Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 12, 2015

Original Article

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

SRI ADI SUMIWI*, OKTAVIA SARMA SIHOMBING, ANAS SUBARNAS, MARLINE ABDASSAH, JUTTI LEVITA

Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy Universitas Padjadjaran, Indonesia, Department of Pharmaceutics and Formulation Technology, Faculty of Pharmacy Universitas Padjadjaran, Indonesia, Department of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy, Universitas Padjadjaran
Email: sumiwi@yahoo.co.id

Received: 10 Jun 2015 Revised and Accepted: 27 Oct 2015

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_{50} = 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 it's *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) CoreTM 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_2. 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 10^6 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 (PGE2), 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 ^oC under 5% CO_2. Acetylsalicylic acid (1 to 64 ul) was used as standard. The production of PGE2 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.

Limonene is the most lipophilic compound compared to others, due to its hetero-aromatic ring and methyl groups. This compound doesn't have polar group such as hydroxyl or carbonyl therefore hydrophobic interaction with the receptor is the only possibility.

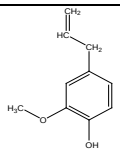
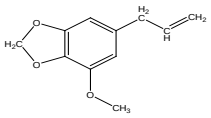
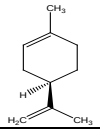
Both enzymes (COX-1 and COX-2) were crystallized with flurbiprofen (resolution 2.00 Å and 2.5 Å). COX-1 enzyme is a homodimer, while COX-2 is a homotetramer (fig. 1).

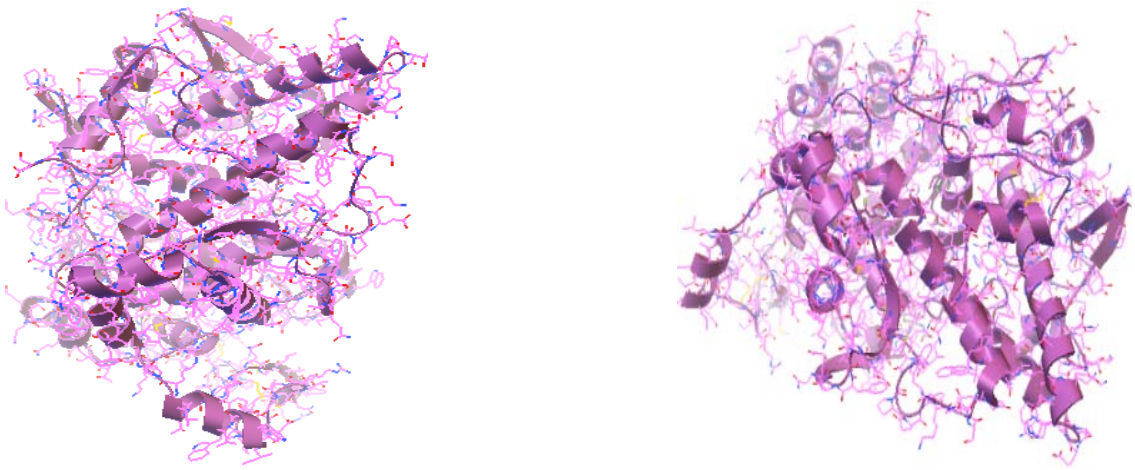
Validation of Auto Dock 4.2 was performed by superimposing flurbiprofen molecules (flurbiprofen obtained by drawing with the one extracted in the enzyme) and redocking (of flurbiprofen to its original place in the enzyme) (fig. 2).

The RMS error value of the superimposing is 1.7582 Å (fig. 2a), means that the softwares used for drawing and geometry optimizing are valid, while docking shows that flurbiprofen could be placed into its original site (fig. 2b). This result defines that software used for docking is valid.

Docking simulation was displayed with VDW scaling factor 1.00. Top score of docking simulation could be seen in fig. 3 (for COX-1), fig. 4 (for COX-2) and table 2.

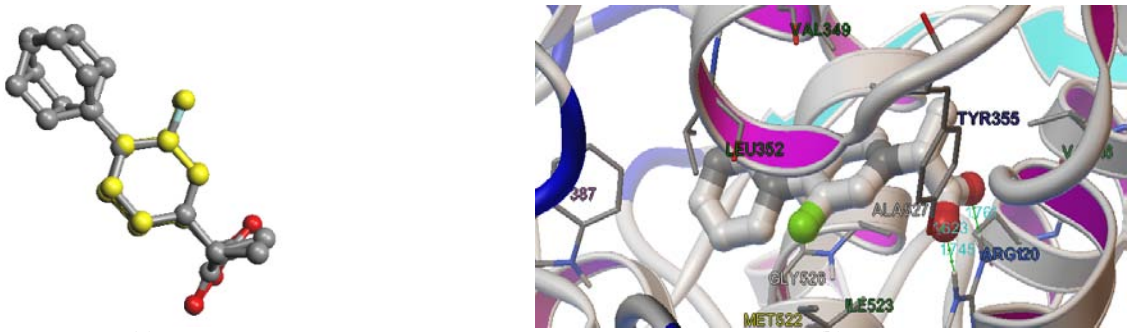
Table 1: QSAR properties of the ligands

Ligand	Structure	Energy optimization (kcal/mol)	Log P	Mass (amu)	Volume (Å^3)
Eugenol		-2496.44	2.55	164.2	563.59
Myristicin		-2747.03	2.52	192.21	615.56
Limonene		-2543.83	2.94	136.24	537.56



(a) (b)

Fig. 1: Monomer of COX-1 (a) and COX-2 (b)



(a) (b)

Fig. 2: Superimpose of flurbiprofen molecules (a), redocking of flurbiprofen to COX-1 (b)

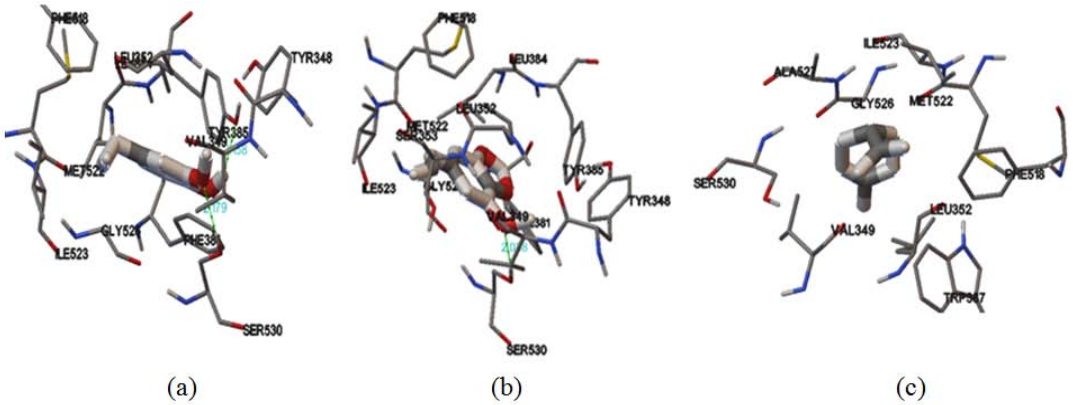


Fig. 3: Docking of eugenol (a), myristicin (b) and limonene (c) to COX-1

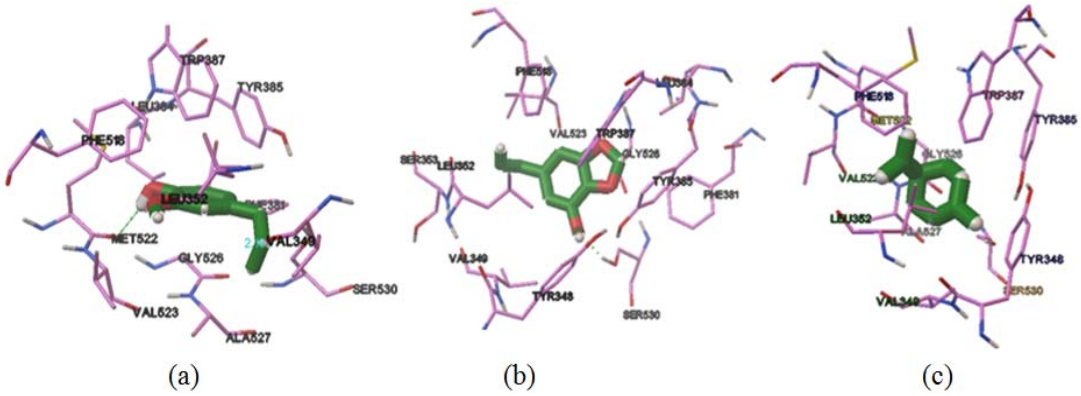


Fig. 4: Docking of eugenol (a), myristicin (b) and limonene (c) to COX-2

Table 2: Docking of ligands to COX enzymes

Ligand	Macromolecule	Energy	Ki (uM)	Hydrogen bonding (HB)
Eugenol	COX-1	-4.27	736.30	Eug H-O…O-H Ser530 (2.179 Å)
	COX-2	-4.11	969.47	-
Myristicin	COX-1	-4.55	462.19	Mir H-O…O-H Ser530 (2.038 Å)
	COX-2	-4.4	592.63	Mir H-O…O-H Ser530 (1.974 Å)
Limonene	COX-1	-4.56	458.12	No HB with both enzymes detected, only hydrophobic interaction
	COX-2	-4.91	251.14	
Flurbiprofen	COX-1	-8.68	0.44	Flur H-O…N-H Arg120 (1.745 Å)
				Flur C=O…N-H Arg120 (1.623 Å)
				Flur H-O…O-H Tyr355 (1.761 Å)
	COX-2	-7.65	2.48	Flur H-O…O-H Tyr355 (1.703 Å)
				Flur H-O…N-H Arg120 (1.952 Å)
Celecoxib	COX-1	-4.38	617.03	No interaction detected
	COX-2	-6.96	7.95	Cel S=O…H-N His90 (2.096 Å)

Determination of calculated selectivity index (cSI) or ratio of Ki COX-2/Ki COX-1 of the ligands was calculated, and the result could be seen in table 3.

Table 3: Selectivity index (cSI) of the ligands

Ligand	Ki COX-1 (uM)	Ki COX-2 (uM)	cSI (Ki COX-2/Ki COX-1)
Eugenol	736.30	969.47	1.32
Myristiscin	462.19	592.63	1.28
Limonene	458.12	251.14	0.55
Flurbiprofen	0.44	2.48	5.64
Celecoxib	617.03	7.95	0.01

Classification cSI are i. e selective COX-2 inhibitor (cSI<0.1), preferential COX-2 inhibitor (0.1<cSI<1.0), and non selective COX-2 inhibitor (cSI>1.0) [8]. Based on cSi value, celecoxib is the best selective inhibitor, followed by limonene, myristicin and eugenol. Limonene is categorized as preferential COX-2 inhibitor, while eugenol and miristicin could be categorized as non-selective inhibitors of COX-2. Flurbiprofen is a non-selective inhibitor.

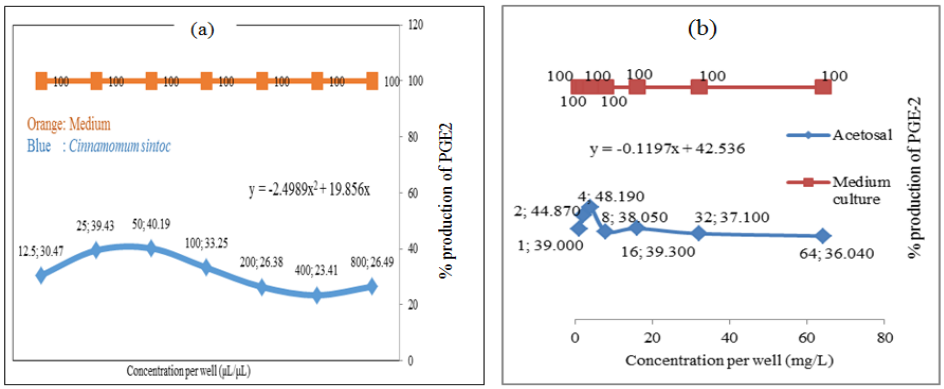


Fig. 5: Colorimetric assay of PGE2 production in LPS-induced fibroblast cells inhibited by (a) *C. sintoc* L.; (b) acetosal

Fig. 5a showed that essential oil of *C. sintoc* L. bark at concentration higher than 50 ul/ul showed a decrease of PGE2 production in human fibroblast cells induced by LPS.

The decrease of PGE2 production could be explained that there is an inhibition of COX-2 expression by compounds contained in volatile oil of *C. sintoc* L. Comparing the phenomenon with that of acetosal (fig. 5b), we observed that the inhibitory activity of *C. sintoc* L. was weaker than acetosal. This result confirmed those of other researchers who concluded that myristicin and limonene showed anti-inflammatory activity [4-6]. Therefore, these compounds could be categorized as COX inhibitors.

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.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Sumiwi SA, Subarnas A, Supriyatna S, Abdasah M, Muchtaridi M. Analysis of chemical composition and its analgesic and anti-inflammatory activity of essential oil of sintoc bark (*Cinnamomum sintoc* Bl.) using *in vivo* methods. J Appl Pharm Sci 2015;5:58-65.
2. Sharma V, Rao LJM. An overview on chemical composition, Bioactivity and processing of leaves of *Cinnamomum tamala*. Crit Rev Food Sci Nutr 2012;54:433-48.
3. Leem JY, Wansu P. Anti-Inflammatory effect of myristicin on RAW 264.7 macrophages stimulated with polyinosinic-polycytidylic acid. Molecules 2011;16:7132-42.
4. Ozaky Y, Soedigdo S, Wattimena YR, Suganda AG. Anti-inflammatory effect of mace, aril of *Myristica fragrans* houtt and its active principles. Jpn J Pharmacol 1989;49:155-63.
5. Yoon, WJ, Lee NH, Hyun CG. Limonene supresses lipopolysaccharide-induced production of nitric oxide, prostaglandin E2 and Pro-Inflammatory cytokines in RAW 264.7 macrophages. J Oleo Sci 2010;59:415-21.
6. Rahman MU, Tahir M, Khan AQ, Khan R, Oday OH, Lateef A, *et al*. D-Limonene suppresses doxorubicin-induced oxidative stress and inflammation via repression of COX-2, iNOS, and NFkB in kidneys of wistar rats. Exp Biol Med 2014;239:465-76.
7. Levita J, Nawawi A, Mutholib A, Ibrahim S. Andrographolide Inhibits COX-2 Expression in human fibroblast cells due to its interaction with arginine and histidine in cyclooxygenase site**.** J Appl Sci 2010;10:1481-4.
8. Nunthanavanit P, Samee W. Molecular docking of natural product-derived compounds: estimation of selectivity on Cyclo-oxygenase-2. Thai Pharm Health Sci J 2011;6:79-85.