

INHIBITORY EFFECT OF *ARTOCARPUS LOWII* KING COMPOUNDS ON COX-2 AND 15-LO ACTIVITIES

Mohamad Norisham Mohamad Rosdi^a, Hasnah Mohd Sirat^b, Siti Awanis Abdullah^b, Shajarahtunnur Jamil^b, Ida Idayu Muhamad^c, Razauden Mohamed Zulkifli^{a*}

Article history

Received
7 November 2014
Received in revised form
30 March 2015
Accepted
1 August 2015

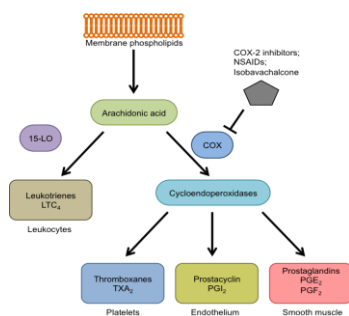
^aDepartment of Bioscience and Health Sciences, Faculty of Bioscience and Medical Engineering, Universiti Teknologi Malaysia

^bDepartment of Chemistry, Faculty of Science, Universiti Teknologi Malaysia

^cIJN-UTM Cardiovascular Engineering Centre, Universiti Teknologi Malaysia

*Corresponding author
razauden@biomedical.utm.my

Graphical abstract



Abstract

Artocarpus lowii King is a rare species of plant in Moraceae family. In this study, the anti-inflammatory activity of cycloheterophyllin, isobavachalcone, 4-hydroxy lonchocarpin and 2',4'-dihydroxy-4-methoxy-3'-prenyldihydroxychalcone isolated from *A. lowii* King were investigated on classical enzymes in arachidonic acid metabolism pathways; cyclooxygenase and lipoxygenase. Isobavachalcone directly inhibited cyclooxygenase-2 enzyme in dose dependent manner, with IC₅₀ value of 0.95 μM. No noticeable effect has been observed with the other tested compounds. In addition, none of the tested compounds displays a direct inhibition on 15-lipoxygenase when compared to the resveratrol as control with the IC₅₀ value of 1.5 μM. Isobavachalcone showed inhibitory effect on cyclooxygenase-2. This study suggests that *A. lowii* King contains potential anti-inflammatory activity.

Keywords: *Artocarpus lowii* King, Anti-inflammatory, Cyclooxygenase-2, Isobavachalcone

Abstrak

Artocarpus lowii King adalah sejenis spesies tumbuhan jarang daripada keluarga Moraceae. Dalam kajian ini, efek anti-inflamatori oleh komponen aktif daripada *A. lowii* King iaitu cycloheterophyllin, isobavachalcone, 4-hydroxy lonchocarpin dan 2',4'-dihydroxy-4-methoxy-3'-prenyldihydroxychalcone ke atas aktiviti enzim cyclooxygenase-2 dan 15-lipoxygenase telah diselidiki. Isobavachalcone secara langsung telah menghalang aktiviti cyclooxygenase-2 dengan nilai IC₅₀ 0.95 μM. Tiada aktiviti memberangsangkan untuk kompaun lain. Selain itu, tiada aktiviti direkodkan untuk efek terhadap 15-lipoxygenase bagi semua kompaun. Hanya kompaun control iaitu resveratrol menunjukkan aktiviti dengan nilai IC₅₀ sebanyak 1.5 μM. Isobavachalcone dicadangkan memiliki efek anti-inflamatori yang menyumbang kepada aktiviti anti-inflamatori sepsis *A. lowii* King.

Kata kunci: *Artocarpus lowii* King, Anti-inflamatori, Cyclooxygenase-2, Isobavachalcone

© 2015 Penerbit UTM Press. All rights reserved

1.0 INTRODUCTION

Globalization has driven a remarkable transformation in living aspect relating to lifestyle, work-nature, and dietary habits. The reflection of this setting can be seen in the rise of degenerative diseases cases in population around the world [1]. The diseases such as cardiovascular diseases (CVD), hypertension, diabetes and obesity have become prominent over the past few years [2]. Study conducted by the International Medical Foundation of Japan revealed that the cases of CVD increased drastically since 1965 until 1997 and the number of cases is approaching alarming state. Recent preliminary report by the World Health Organization (WHO) showed that heart related diseases are the topmost leading causes of death in United States [3]. This situation has alerted medical practitioners and scientists to undergo pathophysiological and therapeutic approaches in heart-related diseases.

Natural products such as coumarin and aspirin are great sources of traditional and modern drugs for treatment of different heart-related diseases [4]. According to the WHO, approximately 80% of the world's populations rely on traditional medicine for their primary health care [5]. Folks applied different plants extracts for treatment of wide variety of diseases including chronic inflammation.

Non-steroidal anti-inflammatory drugs (NSAIDs) are a group of drugs that are capable to provide analgesic, antipyretic and anti-inflammatory effects [6]. Even though NSAIDs are currently used in the treatment of inflammation, these drugs have not shown any significant effect in treating chronic inflammatory disorders. In contrast, several compounds are found to be associated with negative side effects. Thus, there is a need to search for non-poisonous and effective anti-inflammatory compounds [7].

2.0 EXPERIMENTAL

2.1 Sample Preparation

Cycloheterophyllin, isobavachalcone, 4-hydroxylonchocarpin and 2',4'-dihydroxy-4-methoxy-3'-prenyldihydroxychalcone were provided by Jamil et al. [15]. Resveratrol served as positive control, the samples were dissolved in Ethyl Acetate (EtOAc) and diluted ten-fold into five different concentration ranges from 0 μ M to 100 μ M. All samples were stored at 4 °C to avoid decomposition.

2.2 COX-2 Inhibitor Screening Assay Kit

COX-2 inhibitor screening kit (Item No.: 560131; Cayman Chemicals, USA) was used. The assay included two major steps, COX reaction and enzyme immunoassay. In COX reaction, the required reactions such as background reaction, initial activity reaction,

Phytochemicals are reported to be key players in promoting various medicinal activities. Phytochemicals such as flavonoids have demonstrated numerous biological activities such as anticancer, antimicrobial, antiviral, anti-inflammatory, immunomodulatory, and antifibrinolytic activities [8-10]. This type of compounds was also reported to exhibit *in vitro* and *in vivo* anti-inflammatory activities in mice [11-12]. Cohort studies demonstrated that an increased in the consumption of plant-derived foods like fruit and vegetables, nuts, and whole grains is associated with a reduced risk of heart-related diseases [13]. Therefore, flavonoids are presumed to be vital in promoting anti-inflammatory activities, and also in developing a new kind of anti-inflammatory agents [14]. In addition, wide attention of natural-derived medicine occurs probably due to the fact that it possesses less adverse compared to the synthetic counterpart. Natural products also are basically safe and easily obtained.

Artocarpus lowii King is a scarce species of tree in the *Artocarpus* genus of the *Moraceae* family. This plant is widely distributed in South East Asia. In Malaysia, it is locally known as 'miku' while in Indonesia it is known as 'bangsal' [15]. Even though there are numerous biological reports on *Artocarpus* genus, there is little study on anti-inflammatory of *A. lowii* King. Flavonoids from *Artocarpus heterophyllus* and *Artocarpus communis* had shown anti-inflammatory activities through inhibition of superoxide anion formation in fMLP-stimulated (Formyl-Methionyl-Leucyl-Phenylalanine) rat neutrophils and the stimulation of superoxide anion generation [16].

We report herein the investigation on the anti-inflammatory activity of isolated compounds from the leaves of *A. lowii* King on the cyclooxygenase-2 and 15-lipoxygenase enzyme pathways.

and sample reaction were undergone in 100 mM Tris-HCL buffer containing 1 μ M heme and COX-2 (human recombinant). The reactions were pre-incubated for 10 minutes at 37 °C. The reaction was later introduced with 10 μ L of arachidonic acid. After 2 minutes, an amount of 1 M of HCL was added into each sample to terminate the reaction process. Reactions were transferred into 96 well plate coated with mouse anti-rabbit immunoglobulin (IgG). The tracer, prostaglandin acetylcholine esterase, and primary antibody were then inserted into each well. After 24 hrs of incubation at room temperature, the reaction mixtures were removed and all wells were washed with 10 mM potassium phosphate buffer containing 0.05% Tween 20 solution. Then, Ellman's Reagent was added into wells. The plate was incubated for approximately 60 minutes until the maximum binding wells (control) reach the optical density (OD) ranging between 0.3-0.8 at $\lambda_{415\text{nm}}$.

The acquired raw data from plate reader were calculated by applying the data into the equation provided in the manufacturer's protocol below.

$$\text{Percentage of Inhibition (\%)} = \frac{[\text{Total COX activity}] - [\text{Total COX activity (inhibitor)}]}{[\text{Total COX activity}]} \times 100\%$$

The IC₅₀ value is presented; as the value is the amount of inhibitor required to disrupt the enzyme activity by 50%.

2.3 15-Lipoxygenase Inhibitor Assay

Lipoxygenase inhibitor screening assay kit (Item No. 760700) was purchased from Cayman Chemical, USA. All required reagents were prepared according to the provided protocol. An amount of 100 µL of Assay Buffer was added into three wells. In positive control wells (15-LO standard), 90 µL of 15-LO and 10 µL of Assay Buffer were added. Approximately, 90 µL of lipoxygenase enzyme and 10 µL of Ethyl Acetate were inserted to at least two wells (100% Initial Activity wells). An amount of 90 µL of lipoxygenase and 10 µL of inhibitor were added into inhibitor wells. In this experiment, only cycloheterophyllin and isobavachalcone were studied as inhibitors together with the control (resveratrol) on 15-lipoxygenase (15-LO) activity. The wells were incubated for five minutes at room temperature. The reaction was initiated when 10 µL of linoleic acid was added into the wells. Approximately 100 µL of chromogen was pipetted to stop the reaction. The reactions were measured at 515 nm. Percentage of inhibition of sample was calculated by following the equation below as instructed in the manufacturer's protocol:

$$\text{Percentage of inhibition (\%)} =$$

$$\frac{100\% \text{ Initial activity wells} - \text{Inhibitor wells (sample)}}{100\% \text{ Initial Activity wells}} \times 100$$

3.0 RESULTS AND DISCUSSION

3.1 Compounds Effect On COX-2 Inflammatory Mechanism

As shown in Figure 1 below, isobavachalcone showed a significant dose-dependent inhibitory effect on COX-2 activity with IC₅₀ value of 0.95 µM. Meanwhile 4-hydroxylonchocarpin displayed a reverse effect on COX-2 activity. This compound exhibited higher inhibition at lower concentration with 59.18% at 0.1 µM and rose slightly 28.19% at 100 µM. Resveratrol (control) have a steady effect on COX-2 activity even though at 100 µM, the data showed an outlier result.

Isobavachalcone was reported to demonstrate biological activities such as anticancer, antibacterial, antiplatelet, antifungal and anti-tubercular [17-19]. Shin et al. [20] reported that isobavachalcone isolated

from *Angelica keiskei* restrained inducible nitric oxide synthase (iNOS) enzymes expression thus deterring the inflammatory substance, nitric oxide (NO) production. However, the mechanism of action exerted by isobavachalcone remains uncertain. The current study demonstrated that isobavachalcone directly inhibit COX 2 enzyme.

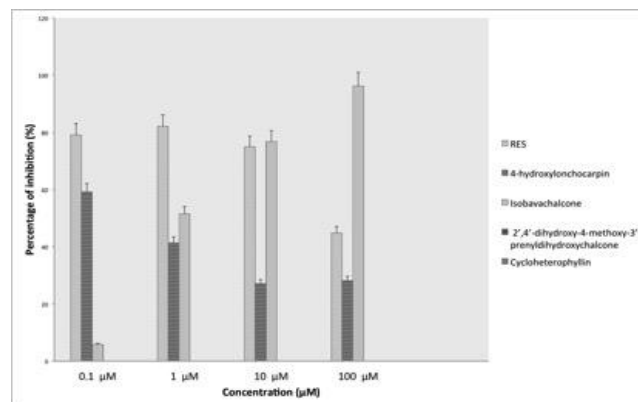


Figure 1 Inhibitory effects of compounds with resveratrol (RES) as control on cyclooxygenase-2 activity

3.2 15-Lipoxygenase Inhibition Activities

In contrast, no inhibitory effect observed on all investigated compounds except for resveratrol (control). In Figure 2, resveratrol has displayed a significant inhibitive activity on 15-lipoxygenase. Cycloheterophyllin observed in this study is contrary to the one isolated from *Artocarpus heterophyllus* LAMK inhibited arachidonate 15-lipoxygenase [21]. We presumed the dissimilar in structure of linoleic acid and arachidonic acid might produce this significant results. Linoleic acid structure contains less double bond compared to arachidonic acid hence the sensitivity of arachidonic acid is higher.

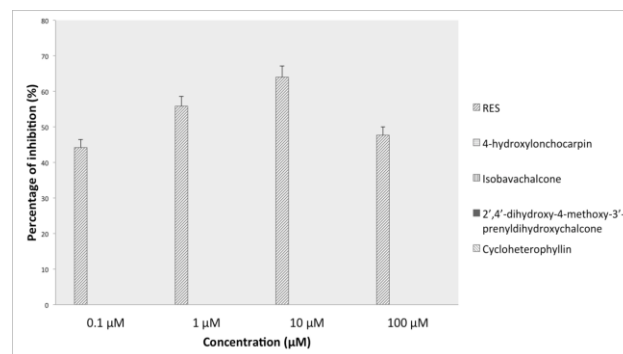


Figure 2 Inhibitory effects of compounds with resveratrol (RES) as control on 15-lipoxygenase activities.

4.0 CONCLUSION

This study shows that isobavachalcone isolated from *A. lowii* King possess potential anti-inflammatory properties via direct COX-2 inhibitory mechanism but not 15-LO. However, no inhibitory effect on both COX-2 and 15-LO by cycloheterophyllin, 4-hydroxyonchocarpin and 2',4'-dihydroxy-4-methoxy-3'-prenyldihydroxychalcone isolated from *A. lowii* King.

Acknowledgement

The authors would like to acknowledge to Research University Grant (GUP) from the Ministry of Higher Education (MOHE) for financial support under vote 01G59 and 07H22 and the Faculty of Biosciences and Medical Engineering, Universiti Teknologi Malaysia for facilities supports.

References

- [1] Popkin, B. M. 1998. The Nutrition Transition And Its Health Implications In Lower-Income Countries. *Public Health Nutr.* 1: 5-21.
- [2] Noor, M. I. 2002. The Nutrition and Health Transition in Malaysia. *Public Health Nutr.* 5: 191-195.
- [3] Hoyerl, D. L., and J. Xu. 2012. Deaths: Preliminary Data for 2011. *Natl. Vital Stat. Rep.* 61: 1-64.
- [4] Yu, J., L. Wang, R. L. Walzem, E. G. Miller, L. M. Pike, and B. S. Patil. 2005. Antioxidant Activity of Citrus Limonoids, Flavonoids, and Coumarins. *J. Agr. Food. Chem.* 53(6): 2009-2014.
- [5] Farnsworth, N. R., O. Akerele, A. Bingel, D. D. Soejarto, and Z. Guo. 1985. Medicinal Plants in Therapy. *Bull World Health Organ.* 63: 965-981.
- [6] Hart, F. D., and E. C. Huskisson. 1984. Non-Steroidal Anti-Inflammatory Drugs. *Drugs.* 3: 232-255.
- [7] Yoon, J. -H., and S. J. Baek. 2005. Molecular Targets of Dietary Polyphenols with Anti-Inflammatory Properties. *Yonsei Med J.* 46: 585-96.
- [8] Robak, J., and R. J. Gryglewski. 1996. Bioactivity of Flavonoids. *Pol J Pharmacol.* 48: 555-64.
- [9] Russo, A., R. Acquaviva, A. Campisi, V. Sorrenti, C. Di Giacomo, and G. Virgata. 2000. Bioflavonoids As Antiradicals, Antioxidants And DNA Cleavage Protectors. *Cell Biol Toxicol.* 16: 91-8.
- [10] Havsteen, B. 2002. The Biochemistry and Medical Significance of the Flavonoids. *Pharmacol Ther.* 96: 67-202.
- [11] Rotelli, A. E., T. Guardia, A. O. Juarez, and N. E. de la Rocha. 2003. Comparative Study Of Flavonoids In Experimental Models of Inflammation. *Pharmacol Res.* 48: 601-6.
- [12] Wang, L., Y. C. Tu, T. W. Lian, J. T. Hung, J. H. Yen, and M. J. Wu. 2006. Distinctive Antioxidant and Anti-Inflammatory Effects of Flavonols. *J Agric Food Chem.* 54: 9798-804.
- [13] Hu, F. B. 2003. Plant-Based Foods and Prevention of Cardiovascular Disease: An Overview. *Am. J. Clin. Nutr.* 78(suppl): 544S-51S.
- [14] Kim, H. P., H. S. Kun, H. W. Chang, S. S. Kang. 2004 Anti-Inflammatory Plant Flavonoids and Cellular Action Mechanisms. *J Pharmacol Sci.* 96: 229-45.
- [15] Jamil, S., H. Mohd Sirat, I. Jantan, N. Aimi, and M. Kitajima. 2008. A New Prenylated Dihydrochalcone from the Leaves of *Artocarpus Lowii*. *J. Nat. Med.* 62: 321-324.
- [16] Wei, B. -L., J. -R. Weng, P. -H. Chiu, C. -F. Hung, J. -P. Wang, and C. N. Lin. 2005. Antiinflammatory Flavonoids from *Artocarpus Heterophyllus* And *Artocarpus Communis*. *J. Agr. Food Chem.* 53(10): 3867-3871.
- [17] Jing, H. X. Zhou, X. Dong, J. Cao, H. Zhu, J. Lou, Y. Hu, Q. He, and B. Yang. 2010. Abrogation of Akt Signalling by Isobavachalcone Contributes to Its Anti-Proliferative Effects Towards Human Cancer Cells. *Cancer Lett.* 294: 167-177.
- [18] Kuetee, V., and L. Sandjo. 2012. Isobavachalcone: An Overview. *Chin. J. Integr. Med.* 18: 543-547.
- [19] Ohno, O., T. Watabe, K. Nakamura, M. Kawagoshi, N. Uotsu, T. Chiba, M. Yamada, K. Yamaguchi, K. Yamada, K. Miyamoto, and D. Uemura. 2010. Inhibitory Effects Of Bakuchiol, Bavachin, Isobavachalcone Isolated From *Piper Longum* On Melanin Production In B16 Mouse Melanoma Cells. *Biosci. Biotech. Bioch.* 74: 1504-1506.
- [20] Shin, H. -J. D. -H. Shon and H. -S. Youn. 2013. Isobavachalcone Suppresses Expression of Inducible Nitric Oxide Synthase Induced by Toll-like Receptor Agonists. *Int Immunopharmacol.* 15(1): 38-41.
- [21] Wang, J. P., S. L. Raung, L. T. Tsao, M. F. Hsu, and C. N. Lin. 1997. Blockade of Protein Kinase C is Involved in the Inhibition by Cycloheterophyllin of Neutrophil Superoxide Anion Generation. *N-S. Arch. Pharmacol.* 355: 551-558.