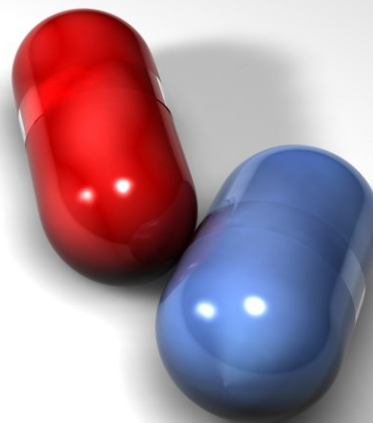


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Letter to the Editor

Anti-inflammatory activity of 9-hydroxycanthin-6-one extracted from hairy-root cultures of *Eurycoma longifolia* potentially via aryl hydrocarbon receptor induction

Sir,

Eurycoma longifolia Jack (Simaroubaceae) is a commercially important herb and is widely used in Southeast Asian countries (Bhat and Karim, 2010; Rehman et al., 2016). The present group and others reported that pharmaceutical secondary metabolites extracted from *E. longifolia* showed anti-inflammatory and anti-cancer properties (Tong et al., 2015; Rehman et al., 2016; Ngoc et al., 2016; Nguyen et al., 2016). The bioactivity of 9-hydroxycanthin-6-one, the main compound in *E. longifolia*, however, is not fully understood.

This study aimed to accelerate 9-hydroxycanthin-6-one production from *E. longifolia* hairy root cultures and to test its anti-inflammatory activity in RAW264.7 macrophage cell line.

We determined the 9-hydroxycanthin-6-one compound extracted from the hairy root of *E. longifolia*. The 9-hydroxycanthin-6-one was obtained as an amorphous yellow powder. Its HR-ESI-MS spectrum revealed the peak at m/z : 237 $[M+H]^+$, corresponding to the molecular formula $C_{14}H_8N_2O_2$. The 1H -NMR spectrum of 9-hydroxycanthin-6-one showed weak signals of the

protons of the aromatic ring and dienes, where the signals characteristic of an ABX aromatic system at δ_H 7.95 (1H, d, $J = 2.0$ Hz, H-8), 6.98 (1H, dd, $J = 2.0; 8.5$ Hz, H-10), 8.11 (1H, d, $J = 8.5$ Hz, H-11). Additionally, two signals of protons at δ_H 8.08 (1H, d, $J = 5.0$ Hz, H-1), 8.72 (1H, d, $J = 5.0$ Hz, H-2) were assigned to two protons in the aromatic ring with one heteroatom of nitrogen. Two doublet signals at δ_H 6.93 (H-3)/8.06 (H-4) correspond to two hydrogen atoms (H-4 and H-5) of the aromatic ring with two heteroatoms of carbon and nitrogen. The singlet signal at δ_H 7.95 is assigned to H-8.

The ^{13}C -NMR and DEPT spectra of this compound indicate the molecule contains 14 carbon atoms, including seven methines and seven quaternary carbons. The signals appearing in the range from δ_C 102.9 to 145.9 indicated the carbon atoms of aromatic rings and dienes. The two signal peaks of the carboxyl group appeared at δ_C 158.9, with this quite low value of δ_C caused by the conjugation effect of the carboxyl group and a diene, and the peak of δ_C 160.5 is assigned to the carbon atom of the hydroxyl group. Therefore, based on the spectral analysis, it could conclude that this compound is 9-hydroxycanthin-6-one.

There was growth of hairy root and accumulation of 9-hydroxycanthin-6-one in Murashige and Skoog (MS), Shenck and Hildebrandt (SH), and McCown's woody plant (WP) liquid culture media supplemented with 3% (w/v) of sucrose. After 30 days, the biomass generated by the SH basal medium was the greatest (0.8 ± 0.0 g,

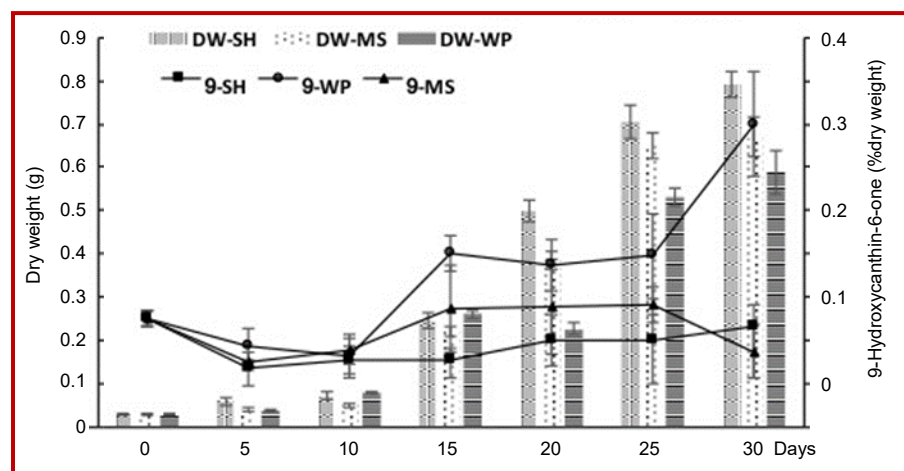


Figure 1: Dry weight and 9-hydroxycanthin-6-one production in hairy root of *E. longifolia* on different basal media (MS, SH, and WP). The mean \pm SD data from three independent experiments are shown



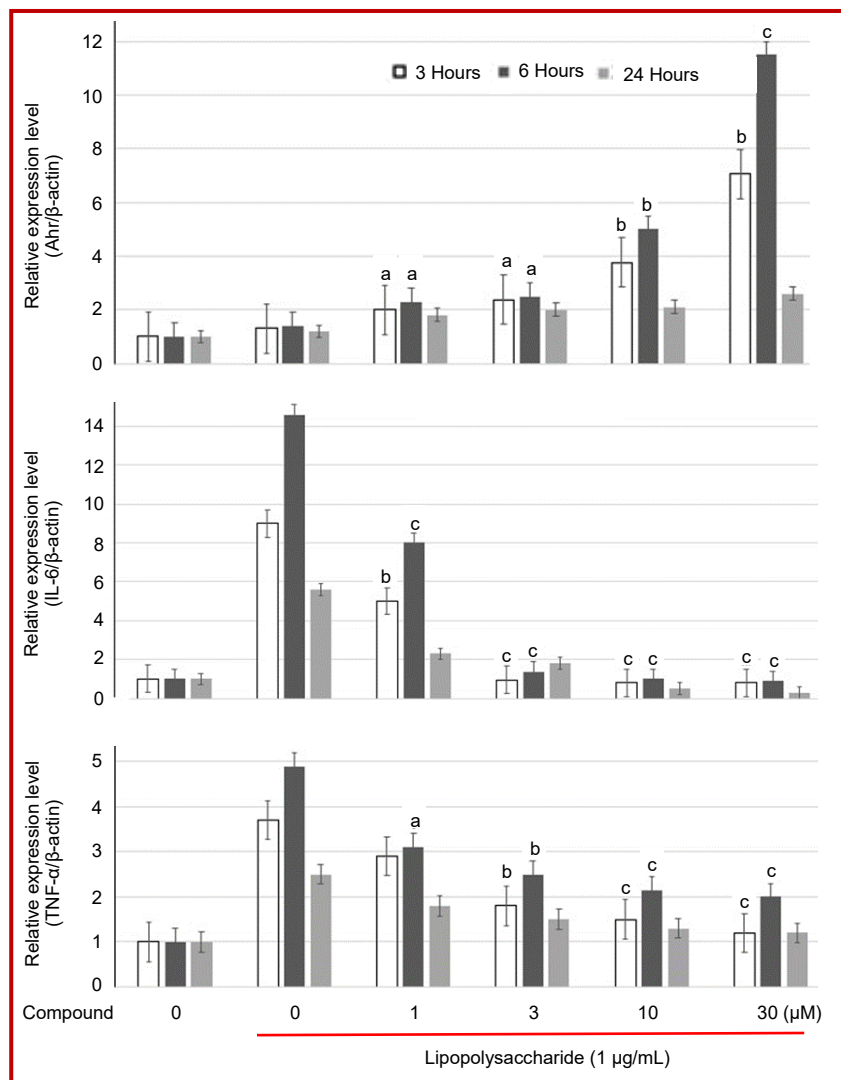


Figure 2: Effect of 9-hydroxycanthin-6-one isolated from the hairy roots of *E. longifolia* on the expression level of Ahr, IL-6, TNF- α in Raw 264.7 cells treated with lipopolysaccharide. RT-qPCR was utilized to measure the expression level. The mean \pm SD data from three independent experiments is shown. ^a $p < 0.05$; ^b $p < 0.01$; ^c $p < 0.005$ compared with lipopolysaccharide only

DW), followed by MS (0.7 ± 0.1 g, DW), and WP (0.6 ± 0.1 g, DW). Meanwhile, 9-hydroxycanthin-6-one production dynamics in WP medium increased after 10 days and reached a maximum value of 0.3 ± 0.0 (% DW) after 30 days. Conversely, 9-hydroxycanthin-6-one production dynamics in the MS and SH media revealed a long lag phase and no growth, with a maximum level of 0.1 ± 0.0 (%DW) after 25 days and 0.1 ± 0.0 (%DW) after 30 days, respectively (Figure 1). These results suggest that the 9-hydroxycanthin-6-one content was greatest in hairy roots cultivated in WP medium, while the maximum biomass of hairy roots was obtained in SH medium.

Besides, the present study reported that aryl hydrocarbon receptor (Ahr), a ligand-dependent transcription factor, played a role as an anti-inflammatory agent via modulating various cytokines in such immune cells as

macrophages, dendritic cells and t cells which involve in the development of inflammatory and autoimmune disorders (Masuda et al., 2011; Zhu et al., 2018; Neavin et al., 2018).

Therefore, looking for new Ahr inducers is crucial for controlling these disorders. This is the first time we showed that Ahr expression was significantly increased in lipopolysaccharide-stimulated RAW264.7 cells under the induction of 9-hydroxycanthin-6-one. The cells were pre-induced with 9-hydroxycanthin-6-one (1, 3, 10, 30 μ M) for 30 min before being stimulated with lipopolysaccharide (1 μ g/mL) for 1, 3, 6, and 24 hours. Interestingly, we found that Ahr gene expression was significantly increased, reaching a maximum of 11.5 fold at a concentration of 30 μ M, compared to untreated cells at 6 hours (Figure 2). Previously, it has been demonstrated that Ahr suppresses pro-inflammatory cytokine production in macrophages by inhibiting nuclear factor- κ B (NF- κ B) activation after lipopolysaccharide stimulation

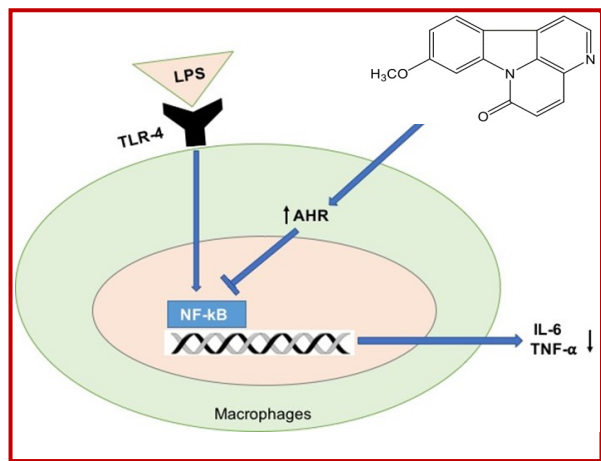


Figure 3: 9-Hydroxycanthin-6-one suppress inflammatory cytokines potentially via Ahr

(Kimura et al., 2009). We, therefore, measure the expression of cytokine genes, including IL-6 and TNF- α during this stimulation. Consistently, inflammatory cytokine genes such as IL-6 and TNF- α were significantly reduced after treatment with various concentrations of 9-hydroxycanthin-6-one (Figure 2). These findings imply that 9-hydroxycanthin-6-one extracted from the hairy roots of *E. longifolia* is a potential activator of Ahr in the context of LPS stimulation that thus might inhibit inflammatory cytokines such as IL-6 and TNF- α in the macrophages (Figure 3).

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