**Table 6:** The $IC_{50}$ values (μg/mL) of *Marantodes pumilum* extracts on cytokine secretion in monosodium urate crystals-stimulated human peripheral blood mononuclear cells

| Specimen | Plant part | Extract | $IC_{50}$ values (μg/mL)±SEM | | | | |
|---|---|---|---|---|---|---|---|
| | | | IL-1α | IL-1β | IL-6 | IL-8 | TNF-α |
| 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 $IC_{50}$ 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 $IC_{50}$ values of active extracts on plasma prostaglandin $E_2$ secretion in monosodium urate crystals-stimulated human whole blood

| Specimen | Plant part | Extract | Percentage of inhibition (%) | $IC_{50}$ (μg/mL) |
|---|---|---|---|---|
| MPA | Roots | DCM | 46.46±0.20 | - |
| | | MeOH | 45.74±1.71 | - |
| | | $H_2O$ | 45.78±1.66 | - |
| | Leaves | DCM | 22.51±2.14 | - |
| | | MeOH | 41.16±0.03 | - |
| | | $H_2O$ | 47.49±1.54 | - |
| MPP | Roots | DCM | 66.08±0.81 | 33.01±0.59 |
| | | MeOH | 54.45±0.96 | 47.14±0.55 |
| | | $H_2O$ | 34.87±0.24 | - |
| | Leaves | DCM | 49.33±5.53 | - |
| | | MeOH | 50.28±3.29 | 47.2±0.58 |
| | | $H_2O$ | 43.60±0.14 | - |
| MPL | Roots | DCM | 72.87±0.44 | 31.58±0.57 |
| | | MeOH | 34.93±1.38 | - |
| | | $H_2O$ | 14.22±0.37 | - |
| | Leaves | DCM | 65.72±0.70 | 35.26±0.57 |
| | | MeOH | 52.08±2.25 | 45.44±0.61 |
| | | $H_2O$ | 37.57±0.86 | - |
| Indomethacin | | | 97.45±0.73 | 0.35±0.47 |
| Negative control | | | 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 indomethacin was 10 μg/mL. -: Not determined as none of tested concentration exceeded 50% inhibition. Percentage inhibition >2.5% was significant at $P$≤0.05 when compared with negative control. [a]$P$≥0.05 was considered not significant compared with indomethacin (positive control). All $IC_{50}$ values of extracts were statistically different compared to indomethacin ($P$≤0.001). SEM: Standard error of mean; DCM: Dichloromethane; MeOH: Methanol; $H_2O$: Water; MPA: *Marantodes pumilum* 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-κB) which upregulates the secretion of inflammatory mediators.[2-4]

NF-κB 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.[39,40] Cell activation with LPS and MSU regulates cytoplasmic levels of NF-κB 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-κB also mediates the expression of rapid response genes in the inflammatory response to injury, including iNOS and COX-2.

$PGE_2$ is an eicosanoid that is biosynthesized from arachidonic acid precursor by the action of COX-2 enzyme and $PGE_2$ synthase in endothelial cells. $PGE_2$ plays a crucial role as a pro-inflammatory mediator in inflammation. Therefore, an inhibitor of the $PGE_2$ secretion may be effective as a therapeutic agent for inflammation.[1]

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 $PGE_2$.[42,43] Thus, the inhibition of COX-2 expression, known to be regulated by NF-κB, would be expected to result in the inhibition of $PGE_2$ secretion and potentially cause anti-inflammatory action.[43,44]

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 $PGE_2$ 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 $IC_{50}$ 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.[14,15] 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-κB 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.[48] Park *et al.*[49] revealed that quercetin and kaempferol scavenged

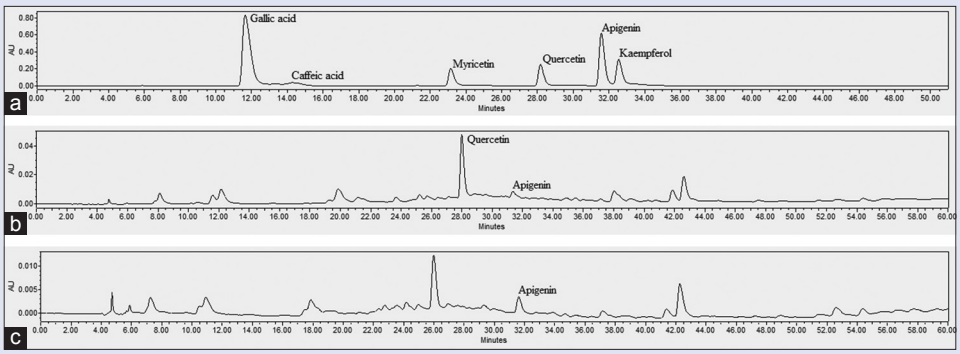


**Figure 2:** High-performance liquid chromatography chromatograms of (a) a mixture of gallic acid, caffeic acid, myricetin, quercetin, apigenin, and kaempferol standards; (b) dichloromethane extract of *Marantodes pumilum* var. *pumila* roots showing peaks corresponding to quercetin (Rt 27.997 min) and apigenin (Rt 31.354 min); and (c) dichloromethane extract of *Marantodes pumilum* var. *lanceolata* roots showing a peak corresponding to apigenin (Rt 31.065 min)

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.[50] Apigenin was reported to inhibit the production of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α in LPS-stimulated PBMCs.[51] The molecular mechanism involves in anti-inflammatory activity of apigenin is suggested to be the inhibition of iNOS, COX-2, IL-6, IL-1β, and TNF-α gene expression and amelioration of p38-MAPK, JNK, and ERK phosphorylation.[45] 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-1α, IL-1β, IL-6, IL-8, TNF-α, and $PGE_2$ could be mainly due to the presence of apigenin as previously reported.[52]

## CONCLUSION

This investigation demonstrated that extracts of *M. pumilum*, especially DCM extract of MPL and MPP roots, possessed inhibitory activity of IL-1α, IL-1β, IL-6, IL-8, TNF-α, 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 antigout activity.

## Financial support and sponsorship

This work was funded by the Ministry of Agriculture and Agro-based Industry Malaysia, under the National Key Economic Areas Research Grant Scheme (NRGS-NH0711D002).

## Conflicts of interest

There are no conflicts of interest.

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