Karoti A, Antoniadis G, Golfinopoulos S, Liakopoulos V, et al. Uric acid induces caspase-1 activation, IL-1β secretion and P2X7 receptor dependent proliferation in primary human lymphocytes. Hippokratia 2013;17:141-5.

Orlovsky EW, Stabler TV, Montell E, Vergés J, Kraus VB. Monosodium urate crystal induced macrophage inflammation is attenuated by chondroitin sulphate: Pre-clinical model for gout prophylaxis? BMC Musculoskelet Disord 2014;15:318.

Saadawi S, Jalil J, Jasama M, Jantani I. Inhibitory effects of acetylmedolodinolin, chrysin and polyacrol from Mitrella kantoi on prostaglandin E2 and thromboxane B2 production and platelet activating factor receptor binding. Molecules 2012;17:4824-35.

Patrignani P, Panara MR, Greco A, Fusco O, Natoli C, Iacobelli S, et al. Biochemical and pharmacological characterization of the cyclooxygenase activity of human blood prostaglandin endoperoxide synthases. J Pharmacol Exp Ther 1994;271:1705-12.

Yin L, Yang YH, Wang MY, Zhang X, Duan JA. Effect of syringing from Phellodendron chinensis on monosodium urate crystal-induced inflammation and intracellular adhesion molecule-1 (ICAM-1) expression. Afr J Pharm Pharmacol 2012;6:1515-9.

Tayal V, Kalra BS. Cytokines and anti-cytokines as therapeutics – An update. Eur J Pharmacol 2008;579:1-12.

Cronstein BN, Tereklaib R. The inflammatory process of gout and its treatment. Arthritis Res Ther 2006;8 Suppl:1 S1-53.

Poulout M, James MJ, McColl SR, Naccache PH, Cleland LG. Monosodium urate microcrystals induce cyclooxygenase-2 in human monocytes. Blood 1998;91:1769-76.

Hougue B, Sanders A, Faber J, Graus YM, van den Berg WB, Garssen J, et al. Decreased pro-inflammatory cytokine production by LPS-stimulated PBMC upon in vitro incubation with the flavonoids apigenin, luteolin or chrysin, due to selective elimination of monocytes/macrophages. Biochem Pharmacol 2005;69:241-8.