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**Thematic Special Issue 2**

Invitation/Call for Special Thematic Issue on "Nanotechnology in Cancer Medicine"

Guest Editor  
Dr. M. Joyce Nirmala  
Aim & Scope

**Vol 7, Issue 12, 2015**

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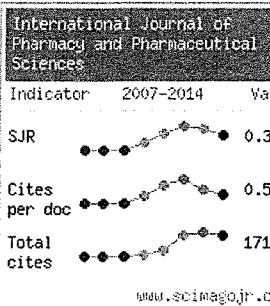
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






















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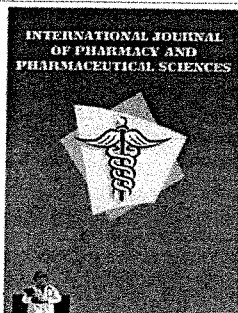
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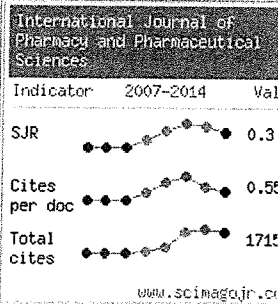
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## A STUDY TO PREDICT ANTI-INFLAMMATORY ACTIVITY OF EUGENOL, MYRISTICIN, AND LIMONENE OF *CINNAMOMUM SINTOC*

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### ABSTRACT

**Objective:** In this work we predicted anti-inflammatory activity of volatile oil of *C. sintoc* L.

**Methods:** Molecular docking was performed to predict the binding modes of eugenol, myristicin, and limonene chemical constituents of *C. sintoc* L. with COX enzymes, using Auto Dock 4.2. COX enzymes were obtained from Protein Data Bank (PDB); COX-1 (PDB code: 2AYL) and COX-2 (PDB code: 3PGH). Flurbiprofen and celecoxib were used as standards. Further assay was carried out on lipopolysaccharide (LPS)-induced fibroblast cells reacted with 800; 400; 200; 100; 50; 25 and 12.5 ul of *C. sintoc* L. bark essential oils. The absorbance of the product was measured using microplate reader at 450 nm. Acetosal was used as the standard drug.

**Results:** Eugenol and myristicin could be categorized as non-selective inhibitors of COX-2, while limonene is categorized as preferential COX-2 inhibitor. The essential oils of *C. sintoc* L. bark reduced PGE2 production on LPS-induced fibroblast cells. The inhibitory activity of *C. sintoc* L. was weaker than acetosal.

**Conclusion:** Bioactive compounds in essential oil of *C. sintoc* L. bark show inhibition on PGE2 production on LPS-induced human fibroblast cells, and could be categorized as COX inhibitors.

**Keywords:** Anti-inflammatory, *Cinnamomum sintoc*, Cyclooxygenase, Eugenol, Limonene, Myristicin.

### INTRODUCTION

Selective inhibition of cyclooxygenase-2 (COX-2) enzyme is a target of anti-inflammatory drugs, due to their property to reduce the side effect of anti-inflammatory non-steroid (AINS). Anti-inflammatory activity of essential oils of *Cinnamomum sintoc* L. (*C. sintoc* L.) bark, belonging to Lauraceae family, had been proven *in vivo* (65.35% oedema-decrease on carrageenan-induced rats at 0.1 ml/200 g of rat body weight) [1]. Other species, *C. tamala*, from the same family, proved anti-inflammatory activity [2].

Leem *et al.* (2011) declared that eugenol has anti-inflammatory activity by inhibition of COX-2 by 58.15% (IC<sub>50</sub> = 8.85 mg/ml *in vitro*), while *in vivo* assay on carrageenan-induced mice gave 0.17 g/kg of body weight [3]. Ozaky and colleagues (1989) concluded that myristicin showed anti-inflammatory activity [4]. Yoon *et al.* (2010) and Rahman *et al.* (2014) found that limonene has inhibitory activity against the production of prostaglandin E2 [5, 6].

The binding site of COX-2 where its selective inhibitor, SC-558, was bound contained His90, Leu117, Val349, Leu352, Ser353, Tyr355, Trp387, Ala516, Phe518, Met522, Val523, Gly526, Ala527, Ser530, Leu534. Based on Levita and her colleagues' work, SC-558 showed hydrogen bond interactions with Arg 513 and Gln 192. The position of Arg 513 is at the lower side of the pocket, which means that the pocket of COX-2 is larger in size than COX-1's. The interaction of SC-558 with Arg 513 might be important because it makes this ligand selective to inhibit COX-2 activity [7]. The molecular mechanism of this plant's anti-inflammatory activity and its *in vitro* assay on fibroblast cells had not been explored yet.

### MATERIALS AND METHODS

Molecular modeling study was performed on personal computer with Intel (R) Core™ i3-2310M @ 2:10 GHz CPU (4CPUs) processor, Windows 7 Home Premium 32-bit operating system, 392.52 GB hard disk capacity, and 4096 MB of RAM. 3D structures of the COX-1 (PDB code: 2AYL) and COX-2 (PDB code: 3PGH) enzymes, which were crystallized with flurbiprofen, were downloaded from Protein Data Bank

(www. pdb. org). Monomers of both proteins were separated and repaired using Swiss PDB viewer v.4.01. Structures of eugenol, myristicin, and limonene were drawn using ChemOffice 2004 (serial number: 202-241479-6622). Energy minimization was carried out by using AM1 semi-empirical method in portable Hyper Chem Release 8.0.7 (verification code: 0-32958). QSAR properties, e. g. log P, mass, and volume, of the ligands were calculated using the same software. Docking of eugenol, myristicin, and limonene to COX enzymes was carried out using Auto Dock v.4.2 at the site where flurbiprofen was co-crystallized.

### Cell culture and differentiation

Human fibroblast cells were obtained from Research Laboratory, Faculty of Dentistry, Universities Indonesia, Jakarta, Indonesia. The cells were grown in high glucose Dulbecco's Modified Eagle Medium (DMEM) which contained D-glucose, L-glutamine, sodium pyruvate (Gibco), supplemented with 10% heat-inactivated FBS (fetal bovine serum), penicillin (100 IU/ml), streptomycin (100 ug/ml), and fungizone, at 37C under 5% CO<sub>2</sub>. The cells were differentiated by incubating them in their culture medium for 48 h and were collected at the third day for further assay.

### Cyclooxygenase inhibition assay

Briefly, fibroblast cells (5.0 x 10<sup>6</sup> cells) in high glucose DMEM supplemented with 10% heat-inactivated FBS, penicillin (100 IU/ml), streptomycin (100 ug/ml), and fungizone, were placed in a 96-wells microplate, and were stimulated with LPS (10 ug/ml) to produce prostaglandin (PGE<sub>2</sub>), a protein which production was catalyzed by cyclooxygenase enzyme. Various volumes of *C. sintoc* L. volatile oils (12.5 to 800 ul) were added into the wells, and the mixtures were incubated for 18 h at 37 °C under 5% CO<sub>2</sub>. Acetylsalicylic acid (1 to 64 ul) was used as standard. The production of PGE<sub>2</sub> was measured using microplate reader at 450 nm.

### RESULTS AND DISCUSSION

QSAR properties of the ligands were calculated and the result could be seen in table 1.