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RESEARCH ARTICLE

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Inhibitory Activity of *Kaempferia galanga* and *Hibiscus sabdariffa* on the Rate of PGH$_2$ Formation

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ABSTRACT

Cyclooxygenase (COX) or Prostaglandin H$_2$ Synthase (PGHS) is the enzyme that catalyzes the first two steps in the biosynthesis of prostaglandins from the substrate, arachidonic acid. The NSAIDs work by inhibiting both COX isoforms, thus the conversion of arachidonic acid into prostaglandin is disturbed. In our country, kaempferia rhizome (*Kaempferia galanga*) and roselle calyx (*Hibiscus sabdariffa*) are used to reduce inflammation by consuming herb tea of these plants. This work studied the inhibitory activity of kaempferia rhizome and roselle calyx on the rate of prostaglandin formation by measuring the absorbance of TMPD (tetramethyl-p-phenyldiamine) oxidized by the extracts, against time. The plants were collected from Manoko plantation in West Java. Phytochemical screening showed that the rhizome contains quinones and terpenes, while the calyx contains polyphenol, flavonoid, quinone and saponin. 100 g of dried rhizome and calyx were separately boiled in 1 L of distilled water for 15 min at 90°C, freeze-dried and dissolved in ethanol 96%. Both *Kaempferia galanga* and *Hibiscus sabdariffa* showed inhibition on PGH$_2$ formations. The rate of PGH$_2$ formations on COX-1 was lower than on COX-2. These plants could be further developed for anti-inflammatory drug.

Key words: Anti-inflammatory agents, cyclooxygenase, *Hibiscus sabdariffa*, *Kaempferia galanga*, PGH$_2$, prostaglandin

INTRODUCTION

Cyclooxygenase (COX) or Prostaglandin H$_2$ Synthase (PGHS) is the enzyme that catalyzes the first two steps in the biosynthesis of prostaglandins from the substrate, arachidonic acid. These are the oxidation of arachidonic acid to the hydroperoxy endoperoxide, PGG$_2$ and its subsequent reduction to the hydroxy endoperoxide, PGH$_2$. The PGH$_2$ is transformed by a range of enzymes and nonenzymatic mechanisms into the primary prostanoids, PGE$_2$, PGF$_{2\alpha}$, PGD$_2$, PGI$_2$ and TXA$_2$ (Vane *et al*., 1998). The main reason for classifying COX-1 and COX-2 as physiological and pathological, respectively, is that COX-2 is only expressed when it is induced by stimuli and therefore, it is associated with inflammation.

NSAIDs work by inhibiting both COX isoforms, thus the conversion of arachidonic acid into prostaglandin is disturbed

(Katzung, 2007). All NSAIDs in clinical use have been shown to inhibit COX, leading to a marked reduction in PG synthesis. The inhibition by aspirin is due to irreversible acetylation of the cyclooxygenase component of COX. In contrast, NSAIDs like indomethacin or ibuprofen inhibit COX reversibly by competing with the substrate, arachidonic acid, for the active site of the enzyme (Vane *et al*., 1990).

Selective inhibition of COX-2 promises to provide NSAIDs with increased safety and has already become a purposeful approach. A publication by Stubanus and colleague provides evidence suggesting that COX-2 inhibitors impair renal function and cause sodium retention in patients with mild pre-existing renal failure and presumably also in some elderly patients with volume depletion (Stubanus *et al*., 2000).

According to Nomura *et al*. (2003) and Jachak *et al*. (2010), plants with secondary metabolites classified as

terpenes, phenolics and flavonoids show anti-inflammatory activity (Nomura *et al*., 2003; Jachak *et al*., 2010). *Kaempferia galanga*, an Indonesian plant, is used empirically to treat inflammation. The rhizome of this plant contains cineol, borneol, 3-carene, camphene, kaempferol, kaempferide, cinnamaldehyde, p-methoxycinnamic acid, ethyl cinnamate and ethyl p-methoxycinnamate. A Thai traditional herbal recipe contains this plant has been proven to inhibit IL-6 and PGE_2 production with IC_{50} value of 0.04±0.01 and 0.08±0.01 µg mL^{-1}, respectively (Aroonrerk and Kamkaen, 2009). Previous *in vivo* study of *Hibiscus sabdariffa* ethanol extract on carrageenan-induced mice showed that there were 22.03, 31.48 and 31.93% reduction of oedema observed at 102, 205, 410 mg of extract dose, respectively (Saptarini *et al*., 2012).

MATERIALS AND METHODS

Instruments and glasswares: The 96-well plate (Cayman Chemical), freeze dryer (Telstar), micropipette (Socorex), microplate reader (MRX TC revelation), Eppendorf tubes, thermometer, digital analytical balance (Sartorius), vortex mixer (VM-300) and analytical glasswares.

Plants and chemicals: Kaempferia rhizome (*K. galanga*) and roselle calyx (*H. sabdariffa*) were obtained from Manoko plantation in West Java, Indonesia. Chemicals are double distilled water (IPHA Laboratories), amyl alcohol (Agung Menara Abadi), ammonia (Agung Menara Abadi), hydrochloric acid (Agung Menara Abadi), acetylsalicylic acid (Bratachem), Colorimetric COX inhibitor screening assay kit No. 701050 (Cayman Chemical).

Extraction: One hundred gram of rhizome and calyx were separately boiled in 1 L of water for 15 min at 90°C as required for infundations. Then, the infused water of both plants were freeze-dried and dissolved in ethanol 96%.

Phytochemical screening and thin layer chromatography: Phytochemical screening for alkaloids, flavonoids, quinones, polyphenols, saponins, tanins, triterpenes, steroids, monoterpenes and sesquiterpenes was carried out to both plants accordingly. The extracts were eluted on silica GF254 plate using a mixture of chloroform-methanol 9:1 (for *K. galanga*) and butanol-acetic acid-water 4:1:5 (for *H. sabdariffa*) for mobile phases.

Sample preparation: The stock solutions of kaempferia rhizome (Fig. 1a) and roselle calyx (Fig. 1b) were prepared by dissolving 100 mg of each plant extract in 1 mL of ethanol 96% and diluted into: 5, 2.5, 1.25 and 0.625 mg mL^{-1} for kaempferia and 4.375, 2.18 and 1.09 mg mL^{-1} for roselle.

Inhibition of the rate of PGH_2 formation by *K. galanga* and *H. sabdariffa*: One hundred micro liter of assay buffer, 10 µL of heme and 7 µL of enzyme (either COX-1 or COX-2) were added into each inhibitor well, followed by the addition of 20 µL of the extracts. The plate was stirred and incubated for 5 min at 25°C. Fifteen microliter of colorimetric substrate solution was added to all wells, followed by 20 µL of arachidonic acid. The plate was stirred and incubated precisely for 2 min at 25°C. The absorbance was measured at 590 nm in 5 min interval.

RESULTS AND DISCUSSION

Monoterpenes/sesquiterpenes and quinones were detected in dried rhizome, while polyphenol, flavonoid, quinone and saponin were indicated positively in dried calyx (Table 1). This result showed correlation with phytochemical studies carried out by Tewtrakul *et al*. (2005), who found out that *K. galanga* revealed many different volatile oils extracted from its dried rhizome. The constituents were ethyl-p-methoxycinnamate (31-77%), methylcinnamate (23.23%), carvone (11.13%), eucalyptol (9.59%) and pentadecane (6.41%), respectively (Tewtrakul *et al*., 2005). The boiling process removed the quinones from the rhizome, probably due to the volatile character of the compounds.

Three spots were observed during chromatographic separation of kaempferia rhizome using CHCl$_3$-methanol (9:1),
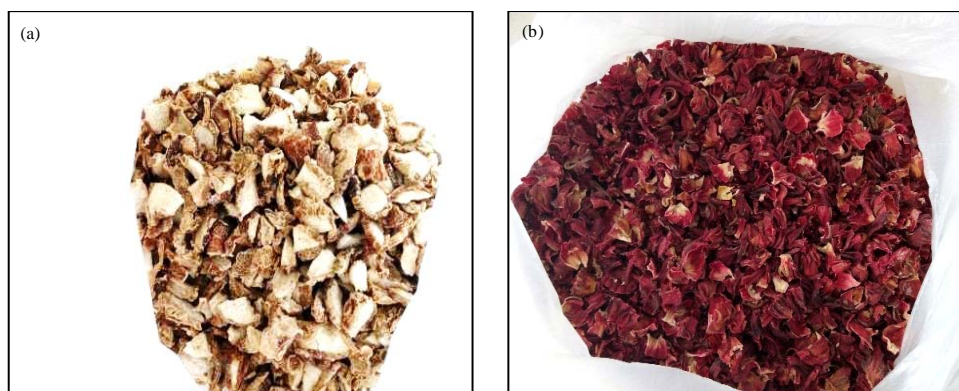

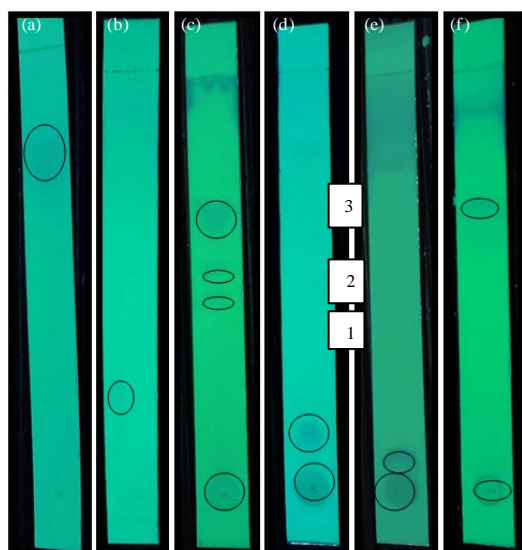Fig. 1(a-b): Dried (a) Kaempferia rhizome and (b) Roselle calyx

Fig. 2(a-f): Chromatograms of *K. galanga* extract eluted on silica GF$_{254}$ using, a: CHCl$_3$-methanol (1:9), b: CHCl$_3$-methanol (19:1), c: CHCl$_3$-methanol (9:1), d: n-hexane-ethyl acetate (4:1), e: Toluene-ethyl acetate (95:5) and f: Ethyl acetate
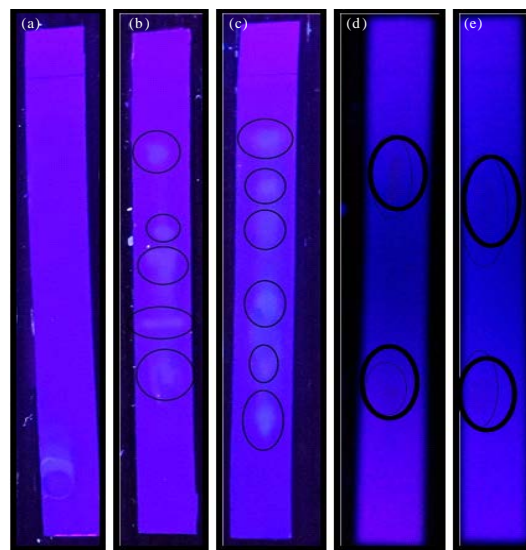

Fig. 3(a-e): Chromatograms of *H. sabdariffa* extract eluted on silica GF$_{254}$ using, a: CHCl$_3$-methanol (9:1), b: Butanol-acetic acid (3:2), c: Butanol-acetic acid-water (4:1:5), d: CHCl$_3$-methanol (1:9) and e: Methanol

Table 1: Phytochemical screeninga

Metabolites	K. galanga		H. sabdariffa	
	Dried rhizome	Water extract	Dried calyx	Water extract
Alkaloids	-	-	-	-
Flavonoids	-	-	+	+
Polyphenols	-	-	+	+
Tannins	-	-	-	-
Monoterpenes/ sesquiterpenes	+	+	-	-
Steroids	-	-	-	-
Triterpenes	-	-	-	-
Quinones	+	-	+	+
Saponins	-	-	+	+

which apparently shows the non-polar character of the compounds (Fig. 2). The Rs values of the spots for spot 1-2 and 2-3 (Fig. 2c), are 1.33 and 1.82, respectively.

Six well separated spots were observed during chromatographic separation of roselle calyx using butanol-acetic acid-water (4:1:5), which apparently shows the polar character of the compounds (Fig. 3). The Rs values of the spots for spot 1-2, 2-3, 3-4, 4-5 and 5-6 (Fig. 3c), are 1.6, 3.0, 3.75, 2.28 and 2.5, respectively.

Inhibition of the rate of PGH$_2$ formation was performed *in vitro* using Colorimetric COX Inhibitor Screening Assay Kit No. 705010 (Cayman Chemical Company, 2013). The basic principle of this kit is the oxidation reaction of the TMPD due to the peroxidase activity of the heme, thus causing the TMPD to release one electron to form a colored compound which absorbs at a wavelength of λ 590 nm (Cayman Chemical Company, 2013). The oxidation reaction of TMPD is equal to the reduction reaction of PGG$_2$ into PGH$_2$ by the enzyme.

Higher the catalysis activity of COX on arachidonic acid, resulted more oxidized TMPD, thus increasing the absorbance.

Description:

- n = 3
- Solvent contains assay buffer, heme, ethanol, arachidonic acid and TMPD

Figure 4 shows the rate of formation of PGH$_2$ on COX-1 and COX-2 affected by *K. galanga* and *H. sabdariffa*. This phenomenon could be explained by correlating it with the biosynthesis of prostaglandins (PGs). In the first step of biosynthesis of PGs, the substrate, arachidonic acid, has to react with COX enzyme and forms enzyme-substrate complex. This reaction takes place in the catalytic site of COX. In the second step, a conformational change occurs in the enzyme-substrate complex to enclose the substrate tightly in the enzyme to form the product. The enzyme then relaxes and releases the product (Fig. 5).

As in the case of the interaction of a substrate with an enzyme, an inhibitor could also form a complex with an enzyme. The equilibrium constant, K$_i$ (k$_2$/k$_1$) is a dissociation constant for cleavaging the enzyme-inhibitor complex, therefore the smaller the K$_i$ value for inhibitor, the more potent the inhibitor is (Silverman, 2004).

When inhibitor binds at the active site, the location where the substrate binds, then it is defined as a competitive inhibitor. Formation of the enzyme-inhibitor complex prevents the binding of the substrate to the enzyme, therefore blocks the catalytic conversion of the substrate, in this case is arachidonic
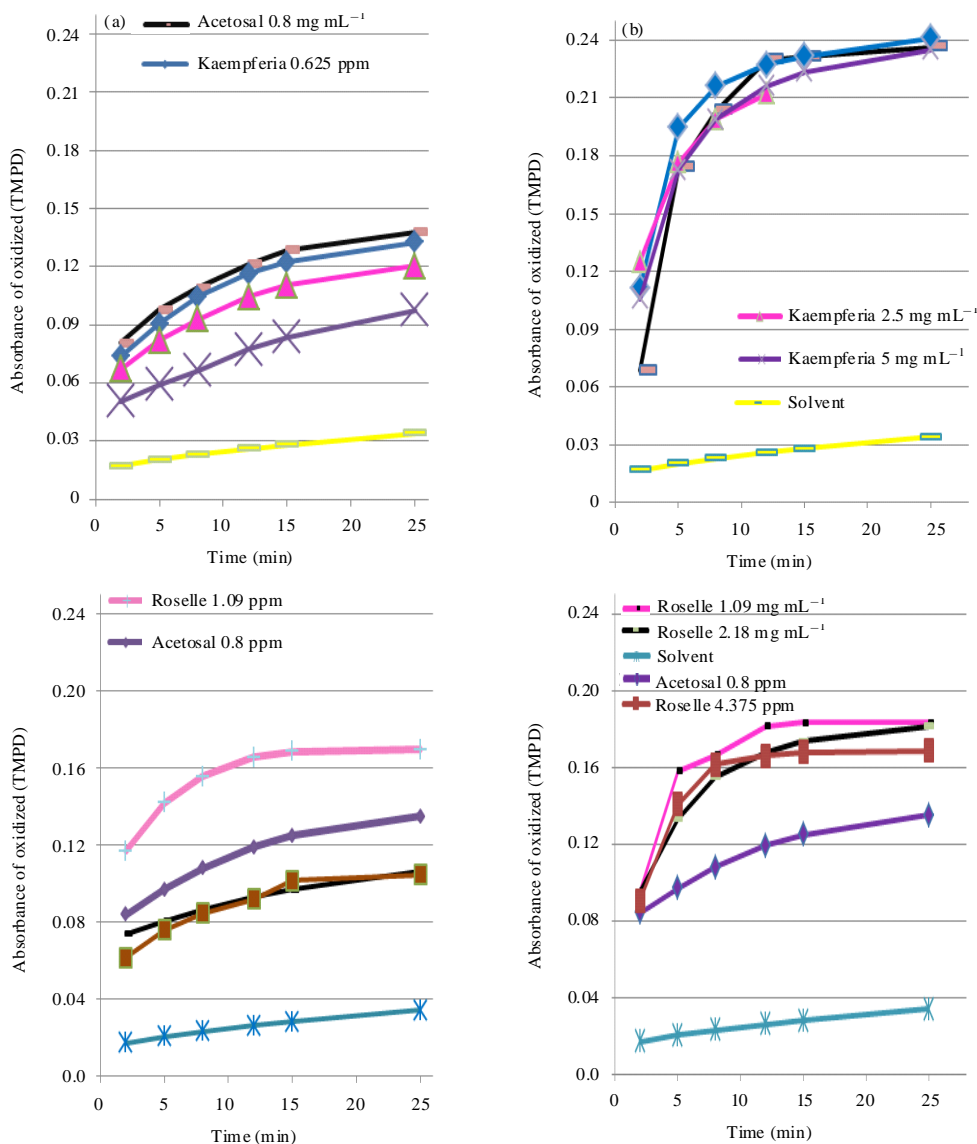
Fig. 4(a-b): Inhibitory activity of *K. galanga* (upper) and *H. sabdariffa* (lower) on the rate of PGH_2 formation on (a) COX-1 and (b) COX-2

acid, to product, which is PGH_2 (Silverman, 2004). In Fig. 4, we could observe that the product PGH_2 on COX-1 was slowly produced as proven by the flat slope of the curve (during measuring time the absorbance values of oxidized TMPD were still low). This means that on COX-1, both extracts shows a higher affinity thus, the substrate arachidonic acid reacts slower with the enzyme (in the performing the assay procedure, the arachidonic acid and TMPD were added after the enzyme was pre-incubated with the extracts). While, on COX-2, the rate of PGH_2 formation is faster (at 5 min the absorbance of oxidized TMPD has already reached higher values). This also applies for acetosal whereby the rate of formation of prostaglandin with COX-1 is slower than with COX-2. It could be concluded that both water extracts

of *K. galanga* and *H. sabdariffa* could inhibit the rate of PGH_2 formation therefore, they might be potential as anti-inflammatory phytomedicines. Their anti-inflammatory activity are weaker than acetosal. These results correlates positively with previous studies on *K. galanga* performed by Ridtitid *et al*. (2008) and Sulaiman *et al*. (2008), who concluded that the crude extract of the plant's rhizome possessed anti-inflammatory and analgesic properties. More recent study which also showed correlation with our result was the work of Umar *et al*. (2014), who concluded that ethyl-p-methoxycinnamate isolated from *K. galanga* inhibited inflammation by suppressing interleukin-1, tumor necrosis factor-α and angiogenesis by blocking endothelial functions.
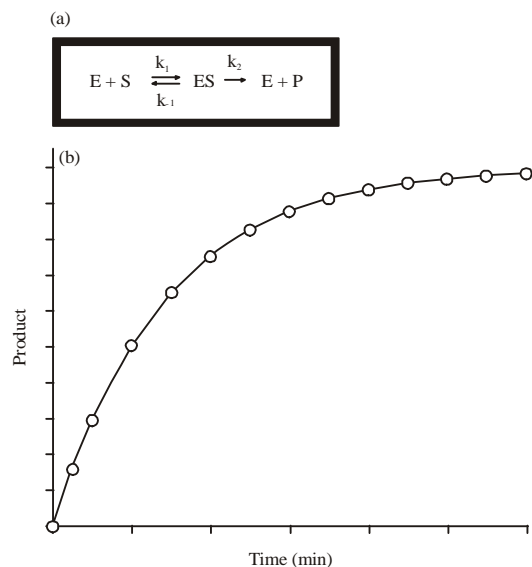
(a)

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \overset{k_2}{\rightarrow} E + P$$

(b)

Fig. 5(a-b): (a) Reaction of enzyme and substrate (Harvey and Ferrier, 2011) and (b) Curve of product against time (Copeland, 2005)

CONCLUSION

Both *K. galanga* and *H. sabdariffa* showed inhibition on the rate of PGH_2 formations. These plants could be further developed for anti-inflammatory drugs.

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REFERENCES

Aroonrerk, N. and N. Kamkaen, 2009. Anti-inflammatory activity of *Quercus infectoria*, *Glycyrrhiza uralensis*, *Kaempferia galanga* and *Coptis chinensis*, the main components of Thai herbal remedies for aphthous ulcer. J. Health Res., 23: 17-22.

Cayman Chemical Company, 2013. COX colorimetric inhibitor screening assay kit. Item No. 701050, Cayman Chemical Company, USA.

Copeland, R.A., 2005. Evaluation of Enzyme Inhibitors in Drug Discovery. John Wiley and Sons, New Jersey, pp: 36.

Harvey, R.A. and D.R. Ferrier, 2011. Biochemistry. 5th Edn., Lippincott Williams and Wilkins Inc., Philadelphia, pp: 58.

Jachak, S.M., R. Gautam, C. Selvam, H. Madhan, A. Srivastava and T. Khan, 2010. Anti-inflammatory, cyclooxygenase inhibitory and antioxidant activities of standardized extracts of *Tridax procumbens* L. Fitoterapia, 82: 173-177.

Katzung, B.G., 2007. Basic and Clinical Pharmacology. 10th Edn., Lange Medical Books/McGraw-Hill, USA., ISBN: 9780071451536, Pages: 1179.

Nomura, M., K. Kasemura, Y. Fujihara and T. Inoue, 2003. Proinflammatory cytokine (TNF-α) suppression of various terpenoids to human monocytic cell. Res. Rep. School Eng., 37: 1-5.

Ridtitid, W., C. Sae-Wong, W. Reanmongkol and M. Wongnawa, 2008. Antinociceptive activity of the methanolic extract of *Kaempferia galanga* Linn. in experimental animals. J. Ethnopharmacol., 118: 225-230.

Saptarini, N.M., D. Fitrianti and P. Bedja, 2012. Anti-inflamatory activity of hibiscus sabdariffa calyx extract. J. Medika Planta, 1: 18-23.

Silverman, R.B., 2004. The Organic Chemistry of Drug Design and Drug Action. 2nd Edn., Elsevier Academic Press, Burlington, MA., USA.

Stubanus, M., G.A. Riegger, M.C. Kammerl, M. Fischereder and B.K. Kramer, 2000. Renal side-effects of cyclo-oxygenase-type-2 inhibitor use. Lancet, 355: 753-753.

Sulaiman, M.R., Z.A. Zakaria, I.A. Daud, F.N. Ng, Y.C. Ng and M.T. Hidayat, 2008. Antinociceptive and anti-inflammatory activities of the aqueous extract of *Kaempferia galanga* leaves in animal models. J. Nat. Med., 62: 221-227.

Tewtrakul, S., S. Yuenyongsawad, S. Kummee and L. Atsawajaruwan, 2005. Chemical components and biological activities of volatile oil of *Kaempferia galanga*. J. Sci. Technol., 27: 503-507.

Umar, M.I., M.Z. Asmawi, A. Sadikun, A.M.S.A. Majid and F.S.R. Al-Suede *et al.*, 2014. Ethyl-p-methoxycinnamate isolated from *Kaempferia galanga* inhibits inflammation by suppressing interleukin-1, tumor necrosis factor-α and angiogenesis by blocking endothelial functions. Clinics, 69: 134-144.

Vane, J.R., R.J. Flower and R.M. Botting, 1990. History of aspirin and its mechanism of action. Stroke, 21: IV12-IV23.

Vane, J.R., Y.S. Bakhle and R.M. Botting, 1998. Cyclooxygenases 1 and 2. Annu. Rev. Pharmacol. Toxicol., 38: 97-120.