

Anti-inflammatory Activity of Isolated Compounds from the Stem Bark of *Garcinia cowa* Roxb

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History

- Submission Date: 15-09-2016;
- Review completed: 30-09-2016;
- Accepted Date: 05-11-2016.

DOI : [10.5530/pj.2017.1.10](https://doi.org/10.5530/pj.2017.1.10)

Article Available online

<http://www.phcogj.com/v9/i1>

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ABSTRACT

Objective: To find the anti-inflammatory active compounds from methanol extract of *Garcinia cowa* Roxb. **Methods:** To evaluate the inhibitory activity of isolated compounds on nitric oxide (NO) production, culture media was assayed using Griess reaction. An equal volume of Griess reagent (1% sulphanilamide and 0.1% N-(L-naphthyl)-ethylene diamine dihydrochloride, dissolved in 2.5% H₃PO₄) was mixed with culture supernatant and color development was measured at 550 nm using a micro plate reader. The amount of nitrite in the culture supernatant was calculated from a standard curve (0–100 μM) of sodium nitrite freshly prepared in deionized water. Percentage of the NO inhibition was calculated by using nitrate level of IFN-γ/LPS-induced group as the control. **Results:** Isolated compounds, tetraprenyltoluquinone, rubraxanthone and α-mangostin from stem bark of *Garcinia cowa* Roxb were evaluated for their anti-inflammatory activity. Only α-mangostin exhibited strong anti-inflammatory activity with 83.42 % inhibition of NO and without inducing severe cytotoxicity at 50 μM. Rubraxanthone showed weak inhibition of NO with 23.86 % inhibition of NO while maintained 77.32 % of cell viability. TPTQ also showed the strong inhibition of NO with 80.98 % inhibition but unfortunately this compound also induced severe cytotoxicity with 39.62% viability. **Conclusion:** α-Mangostin exhibited strong anti-inflammatory activity without inducing severe cytotoxicity at 50 μM. Rubraxanthone showed weak inhibition of NO while Tetraprenyltoluquinone also showed the strong inhibition of NO however this compound also induced severe cytotoxicity.

Key words: Nitric oxide, Anti-inflammatory, *Garcinia cowa*, Rubraxanthone, α-mangostin, tetraprenyltoluquinone

INTRODUCTION

The inflammatory disease as manifested in rheumatism, allergies and asthma is increasing. About 10% of the children are said to have asthmatic disorders. Although several different steroids and NSAIDS such as celecoxib, aspirin, ibuprofen, and phenylbutazole have been approved for treatment of inflammatory conditions, most of them have side effects, especially when consumed over long periods of time.¹ Therefore, the empirical approach to discover new drugs with less side effects via the systematic screening of plants extracts still remains an interesting strategy to find new lead compounds.²

In our preliminary study, the methanol extracts of eight species of different parts of *Garcinia spp.* were evaluated for their cytotoxicity and anti-inflammatory activity using MTT and Griess assay method, respectively.³ Ethanol extract of the stem bark of *G. cowa* also active on T47D breast cancer cell. Several compounds, [2E,6E,10E]-(+)-4b-hydroxy-3-methyl-5b-(3,7,11,15-tetramethyl-2,6,10,14-hexadecatetraenyl-2-cyclohexen-1-one (1), 6-hydroxycalabaxanthone (2), 2-(3-methyl-2-butenyl)-1,5,6-trihydroxy-3-methoxy-4-(1,1-dimethyl-2-propenyl)-9H-xanthen-

9-one (3), rubraxanthone (4), α-mangostin (5), a new compound 1,3,6-trihydroxy-7-methoxy-4-(4-acetoxy-3-methyl-2-butenyl)-8-(3,7-dimethyl-2,6-octadienyl)xanthone (6) and cowanin (7) were isolated from this part.^{5,6}In this paper anti-inflammatory study of isolated compound from the stem bark of *G. cowa* was reported.

MATERIALS AND METHODS

Instruments

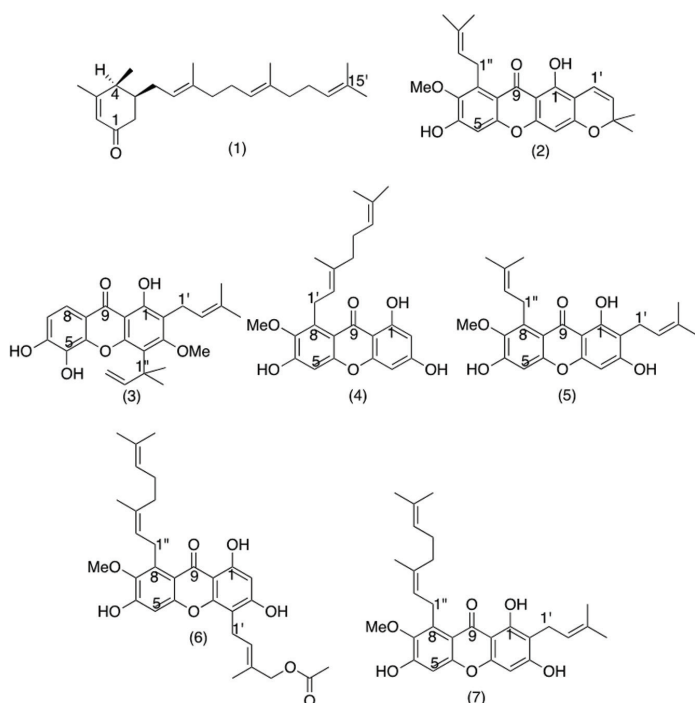
Holten Laminar microbiological safety cabinet class II was obtained from Heto-Holten (Allerød, Denmark). Galaxy R CO₂ incubator was from RS Biotech (Ayrshire, Scotland). A micro plate reader with SOFTmax[®] Pro software (Versamax, Molecular Devices, California, USA) was used to measure the absorbance of formazan solution.

Plant material and compound isolation

Plant material:

Garcinia cowa Roxb (stem bark, leaves and roots) was collected at Sarasah Bonta, Harau Valley, and West Sumatra at an altitude of 500 m. The voucher specimens (DR-180)

Cite this article : Wahyuni FS, Ali DAI, Lajis NH, Dachriyanus. Anti-inflammatory activity of isolated compounds from the stem bark of *Garcinia cowa* Roxb. *Pharmacogn J.* 2017;9(1):55-7.



were identified by Dr Rusdi Tamin and were deposited in the herbarium of Andalas University, Padang, Indonesia. Plant materials were cut into small pieces (3-5 mm thickness) and air-dried under shade. The dried plant materials were ground to powder before extraction. Tetraprenyltoluquinone, α-mangostin, and rubraxanthone were isolated from the stem bark of *G. cowa* according to the method described earlier.⁶

Preparation of extracts and compounds

The methanol extracts and compounds were prepared by dissolving in DMSO to make stock solutions of 100 mg/mL and 100mM, respectively. All drug solutions were aliquot into appropriate volume and stored refrigerated at -20°C prior to use.

Cell culture

RAW264.7 cell line was obtained from American Type Culture Collection (ATCC), USA. Dulbecco's Modified Eagle Medium (DMEM) both with and without phenol red, phosphate buffered saline and Hanks' balanced salt solution (HBSS), 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT), phosphate buffered saline (PBS) and Griess reagent were from Invitrogen (Carlsbad, USA). Foetal bovine serum (FBS), LPS from *E. coli* serotype O111:B4, Indomethacin, L-NAME [L-NG-nitroarginine methyl ester (hydrochloride)], dimethyl sulfoxide (DMSO), and sodium nitrite were obtained from Sigma (St Louis, USA). Interferon gamma (IFNγ) was from BD Biosciences (New Jersey, USA). NF-κB translocation kit was from Cellomics (Pittsburgh, USA). All other chemicals and reagents used were of HPLC grade.

The murine monocyte macrophage cell line (RAW 264.7) was maintained in DMEM supplemented with 10% FBS, 4.5 g/l glucose, sodium pyruvate (1 mM), L-glutamine (2 mM), streptomycin (50 µg/ml) and penicillin (50 U/ml) at 37°C and 5% CO₂. Cells at confluence of 80-90% were centrifuged at 120×g at 4°C for 10 min and cell concentration was adjusted to (2×10⁶) cells/ml, whereby the cell viability always more than 90%, as determined by trypan blue exclusion. A total of 50 µL of cell suspension was seeded into a tissue culture grade 96-well plate (4×10⁵ cells/well) and incubate for 2 h at 37°C, 5% CO₂ for cells attachment. Then, the cells were stimulated by using 100 U/ml of IFN-γ and 5µg/ml of

LPS with or without the presence of columbine tested at the final volume of 100µL/well. DMSO was used as vehicle, where the final concentration of DMSO was maintained at 0.1% of all cultures. Cells were further incubated at 37°C, 5% CO₂ for 20 h. The culture supernatant was subjected to Griess assay for nitrite determination and the cells remaining in the well were tested for cell viability assay by using MTT reagent.

In vitro test for anti-inflammatory activity - Griess assay

To evaluate the activity of extracts in inhibiting NO production, the Griess assay was employed (modified method of Dirsch *et al.*, 1988).⁷ Briefly, RAW 264.7 cells (murine monocytes macrophage) were stimulated to produce inflammation using recombinant mouse IFN-γ (BD Pharmingen, USA) and lipopolysaccharide (LPS) (from *Escherichia coli*). To evaluate the NO inhibitory activity of extracts, Griess reagent (1% sulfanilamide/ 0.1% N-(1-naphthyl) ethylene diamine dihydrochloride in 2.5% H₃PO₄) was mixed with an equal part of cell culture medium of control and extracts treated RAW 264.7 cells. The color development corresponding to NO level was assessed at 550 nm with a micro plate reader (Spectramax, Plus 384, Molecular Devices, Inc., USA). The percentage NO inhibition was determined according to the formula below. This was followed by cell viability determination using the MTT assay as describe above:

$$\frac{\text{No level of control} - \text{No level of extract treated cells}}{\text{No level of control cells}} \times 100\%$$

RESULTS

Isolated compounds, tetraprenyltoluquinone, rubraxanthone and α-mangostin from stem bark of *Garcinia cowa* Roxb were evaluated for their anti-inflammatory activity. The result can be showed in Table 1.

DISCUSSION

Inflammation is a pathophysiological process mediated by a variety of signaling molecules produced mainly by leukocytes, macrophages and plasma cells. Macrophages play a crucial role in the generation of the pro-inflammatory molecule nitric oxide (NO). NO is generated at high levels during human inflammatory reaction. NO synthesized by the enzyme inducible nitric oxide synthase (iNOS) has been reported as a mediator of acute and chronic inflammations.⁸ Studies have shown that macrophages upon stimulation with bacterial lipopolysaccharide (LPS) express iNOS to produce large amount of NO. iNOS is one of the essential components of the inflammatory response and is involved in the pathogenesis of several inflammatory diseases such as asthma and rheumatoid arthritis. In this study, using Griess assay to measure the level of NO produced by activated murine RAW 264.7 macrophage cells, plants extracts were evaluated for their anti inflammatory activity.

NO may induce toxic reactions against other tissues of the host when it is generated at high levels in certain types of inflammation, for example asthma. NO also has been implicated as a pro-inflammatory agent.⁹ Nitric oxide (NO) is a signal molecule with functions such as neurotransmission, local vascular relaxation and anti-inflammation in many

Table 1: No inhibitory activity of isolated compounds

No	Compounds	% inhibition of NO (50µM)	% viability of RAW cell (50µM)
1.	Tetraprenyltoluquinone (1)	81	40
2.	rubraxanthone (4)	24	77
3.	α-mangostin(5)	83	82

physiological and pathological processes. Due to its high reactivity in biological systems, it is transformed in the bloodstream into nitrates (NO₃⁻) by oxyhemoglobin. The Griess reaction is a technically simple method (spectrophotometric, 540 nm) for the analysis of nitrites (NO₂⁻) in aqueous solutions.¹⁰

Three compounds were evaluated for the inhibition of NO production. Only α -mangostin (5) exhibited strong anti-inflammatory activity with 83.42% inhibition of NO and 82.36% cell viability. Anti-inflammatory activity of α -mangostin (5) has been reported before by Chen *et al.*, (2008).⁷ Rubraxanthone (4) showed weak inhibition of NO with 23.86 % inhibition value of NO and 77.32 % of cell viability. Compound (1) also showed the strong inhibition of NO with 80.98% inhibition but it also kill the RAW cell with 39.62 % viability.

CONCLUSION

In conclusion, α -mangostin (5) exhibited strong anti-inflammatory activity without inducing severe cytotoxicity. Rubraxanthone (4) showed weak inhibition of NO while tetraprenyltoluquinone (1) showed the strong inhibition of NO inhibition however this compound also induced severe cytotoxicity.

ACKNOWLEDGEMENTS

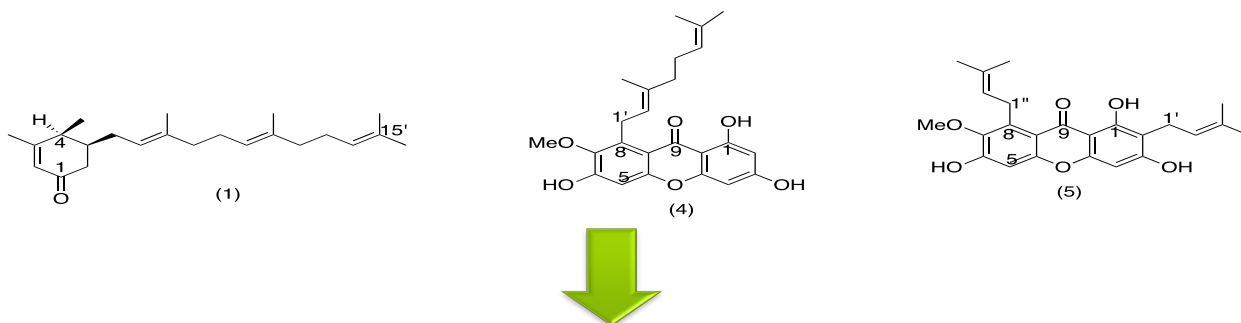
The author thank the Malaysian Ministry of Science, Technology and the Environment are thanked for financial support under the Intensified Research in Priority Areas Research Grant (IRPA, EAR No. 09-02-04-0313), International Foundation for Science (IFS no F/3967-1), National L'Oreal Fellowship and Higher Education Grant from Ministry of Education Indonesia

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PICTORIAL ABSTRACT

Isolated Compounds from *Garcinia cowa*



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