Original Article

**In Vitro Cytotoxicity and Anti-inflammatory Cytokinine Activity Study of Three Isolated Novel Compounds of Prismatomeris glabra**

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Submitted : 07-Nov-2019  
Revised : 09-Mar-2020  
Accepted : 24-Aug-2020  
Published : 21-Dec-2020

**ABSTRACT**

**Objectives:** The aim of the present study was to isolate and evaluate cytotoxicity and anti-inflammatory activities of new novel compounds isolated from *Prismatomeris glabra*. **Materials and Methods:** Dried root of *P. glabra* was extracted under reflux with methyl alcohol, fractionated through the vacuum liquid chromatography technique, and evaporated and then purified the compounds using column chromatography and preparative thin-layer chromatography. THP-1 cells were treated with amentoflavone, 5,7,4′-hydroxyflavonoid, and stigmasterol with various concentrations (0–30 µg/mL) and then incubated with MTS reagent for 2h. Treatment was done for 24, 48, and 72h. Then, effects of these compounds were also tested on PGE₂, TNF-α, and IL-6 expression in human THP-1-derived macrophage cells for 24h. **Results:** Three new compounds such as amentoflavone, 5,7,4′-hydroxyflavonoid, and stigmasterol were isolated. After 24h of incubation, a significant decrease in cell viability was reported with IC₅₀ values of amentoflavone, 5,7,4′-hydroxyflavonoid, and stigmasterol (21 µg/mL = 38 M), (18 µg/mL = 66 M) and (20 µg/mL = 48.5 M), respectively. Whereas for 48 and 72h treatment showed a less decreased cell viability compared with 24h treatment. These compounds also showed a significant reduction in the production of TNF-α, IL-6, and PGE₂ in a dose-dependent manner. **Conclusions:** The isolated new compounds showed significant cytotoxicity and anti-inflammatory effects.

**KEYWORDS:** Amentoflavone, anti-inflammatory, cytotoxicity, *Prismatomeris glabra*

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**Introduction**

The medicinal plant has been used for many years in the treatment of various diseases and still endures to provide fresh medications to our community. In recent days, one plant can be used to treat different types of diseases because there is always discovery of new characteristics in the medicinal plant. Consequently, screening of plants for biological activity for more than one pharmacological activity is significant. Hence, testing and screening of raw extract of the plants are required for the development of new.

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How to cite this article: Alkadi KAA, Ashraf K, Adam A, Shah SAA, Taha M, Hasan MH, et al. In vitro cytotoxicity and anti-inflammatory cytokinin activity study of three isolated novel compounds of *Prismatomeris glabra*. J Pharm Bioall Sci 2021;13:116-22.
plant-based entities with high effectiveness and lower side effect in the treatment of various diseases and cancer in particular.[1-3]

P. glabra (Rubiaceae) or locally known as “Haji Samat,” is usually found in the mixed dipterocarp and keranga forests of up to 700 m altitude in Peninsular Malaysia, Sumatra, and Borneo.[4-5] P. glabra has simple and oppositely arranged leaves with white-purplish flowers and green-whitish fruits in the form of berries. In Malaysia, the aqueous extracts of the roots of P. glabra have been used traditionally by the farmers and certain rural Malays for wellness, enhancing stamina, and for its ergogenic effects. In addition, as stated by a local taxonomist, this plant has been used as an aphrodisiac for generations in Kelantan and Terengganu.[6-9]

Materials and Methods

General experimental procedures

Melting points were determined on a Yanaco MP-S3 apparatus. UV spectra were measured on a Shimadzu UV 240 spectrophotometer. A JASCO DIP–360 Digital polarimeter was used to measure the optical rotations in chloroform by using a 10-cm cell tube. The 1H-NMR and 2D NMR spectra were recorded on a Bruker Avance III 600 Ascend spectrometer using the BBO probe, while 13C-NMR spectra were recorded on a Bruker Avance III 600 Ascend spectrometer operating at 125 MHz using CDCl3 as a solvent. Chemical shifts were reported in δ (ppm), relative to SiMe4 as internal standard, and coupling constants (J) were measured in Hz. The EI-MS and HREIMS were measured on the Jeol HX 110 mass spectrometer. Thin-layer chromatography (TLC) was performed on silica gel pre-coated plates (PF 254 20 × 20, 0.25 mm, Merck, Germany). Ceric sulfate in 10% H2SO4 has been used as the staining reagent for the staining of compounds on TLC. All reagents used were of analytical grades.

Plant material and extraction

The root of P. glabra species was collected from Pasoh-Negeri Sembilan. Voucher specimens were prepared, identified, and deposited at the Herbarium of the Forest Research Institute Malaysia (FRIM), Kepang.

The dried root of P. glabra was extracted under reflux with MeOH for 6–8 h. The obtained MeOH extract was fractionated by the vacuum liquid chromatography (VLC) technique using different polarities. Evaporation of the respective solvents and purified the compounds through column chromatography and preparative TLC.[4,10,11]

MTS method

Cell culture

THP-1 cells were grown in T75 cell culture flasks in a total volume of 20 mL of complete cell culture medium. Cells were kept in an incubator at 37°C and 5% CO2 in humidified air.

Cell seeding into 96-well plate

Confluent (80–90%) cells were taken out from the incubator for plating. Cells were viewed under an inverted microscope to check the percentage of confluence and to confirm the absence of bacterial and fungal contaminations. The cells were centrifuged for 5 min at 4000 rpm, the pellet was formed, the supernatant was removed, and 1 mL of complete medium was added into the centrifuge tube. The cells were re-suspended and 10 μL was taken and added into an Eppendorf tube containing 100 μL of trypan blue for the determination of cell viability. Then, 30 μL of the mixture was pipetted onto both sides of a hemocytometer and the number of cells was counted. Cells were seeded at a density of 3 × 104 per well in a 96-well plate, and incubated with the desired drug concentration in triplicates, to a total of 200 μL, at 37°C in a humidified atmosphere of 5% CO2 for 24 h. We used two negative controls for the tests: (1) THP-1 cells in drug-free culture media. (2) THP-1 cells treated for 24, 48, and 72 h with the maximum concentration of the drug’s solvent used in the experiments (4% DMSO).[10,12-14]

The following equation was used to calculate the volume of cells and the volume of media needed to get 2 × 104 cells/mL in each well plate.

\[
\frac{\text{Volume of cells per well}}{\text{number of counted cells} \times 10^4 \times \text{d.f.}} = 2 \times 10^4 \frac{\text{volume of media}}{\text{number of counted cells} \times 10^4 \times \text{d.f.}}
\]

where d.f. is the dilution factor.

The volume of media per well to get 2 × 104 cells/mL = 50 μL – volume of the cells per well.

Cytokine assays

The levels of cytokines TNF-α, IL-6, and PGs were determined by specific enzyme-linked immunosorbent assay (ELISA) techniques according to the manufacturer’s instructions (R&D Systems Europe, France). The minimum detectable doses were less than 5 pg/mL for TNF-a and IL-1b, and less than 20 pg/mL for IL-6. The serum samples were incubated without dilution. In comparison with a standard cytokine curve, the concentrations of cytokines in serum or tissue extracts were determined spectrophotometrically (Bio-Tek EL 808, Bio-Tek Instruments Inc., Colmar, France) by reading the absorbance at 420 nm.[15-17]

Statistical analysis

All data were expressed as mean ± SD. The statistical analysis was performed using Sigma Stat software.
(SPSS Science, NC, USA). The global course of each parameter was evaluated by an ANOVA ranks test (Kruskal–Wallis test) and Dunnett’s test, which is used for multiple comparison procedures, to compare each of many treatments with a single control.

**Results and Discussion**

**Phytochemical investigation**

The dried stem root of *P. glabra* weighing 800 g was turned into the powdered form on a dry grinder successively. Extraction with a maceration was done using 500 g of the powder in 1 L of methanol and kept the mixture on a sealed tank for 3 days. The MeOH extracted solution was evaporated under pressure to yield a gummy extract (45 g). Twenty grams from this extract was subjected to vacuum liquid chromatography on a Sephadex LH-20 eluted with *n*-hexane–EtOAc (50:50→0:100) system and MeOH to give six fractions: fr.1, 1.5 g; fr. II, 2.8 g; fr. III, 4.5 g; fr. IV, 5.2 g; fr. V, 4.1 g; fr. VI, 1.7 g. Fractions III (4.5 g), fr. IV (5.2 g) and fr. V (4.1 g) were further separately subjected to silica gel and a Sephadex LH-20 column eluted with *n*-hexane–EtOAc and MeOH system. Evaporation of the respective solvents and purification through preparative TLC and Sephadex LH-20 provided β-stigmasterol (1) (7 mg), apigenin (2) (4 mg), and amentoflavone (3) (6 mg) [Figure 1].

The 1H and 13C assignments of compound 1 were verified by 2D Heteronuclear Single Quantum Coherence (HSQC) experiments, while connectivities were verified by Heteronuclear Multi Bond Coherence (HMBC). The hydroxyl group was attached to C-3 due to long-range correlations between this carbon and the methylene protons at H-1 and H-2. One of the double bonds was placed at C-5 because long-range correlations were observed between this carbon and H-4 and H-19. The second double bond was placed at C-22 due to long-range correlations between this carbon and H-20 and H-21. The position of the isopropyl group was deduced from the correlations between C-24 with H-28 and H-29. All long-range correlations observed are consistent with the structure of compound 1.

The relative stereochemistry of compound 1 was deduced from the NOESY spectrum and is as drawn in the structure. NOESY correlations indicate that the protons in the β-position (above the plane) are as follows: H-8/H-18/H-19/H-20/H-21/H-23, while for the protons in the α-position (below the plane) are as follows: H Ala/H-3/H-9/H-14/H-17/H-22/H-24. The literature search revealed that compound 1 is β-stigmasterol as evident by its similar 1H and 13C NMR spectral data. Compound 2 was obtained as a yellow powder and its molecular formula was established as C15H10O5 from its molecular spectrum (MS) data, which showed [M+1] 

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**Figure 1:** Compounds isolated from *Prismatomeris glabra* β-stigmasterol (1), apigenin (2), and amentoflavone (3)

**Figure 2:** HMBC correlations of compound 1 and COSY and HMBC correlation of compound 2
Alkadi, et al.: In vitro cytotoxicity and anti-inflammatory cytokinine activity of *P. glabra*

+ H]+ ion at *m/z* 271. The molecular formula of 2 was further supported by its 13C NMR spectral data. The UV spectrum of 2 also showed absorption maxima at 268, and 337 nm, suggesting a flavonoid structure. The 1H NMR spectrum of 2 showed the presence of two meta coupled aromatic doublets at δ 6.68 and 6.88 corresponding to H-6 and H-8 protons, two doublet of doublets at δ 6.95 and 7.81 for H-3'/H-5' and H-2'/H-6' protons of ring B, and a singlet at δ 6.62 corresponding to H-3 protons, characteristic for a 5,7,4'-trisubstituted flavone. The 13C NMR spectrum showed the presence of 12 aromatic carbons; 7 quaternary carbons, 5 methine carbons, and an unsaturated carbonyl carbon. The 1H and 13C NMR values for all the carbons were assigned based on HSQC and HMBC correlations. A search in the literature suggested that the spectral data of 2 were consistent to 4',5,7-trihydroxyflavone, also known as apigenin. The structure was further supported by the COSY and HMBC correlations as shown in Figure 2.

Compound 3 was obtained as a colorless powder and its molecular formula was established as C30H18O10 from its MS data that showed [M + H]+ ion at *m/z* 539. 1H NMR spectrum of 3 showed two signals at δ H 13.09 and 12.96.

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**Figure 3:** Cytotoxicity of the compounds with various concentrations (0–30 μg/mL) on THP-1 cells for 24 h incubation

**Figure 4:** Effect of stigmasterol (1), 5,7,4'-hydroxyflavonoid (2), and amentoflavone (3) on PGE2 expression in human THP-1-derived macrophage cells were pretreated with different concentrations (0–30 μg/mL) for 24 h
due to chelated phenolic hydroxyls, and two doublets at \( \delta 7.56 (J = 9.0 \text{ Hz}, H-2^‴, H-6^‴) \), and \( 6.70 (J = 9.0 \text{ Hz}, H-3^‴, H-5^‴) \) assigned to an AA’BB’ aromatic system. Signals of a trisubstituted aromatic ring containing one oxygenated carbon were observed at \( \delta H 8.00 (d, J = 2.5 \text{ Hz}, H-2') \), \( 7.14 (d, J = 9.0 \text{ Hz}, H-5') \), and \( 7.99 (dd, J = 9.0; 2.5 \text{ Hz} H-6') \), along with doublets of a tetrasubstituted aromatic ring containing three oxygenated carbons at \( \delta H 6.18 (J = 2.5 \text{ Hz}, H-6) \) and \( 6.45 (J = 2.5 \text{ Hz}, H-8) \). Analysis of the HMBC spectra evidenced correlations of the hydroxyl group with hydrogen bonded by a cross peak at \( \delta H 13.09 (OH-5) \) with C-5'' (\( \delta 161.1 \)), C-6'' (\( \delta 98.7 \)), and C-10'' (\( \delta 103.6 \)) and \( \delta H 12.96 (OH-5'') \) with C-5 (\( \delta 162.1 \)), C-6 (\( \delta 98.9 \)) and C-10 (\( \delta 103.7 \)), which are in agreement to those of amentoflavone.\(^{[17]}\)

**Cytotoxicity of the compounds isolated from *P. glabra* using THP-1 macrophage cell line**

Cytotoxicity of stigmasterol (1), 5,7,4’-hydroxyflavonoid (2), and amentoflavone (3), with various concentration (0–30 µg/mL) on THP-1 cells for 24h incubation, \( \bar{x} \pm \text{S.D.} \) (\( n = 3 \)). IC\textsubscript{50} values for stigmasterol was 20 µg/mL (\( \equiv 48.5 \text{ M} \)), 5,7,4’-hydroxyflavonoid was 18 µg/mL (\( \equiv 66 \text{ M} \)), and amentoflavone was 21 µg/mL (\( \equiv 38 \text{ M} \)), as shown in Figure 3.\(^{[18]}\)

**Activity of proinflammatory cytokines (PGE\textsubscript{2}, IL-6, and TNF-\textalpha)**

**Prostaglandin E2 (PGE2)**

Effect of stigmasterol, 5,7,4’-hydroxyflavonoid, and amentoflavone on PGE\textsubscript{2} expression in human THP-1-derived macrophages cells were pretreated with
different concentrations (0–30 µg/mL) for 24h. At the end of the treatment, the cell culture medium was collected. PGE, was measured using an ELISA kit. \( \bar{x} \pm S.E.N (n = 3) \). Significantly different \( *P < 0.0001 \) (ANOVA + Dunnett’s tests) [Figure 4].

**Interleukin-6 (IL-6)**

The effect of stigmasterol, 5,7,4′-hydroxyflavonoid, and amentoflavone on IL-6 expression in human THP-1-derived macrophages cells was pretreated with different concentrations (0–30 µg/mL) for 24h. At the end of treatment, the cell culture medium was collected. IL-6 was measured using an ELISA kit. \( \bar{x} \pm S.E.N (n = 3) \). Significantly different \( *P < 0.0001 \) (ANOVA + Dunnett’s tests) [Figure 5].

**Tumor necrosis factor (TNF-α)**

The effect of stigmasterol, 5,7,4′-hydroxyflavonoid, and amentoflavone on TNF-α expression in human THP-1-derived macrophages cells were pretreated with different concentrations (0–30 µg/mL) for 24h. At the end of the treatment, the cell culture medium was collected. TNF-α was measured using an ELISA kit. \( \bar{x} \pm S.E.N (n = 3) \). Significantly different \( *P < 0.0001 \) (ANOVA + Dunnett’s tests) [Figure 6].

The effects of the compounds on PGE, TNF-α, and IL-6 expression in human THP-1-derived macrophage cells were pretreated with different concentrations showed significantly reduced production of TNF-α, IL-6, and PGE, in a dose-dependent manner.

**Prismatomeris glabra** is a well-known Malaysian plant. In this research, we isolated and identified three novel compounds with good cytotoxicity and anti-inflammatory activity. Some literature also showed the presence of saponins, flavonoids, alkaloids, terpenoids, tannins, and glycosides in *P. glabra*. Burkill also identified the ergogenic effects of *P. glabra*. As the study showed, pro-inflammatory cytokines such as TNF-α, IL-6, PGs play a key role in the process of inflammatory diseases. Too much production of these pro-inflammatory cytokines may cause a systemic inflammatory response syndrome, shock. The present study could help open a window for the development of a new drug entity.

**Conclusions**

The effects of the compounds (5,7,4′-hydroxyflavonoid, amentoflavone, and stigmasterol) on PGE, TNF-α and IL-6 expression in human THP-1-derived macrophage cells showed significantly reduced production of TNF-α, IL-6, and PGE in a dose-dependent manner. Thus, the isolated compounds showed significant cytotoxicity and anti-inflammatory effects and it can be used as an entity for drug discovery in the future.

**Acknowledgments**

Authors would like to acknowledge the Ministry of Higher Education (MoHE) for financial support under the Fundamental Research Grant Scheme (FRGS) with sponsorship reference numbers FRGS/1/2019/STG05/UITM/02/9. The author would also like to acknowledge Universiti Teknologi MARA for the financial support under the reference number 600-IRMI/FRGS 5/3 (424/2019).

**Financial support and sponsorship**

Nil.

**Conflict of interests**

The authors declare no conflict of interests.

**REFERENCES**

1. Rafieian-Kopaei M, Nasr H. On the occasion of World Cancer Day 2015: the possibility of cancer prevention or treatment with antioxidants: the ongoing cancer prevention researches. Int J Prev Med 2015;6:108.
2. The Son N. An overview of the genus *Prismatomeris*: phytochemistry and biological activity. Bull Facult Pharm Cairo Univ 2017;55:11-8.
3. Abdullah NH. Chemicals constituents of *Prismatomeris malayana* Ridley and quantitative structure activity relationship study on anti-inflammatory and their analogues (Doctoral thesis in chemical sciences). Kuala Lumpur: University of Malaya; 2014.
4. Wang C, Ding X, Feng SX, Guan Q, Zhang XP, Du C, et al. New tetrahydroantraquinones from the root of *Prismatomeris connata* and their cytotoxicity against lung tumor cell growth. Molecules 2015;20:22565-77.
5. Kanokmedhakul KS, Kanokmedhakul R, Phatchana biological activity of antraquinones and triterpenoids from *Prismatomeris fragrans* J Ethnopharmacol 2005;100:284-8.
6. Feng S, Bai J, Qi S, Li Y, Chen T. Iridoid and phenolic glycosides from the roots of *Prismatomeris connata*. Nat. Prod Commun 2012;7:561-2.
7. Conserva LM, Junior JCF Borreria and Spermacoce species (Rubiaceae): a review of their ethnomedicinal properties, chemical constituents, and biological activities. Pharmacogn Rev 2012;6:46-55.
8. Zen RY. Notes on the genus *Prismatomeris* Thw. (Rubiaceae) of China. J Syst Evol Acta Phytotaxonomica Sin 1988;26:443-9.
9. Shuang JJ, Ming FZ, Cheng ZP. Chemical constituents from the root of *Prismatomeris tetrandra*. China J Chin Mater Med 2005;30:1751-3.
10. Krohn K, Gehle D, Dey SK, Nahar N, Moshiuzzaman M, Sultana N, et al. Prismatomerin, a new iridoid from *Prismatomeris tetrandra*. Structure elucidation, determination of absolute configuration, and cytotoxicity. J Nat Prod 2007;70:1339-43.
11. Kanokmedhakul K, Kanokmedhakul S, Phatchana R. Biological activity of anthraquinones and triterpenoids from *Prismatomeris fragrans*. J Ethnopharmacol 2005;100: 284-8.
12. Salleh RM, Hasan MH, Adam A. Phenolic compound and antioxidant levels of *Prismatomeris raijglabra*. J Pharmacogn Phytochem 2015;3:05-11.
13. Marano MA, Moldawer LL, Fong Y. Cachectin/TNF production in experimental burns and *Pseudomonas* infection. Arch Surg 1988;123:1383.
14. Faunce DE, Gregory MS, Ovacs EJK. Acute ethanol exposure prior to thermal injury results in decreased T-cell responses mediated in part by increased production of IL-6. Shock 1988;10:135.
15. BandtDe JP, Martin Chollet S, Hermann A. Cytokine response to burn injury: relationship with protein metabolism. J Trauma 1994;36:624.
16. Dehne MG, Sablotzki A, Hoffmann A. Alterations of acute phase reaction and cytokine production in patients following severe burn injury. Burns 2000;28:535.
17. Deveci M, Eski M, Sengezer M. Effects of cerium nitrate bathing and prompt burn wound excision in IL-6 and TNF-α levels in burned rats. Burns 2000;26:41.
18. Kenneth R, Markham, Carolyn S, Hans G. 13C NMR studies of some naturally occurring amentoflavone and hinokiflavone bioflavonoids. Phytochem 1987;26:3335-7.
19. Burkhill IH. Dictionary of the economic products of the Malay Peninsula. Vol. 2. Kuala Lumpur: Ministry of Agriculture Malaysia; 1935.
20. Wass L, Soromou LW, Zhang Z, Li R, Chen N, Guo W, *et al*. Regulation of inflammatory cytokines in lipopolysaccharide-stimulated RAW 264.7 murine macrophage by 7-O-methyl-naringenin. Molecules 2012;17:3574-85.