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Anti-inflammatory effect and mechanism of action of ellagic acid-3,3',4-trimethoxy-4'-*O*-α-L-rhamnopyranoside isolated from *Hopea parviflora* in lipopolysaccharide-stimulated RAW 264.7 macrophages

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SHORT COMMUNICATION

Anti-inflammatory effect and mechanism of action of ellagic acid-3,3′,4-trimethoxy-4′-*O*-α-L-rhamnopyranoside isolated from *Hopea parviflora* in lipopolysaccharide-stimulated RAW 264.7 macrophages

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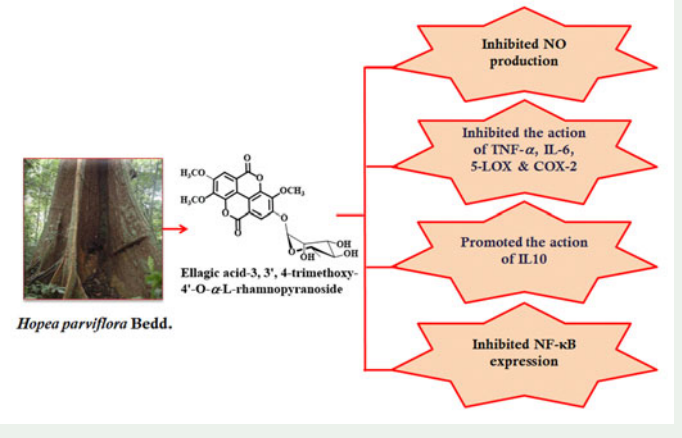
ABSTRACT

Phytochemical investigation of the stem bark of *Hopea parviflora* resulted in the isolation of **9** compounds; which includes friedelin (**1**), friedelin-3β-ol (**2**), (-)-ampelopsin A (**3**), (-)-ε-viniferin (**4**), (-)-hopeaphenol (**5**), vaticaphenol A (**6**), 2,4,8-trihydroxyphenanthrene-2-*O*-glucoside (**7**), ellagic acid-3,3′,4-trimethoxy-4′-*O*-α-L-rhamnopyranoside (**8**) and β-sitosterol-β-D-glucoside (**9**). Among them, compounds **1**, **2**, **6**, **7**, **8** and **9** are isolated for the first time from this species. Further, we evaluated the anti-inflammatory activity of compounds **4**, **5**, **6**, **7** and **8**. In this study, compound **8** inhibited the activity of proinflammatory mediators like NO, TNF-α, IL-6, 5-LOX and COX-2, also promoted the action of anti-inflammatory mediator like IL-10 *via* inhibition of the NF-κB pathway in LPS-stimulated RAW 264.7 macrophages.



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1. Introduction

Hopea parviflora Bedd. (*H. parviflora*, Dipterocarpaceae); an endangered species and is commonly recognized as "Iron wood of Malabar" or "White Kongu". The first report on isolation of phytochemicals from this species was performed by the group of Tanaka, where they isolated (+)-parviforol, (-)-ampelopsin A, (+)-balanocarpol, (-)-ε-viniferin and (-)-hopeaphenol from the stem bark of *H. parviflora* (Tanaka et al. 2000). After that, Naohito et al. reported the isolation of hopeasides A, B, C and D, malibatol, piceid, vateriaphenol B, pauciflorol C, grandiphenol A and vatalbinoside A from this species (Naohito et al. 2011). There is no detailed report on the phytochemical and pharmacological evaluations of *H. parviflora* except these studies. Herein, we describe isolation, characterization and anti-inflammatory activity of the compounds isolated from the stem bark of *H. parviflora*.

2. Results and discussion

2.1. Isolation and characterization

The purification of acetone extract of dried and milled stem bark of *H. parviflora* led to the isolation of **9** compounds. They were identified as friedelin (**1**) (Queiroga et al. 2000), friedelin-3β-ol (**2**) (Queiroga et al. 2000), (-)-ampelopsin A (**3**) (Reniero et al. 1996), (-)-ε-viniferin (**4**) (Reniero et al. 1996), (-)-hopeaphenol (**5**) (Reniero et al. 1996), vaticaphenol A (**6**) (Eun-Kyoung et al. 1999), 2,4,8-trihydroxyphenanthrene-2-*O*-glucoside (7) (Baderschneider and Winterhalter 2000), ellagic acid-3,3′,4-trimethoxy-4′-*O*-α-L-rhamnopyranoside (**8**) (Hoang et al. 2012) and β-sitosterol-β-D-glucoside (**9**) (Rahman et al. 2009), of which **1**, **2**, **6**, **7**, **8** and **9** are reported for first time from this species. Structures of the isolated compounds are shown in Figure 1 and the spectral details of the compounds **1–9** are depicted in SI (S1–S23).

2.2. Cytotoxic effect of compounds 4–8 in RAW 264.7 macrophages

Among the isolated phytochemicals, based on the amount obtained, we chose compounds **4**–**8** for the analyses of cytotoxic effects in RAW 264.7 macrophages by means of MTT assay. The cells were treated with 1 μM, 5 μM, 10 μM, 25 μM, 50 μM and 100 μM concentrations of compounds **4–8**. From the Figure S24, it is clear that compound **8** showed less toxicity in 1 μM and 5 μM concentrations. Therefore, 1 and 5 μM concentrations of compound **8** were taken for further studies.

2.3. NO production in LPS-stimulated RAW 264.7 cell lines

Nitric oxide (NO) plays a crucial role in the host defence response against various pathogens. However, over-production of NO induces tissue damage associated with inflammations. Therefore, more attention is now being paid to the development of new drug leads as potent inhibitors of NO production concerning the treatment of inflammatory diseases (Pacher et al. 2007). Herein, we investigated the effects of compound **8** in LPS-induced RAW 264.7 cells (Figure S25). The result showed that NO

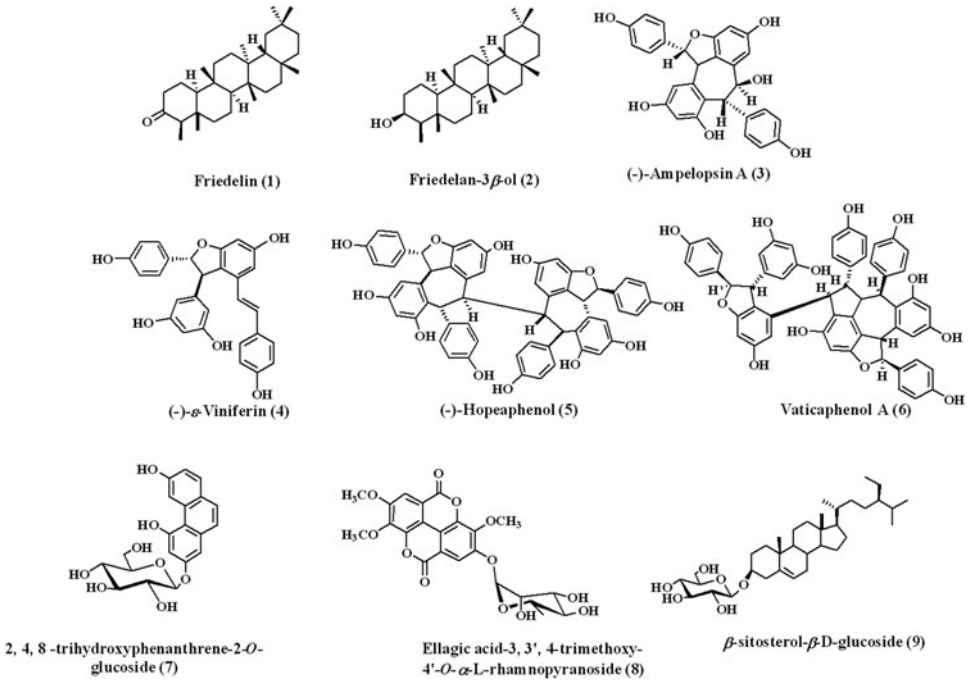

Figure 1. Compounds isolated from the stem bark of *H. parviflora*.

production remarkably increased in LPS- stimulated RAW 264.7 cells. Cells pre-treated with compound **8** showed a significant inhibition (∼2 & 4 fold decrease at 1 & 5 μM respectively) in NO production as compared to the positive control (dexamethasone).

2.4. TNF-α, IL-6, 5-LOX, COX-2 and IL-10 production in LPS-stimulated RAW 264.7 macrophages

In the present study, we investigated the effects of compound **8** on LPS-induced TNF-α, IL-6, 5-LOX, COX-2 and IL-10 production in LPS-induced RAW 264.7 cells (Figure S26). Proinflammatory mediators like TNF-α, IL-6, 5-LOX and COX-2 were found to be decreased in RAW 264.7 cells in a dose dependent manner, when compared with LPS and dexamethasone (positive control). It is also noted that anti-inflammatory cytokine IL-10 was found to be increased in cell treated with compound **8** when compared with LPS and dexamethasone (positive control) in a dose dependent manner.

2.5. Compound 8 inhibited NF-κB expression in LPS-stimulated RAW 264.7 macrophages

NF-κB is a key transcriptional factor and mainly involved in immune and inflammatory responses. Upon activation by external stimuli, the IκB protein is phosphorylated and degraded and translocated into the nucleus. Translocated NF-κB interacts with κB elements in the promoter region of several inflammatory genes, leading to the transcription of pro-inflammatory mediators and cytokines. Thus, NF-κB has been regarded as the main molecular target in the development of therapies for inflammatory diseases

(Ghosh and Hayden 2008). We herein found out that compound **8** inhibited NF-κB productions in a dose dependent manner (∼1 & 2 fold decrease at 1 & 5 μM respectively) in LPS-induced RAW 264.7 macrophages (Figure S27).

3. Conclusion

In summary, we have investigated the anti-inflammatory activity of compounds isolated from the stem bark of *H. parviflora*. Among the isolated compounds, ellagic acid-3,3′,4-trimethoxy-4′-*O*-α-L-rhamnopyranoside (**8**) exhibited less toxicity in RAW 264.7 macrophages. Compound **8** inhibited the action of pro-inflammatory mediators like NO, TNF-α, IL-6, 5-LOX and COX-2 and promoted the action of anti-inflammatory mediator IL-10 *via* inhibition of NF-κB pathway in LPS-stimulated RAW264.7 macrophages. Further studies on the anti-inflammatory effects of ellagic acid-3,3′,4-trimethoxy-4′-*O*-α-L-rhamnopyranoside (**8**) are warranted in future.

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Disclosure statement

The authors confirm that this article content has no conflict of interest.

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