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Pharmacological Activities of *Plectranthus scutellarioides* (L.) R.Br. Leaves Extract on Cyclooxygenase and Xanthine Oxidase Enzymes

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***Plectranthus scutellarioides* (L.) R.Br. (family Lamiaceae) has been widely used in West Java, Indonesia, to cure various diseases. People boiled the leaves of the plant in water and consumed the tea daily until the symptoms reduced. This work was conducted to study the pharmacological activity of *P. scutellarioides* (L.) R.Br. extract on cyclooxygenases (COXs) and xanthine oxidase (XO) enzymes. The plant was purchased from Manoko plantation in Lembang, West Java, Indonesia. The leaves were sundried, crushed, and soaked in ethanol for 3 × 24 h, prior to be used. The extraction was continued further using ethyl acetate and water. Inhibitory activity of the extract on COXs was performed by measuring the absorbance of reduced-tetramethyl-*p*-phenylendiamine (TMPD) at 590 nm, which correlates to the level of PGH_2 production, while its inhibitory on XO was measured at 290 nm. *P. scutellarioides* (L.) R.Br. leaves extracts (ethanolic, ethyl acetate, and water) showed inhibition on COX-1 and COX-2 enzymes (40.43% for COX-1 and 97.04% for COX-2), while on XO, the water extract showed the highest inhibition (IC_{50} water extract = 6 µg/ml; IC_{50} allopurinol = 0.15 µg/ml). This plant could be further proposed as XO and nonselective COX inhibitors.**

Key words: Anti-inflammatory, cyclooxygenase (COX), gout, non-steroidal anti-inflammatory drugs (NSAIDs), prostaglandin, prostaglandin H_2 (PGH_2), xanthine oxidase (XO).

INTRODUCTION

The inflammatory response protects the body against infection and injury but it could become disregulated with deleterious consequences to the host. It is now evident that endogenous biochemical pathways activated during defense reactions can counter-regulate inflammation and promote resolution. Hence, resolution is an active rather

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than a passive process, as once believed, which now promises novel approaches for the treatment of inflammation-associated diseases based on endogenous agonists of resolution (Serhan et al., 2007).

Plectranthus scutellarioides (L.) R.Br. which belongs to Lamiaceae or Labiatae family, is a native plant of Southeast Asia, including Indonesia. This plant could also be cultivated in tropical and temperate regions around the world (Hanelt et al., 2001; Acevedo-Rodriguez and Strong, 2012). *P. scutellarioides* (or *Coleus scutellarioides*) has been widely used in West Java, Indonesia, to cure various diseases (Roosita et al., 2008). People boiled the leaves of the plant in water and consumed the tea daily until the symptoms reduced.

Other species of the same family, known as *Plectranthus amboinicus* or Indian borage, exhibited antiplatelet aggregation ability, antibacterial activity, and antiproliferative effect against Caco-2, HCT-15, and MCF-7 cell lines (Bhatt et al., 2013).

Iranian researchers, Saghafi et al. (2013) reported that *Teucrium polium* extract in different regions is a rich source of antioxidant and showed inhibitory effect on xanthine oxidase.

Some drugs, such as the widely used cyclooxygenase-2 (COX-2) inhibitors, have been proven to be toxic (Gilroy et al., 1999; Bannenberg et al., 2005; Serhan et al., 2007), whereas others possess pro-resolving actions, such as glucocorticoids (Rossi and Sawatzky, 2007), cyclin-dependent kinase inhibitors (Rossi et al., 2006), and aspirin (Serhan, 2007). Non-steroidal anti-inflammatory drugs (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). Aspirin is the most commonly non-steroidal anti-inflammatory drug, which low doses could be used to prevent and treat cardiovascular diseases. Recent studies showed that there is increasing evidence that aspirin initiates biosynthesis of novel anti-inflammatory mediators by means of interactions between endothelial cells and leukocytes. These mediators are classified as aspirin-triggered 15-epi-lipoxins (Chiang et al., 2004).

Xanthine oxidase (XO) is a member of group of enzymes known as molybdenum iron-sulphur flavin hydroxylases (Symons et al., 1989). It catalyses the oxidation of hypoxanthine to xanthine and then to uric acid, the final reactions in the metabolism of purine bases (Zarepour et al., 2010). Xanthine oxidase inhibitors (XOI) are much useful, since they possess lesser side effects compared to uricosuric and anti-inflammatory agents. Allopurinol is the only clinically available XOI, which also suffers from many side effects such as hypersensitivity syndrome, Steven's Johnson syndrome and renal toxicity. Thus, it is necessary to develop compounds with XOI activity with lesser side effects compared to allopurinol. Flavonoids and polyphenols have been reported to possess xanthine oxidase inhibitory activity. In addition, flavonoids also have anti-inflammatory and antitumor properties (Umamaheswari et al., 2013; Lio et al., 1985).

This work was aimed to study the pharmacological activity of *P. scutellarioides* (L.) R.Br. leaves extracts (ethanolic, ethyl acetate, and water) on cyclooxygenases (COXs) and xanthine oxidase (XO) enzymes.

MATERIALS AND METHODS

Plant

The fresh plant was purchased from Manoko plantation at Lembang, West Java, Indonesia, in November 2015. The specimen (No. 1011/11.CO2.2/PL/2015) was determined at Laboratory of Identification and Determination, School of Life Sciences and Technology, Bandung Institute of Technology, Indonesia, and confirmed as *P. scutellarioides* (L.) R.Br. (family Lamiaceae).

Preparation of extracts

The leaves were sundried in a glass-roofed room for 5 days, and then 1.2 kg of the dried leaves were crushed to powder and soaked in 1 L of 70 % ethanol for 3 × 24 h at room temperature. The extraction was continued sequentially using ethyl acetate and water. The extracts were filtered through Whatman No. 41, the solvent was vacuum-evaporated at 40 to 60°C, followed by freeze-drying process, prior to be further used.

Phytochemical screening

Phytochemical screening was performed according to standard method using specific reagents to detect secondary metabolites (alkaloids, flavonoids, polyphenols/tannins, terpenoids, quinones, and saponins) in *P. scutellarioides* (L.) R.Br. leaves extracts.

Thin layer chromatography (TLC) analysis

TLC was performed on silica GF_{254} plate using a mixture of *n*-butanol, acetic acid, and water (4:1:3) was used as eluent for ethanol and water extracts, whereas a mixture of toluene, ethyl acetate, and acetic acid (7:2:1) was used for ethyl acetate extract. The spots were observed using $AlCl_3$ as spray reagent.

Spectrophotometry analysis

Spectrophotometry analysis was performed to the ethanol extract (with and without $AlCl_3$) at 220 to 450 nm. Quercetin was used as standard.

High performance liquid chromatography (HPLC)

Reversed-phase HPLC was performed on LC-10AT VP (Shimadzu),

using Atlantis Hilicsilica C18 (Waters®) column, 150 mm × 4.6 mm × 5 µm, as stationary phase, and a mixture of acetonitrile, phosphoric acid, and methanol (40:50:10) as mobile phase. Flow rate was 0.8 ml/min, and detection was set at 339 nm. The chromatographic peak was confirmed by comparing the retention time of *P. scutellarioides* (L.) R.Br. leaves extract with that of quercetin standard.

Pharmacological assay

Inhibitory activity on COX enzymes

100 mg of freeze-dried extracts were dissolved in 50 ml of ethanol. The solution was diluted until three concentrations were obtained.

This assay was performed using Colorimetric COX Inhibitor Screening Assay No.705010 (Cayman Chemical Company): 150 µl 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. Then, 15 µl 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 minp at 25°C. Finally, the absorbance was measured at 590 nm in 5 min interval. Acetosal was used as drug standard.

Inhibitory activity on XO enzyme

Freeze-dried extracts (50 mg) were dissolved in 25 ml of dimethyl sulfoxide (DMSO). The solution was diluted until various concentrations were obtained.

This assay was performed using Xanthine Oxidase Inhibitor Screening Assay (Sigma Aldrich, USA): 1 ml of extract solution was added by 3 ml of phosphate buffer and 2 ml of xanthine substrate solution. The mixture was preincubated at 30°C for 10 min, and was added by 0.1 ml of xanthine oxidase enzyme solution. The mixture was homogenized and incubated at 30°C for 30 min. The reaction was stopped by using 1 ml of HCl 1 N and the absorbance of uric acid was measured at 290 nm. Allopurinol was used as standard. The percentage of inhibition was calculated by

$$\text{Percentage of inhibition} = \{ (A-B) - (C-D)\}/(A-B)\} \times 100$$

where A is the activity of the enzyme without the compound, B is the control of A without the compound and enzyme, C and D are the activities of the compound with or without XO ,respectively. The assay was done in triplicate and IC_{50} values were calculated from the percentage of inhibition (Sahgal et al., 2009).

RESULTS AND DISCUSSION

Preparation of extracts

Dried leaves extraction (1.2 kg) resulted in 234.61 g of ethanol extract (19.55%), whereas the ethyl acetate and the water extracts were 9.71 and 26.06 g, respectively.

Phytochemical screening

Qualitative analysis by phytochemical screening of *P. scutellarioides* (L.) R.Br. leaves extracts revealed the presence of polyphenols, flavonoids, saponins, and quinones (Table 1)

Table 1 showed the results of phytochemical screening. Both the dried leaves and the extracts of *P. scutellarioides* (L.) R.Br. contain polyphenols, flavonoids, saponins, and quinones (indicated with positive mark in the table). No alkaloids and terpenes are observed in either the dried leaves or extracts. These results were compared with those of Bhatt et al. (2013) who had worked on *P. amboinicus* or Indian borage of the same family, Lamiaceae. They reported that this plant contained phenolics (49.91 mg GAE/g extract), flavonoids (26.6 mg RE/g extract), and condensed tannins (0.7 mg TAE/g extract) (Bhatt et al., 2013).

Thin layer chromatography (TLC) analysis

TLC analysis of the ethanol extract, ethyl acetate fraction, and water fraction, resulted 3, 6, and 6 spots, respectively (Rf value ranged between 0.29 and 0.91), which gave colour with $AlCl_3$ (Figure 1).

Spectrophotometry analysis

Spectrophotometry analysis result is as shown in Figure 2. Figure 2 shows that quercetin (red) has two bands which maximas are detected at 258 and 375 nm, whilst the extract indicated peaks at 260 and 335 nm.

The UV lambda maxima of quercetin-3-*O*-rhamnoside and quercetin-uronic acid were 256 and 352 nm (Plazonić et al., 2009), which are closely similar with those of our extract.

According to Sisa (2010), all flavonoids have aromatic chromophores, as indicated by UV absorptions in the 250 nm region of their UV spectra. These compounds may undergo π,π* excitation and react from π,π* excited states. Certain flavonoids contain carbonyl chromophores and absorb light in the 300 nm region. They may undergo n,π* excitation to react from n,π* excited states. Carbonyl chromophores that are conjugated with the aromatic ring (e.g., acetophenones and chalcones) absorb UV light in the 350 nm region. The n,π* and π,π* excited states of these compounds are almost degenerate and the state from which their reactions originates is sometimes controversial. Polyphenolic chalcones may absorb light in the visible region as is evident by their colours (Sisa et al., 2010).

There is an occurance of batochromic shift (a shift of the maxima to longer wavelength) of the first bands of both extract and quercetin. The shift confirmed the presence of flavonoids in *P. scutellarioides* (L.) R.Br. leaves extract.

High performance liquid chromatography (HPLC)

HPLC analysis result is as shown in Figure 3. Figure 3b

Table 1. Phytochemical Screening of *P. scutellarioides* (L.) R.Br. leaves extracts.

Secondary metabolite	Dried leaves	Extract		
		Ethanol	Ethyl acetate	Water
Alkaloids	-	-	-	-
Polyphenols	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	+	+	+
Quinones	+	+	+	+
Terpenes	-	-	-	-

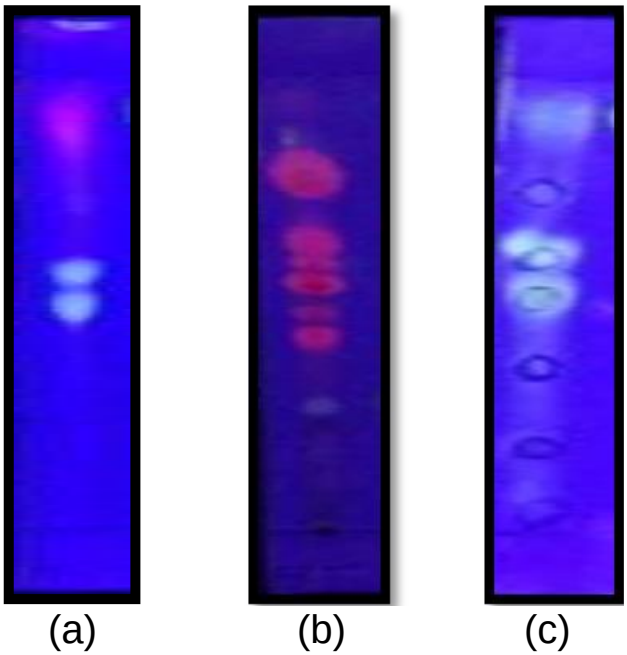

Figure 1. TLCs of (a) ethanol extract; (b) ethyl acetate fraction; (c) water fraction of *P. scutellarioides* (L.) R.Br.

shows a dominant peak in *P. scutellarioides* (L.) R.Br. leaves extract, which eluted at 4.91 min (Rt of quercetin standard = 4.88 min as shown in Figure 3a).

Inhibitory activity on COX enzymes

The basic principle of this kit is the oxidation of TMPD by the peroxidase activity of the heme, to form a colored compound which absorbs at λ 590 nm.

Figures 4 and 5 show that all extracts possess inhibitory activity on both COX-1 and COX-2 enzymes, although it is weaker when compared with acetosal. The inhibitory activity of *P. scutellarioides* (L.) R.Br. leaves extract on COX-2 is stronger than on COX-1 (40.43% for COX-1 and 97.04% for COX-2), thus this plant could be further proposed as a nonselective COX inhibitor.

We compared the results with those of other researchers. Ravikumar and colleagues concluded that aqueous and ethanolic extracts of leaves of the same genus, *Plectranthus amboinicus* (Lour.) Spreng, showed anti-inflammatory activity (Ravikumar et al., 2009; Devi and Periyanayagam, 2010).

Inhibitory activity on XO enzyme

Inhibitory activity of *P. scutellarioides* (L.) R.Br. leaves extract on XO enzyme is as shown in Figure 6. Figure 6 shows that all *P. scutellarioides* (L.) R.Br. leaves extracts, which contained flavonoids (Table 1), possess inhibitory activity on XO enzyme. According to Umamaheswari et al. (2009), inhibitory activity on XO enzyme might be attributed to the presence of benzopyran ring in the flavonoids. They concluded that flavonoids could be a promising remedy for the treatment of gout and related

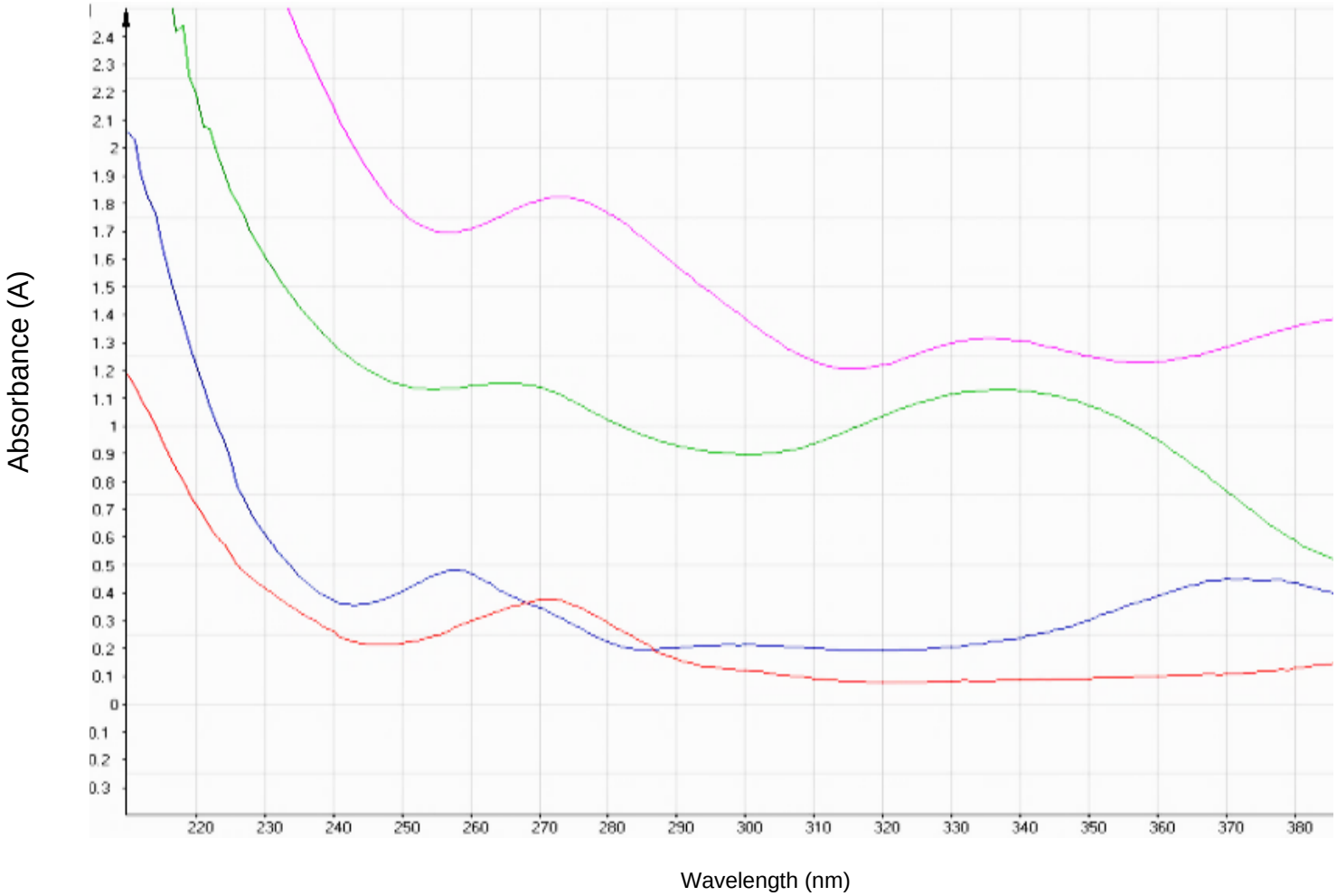

Figure 2. Spectra of quercetin standard + $AlCl_3$ (red); quercetin standard (blue); ethanol extract (green); and ethanol extract + $AlCl_3$ (pink).

inflammatory disorders (Umamaheswari et al, 2013). The water extract revealed the highest inhibition although it is weaker when compared with allopurinol (IC_{50} water extract = 6 µg/ml; IC_{50} allopurinol = 0.15 µg/ml), therefore this plant could be further proposed as XO inhibitor.

Conclusion

P. scutellarioides (L.) R.Br. leaves extracts (ethanolic, ethyl acetate, and water) show inhibition on COX-1 and COX-2 enzymes, while on XO, water extract showed the highest inhibition. This plant could be further proposed as both XO and COX inhibitors.

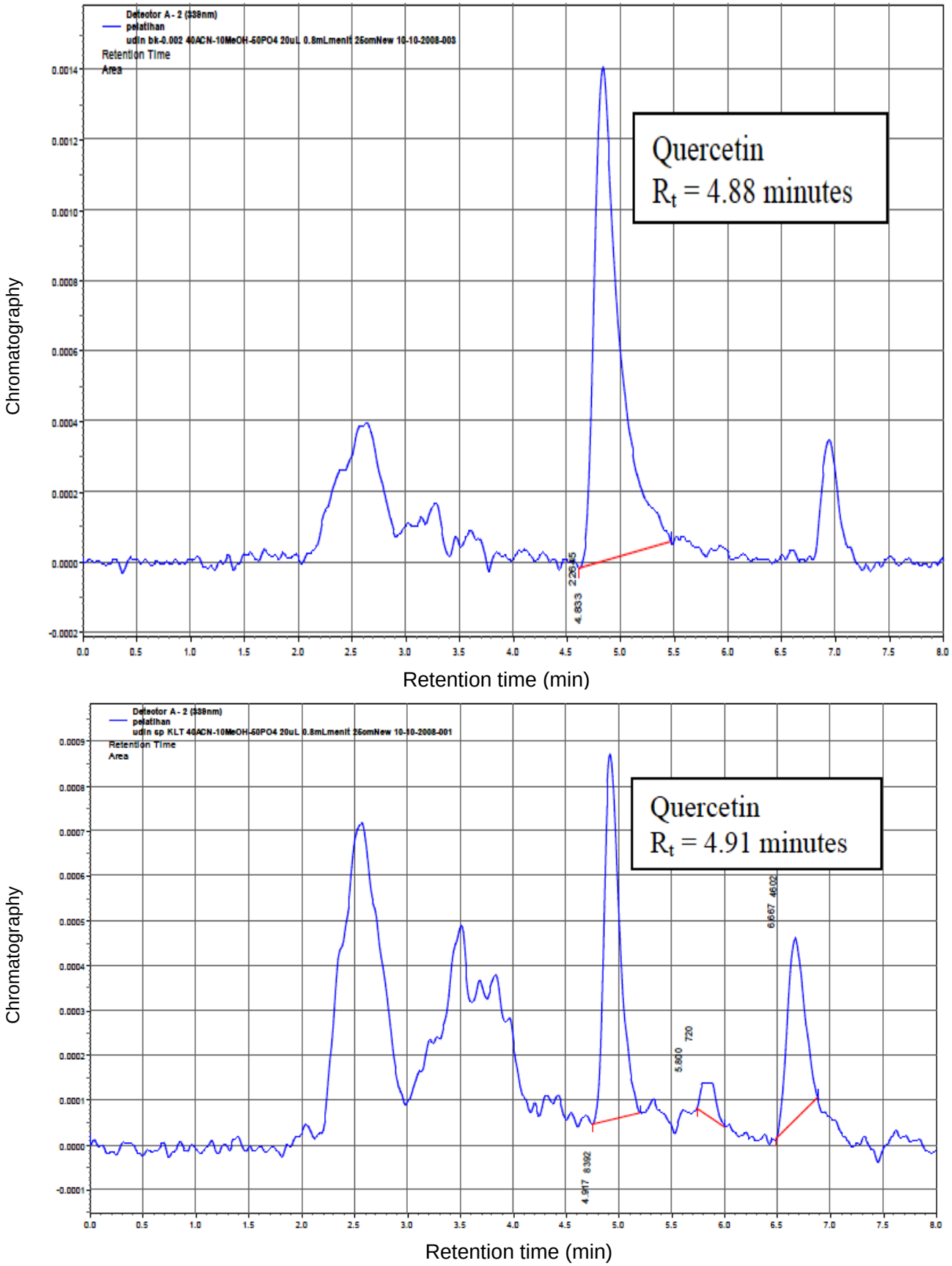

Figure 3. HPLC chromatograms of (a) quercetin standard and (b) ethanol extract.

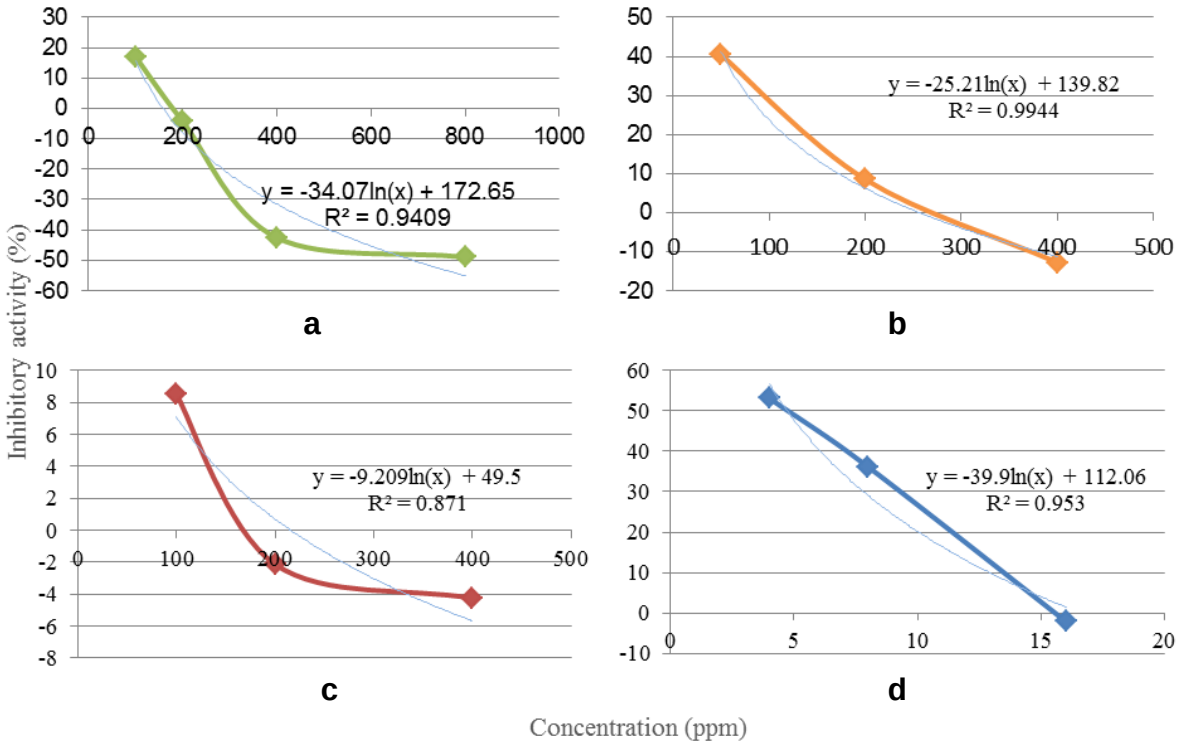

Figure 4. Inhibitory activity of (a) ethanol extract; (b) ethyl acetate fraction; (c) water fraction; (d) acetosal on COX-1.

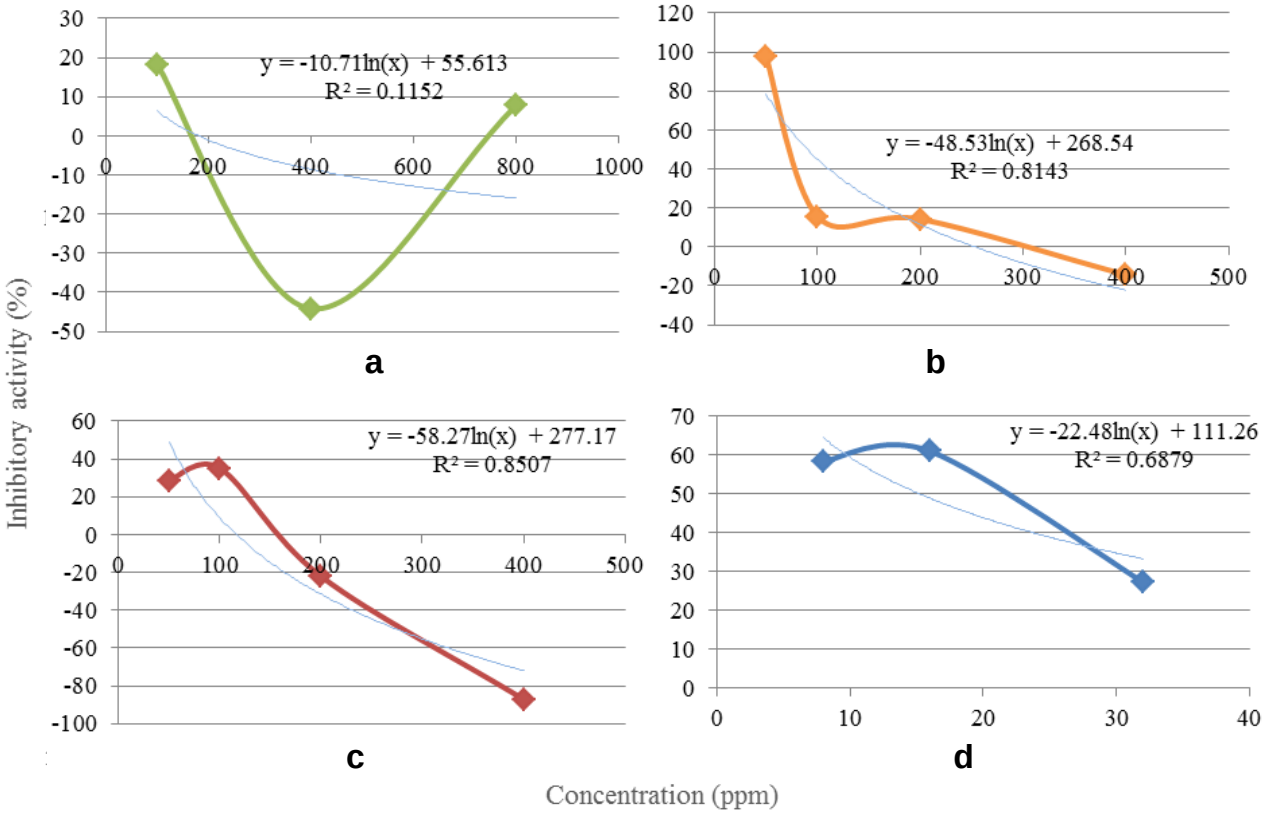

Figure 5. Inhibitory activity of (a) ethanol extract; (b) ethyl acetate fraction; (c) water fraction; (d) acetosal on COX-2.

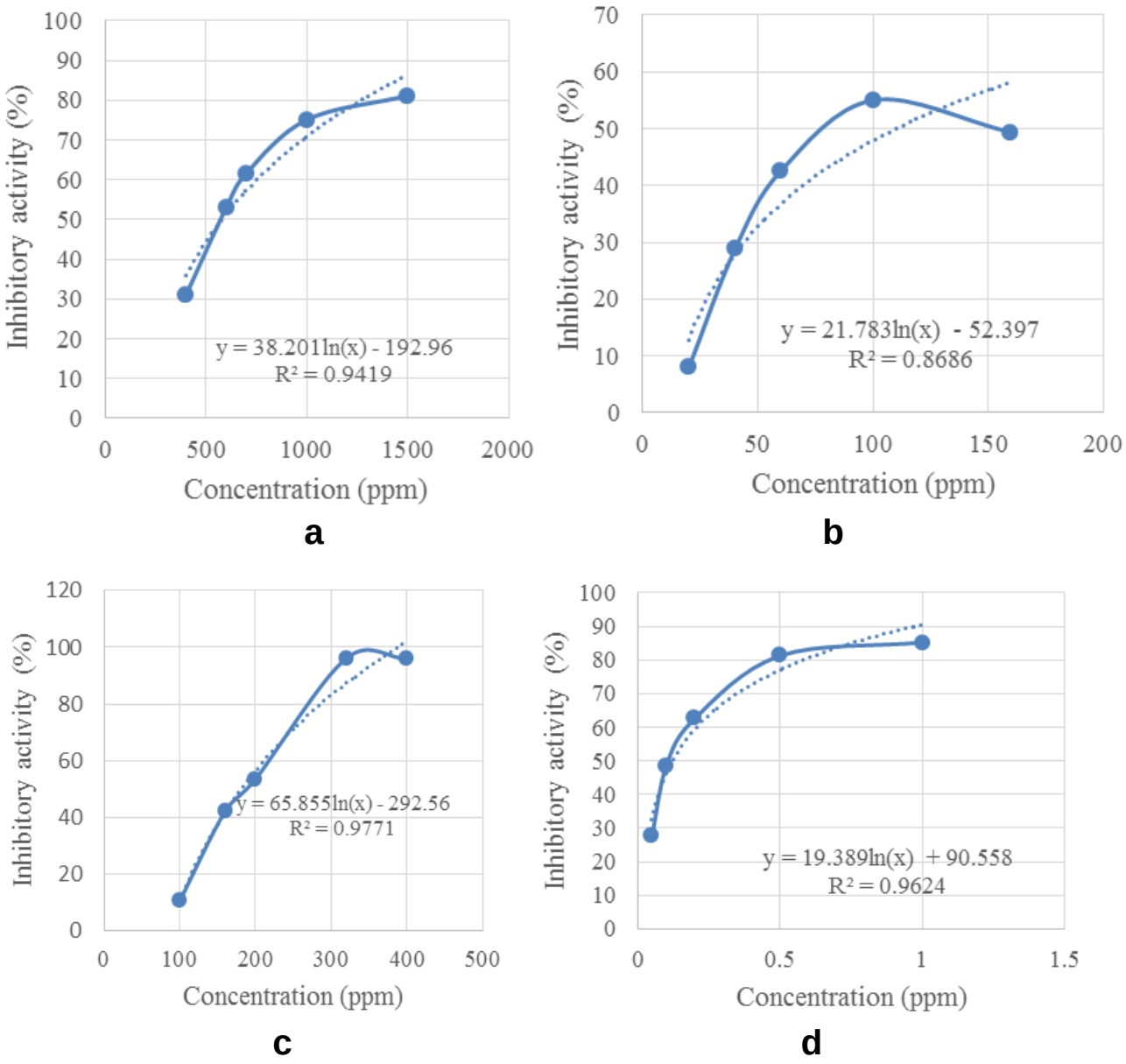

Figure 6. Inhibitory activity of (a) ethanol extract; (b) ethyl acetate fraction; (c) water fraction; (d) allopurinol on XO.

Conflicts of interests

The authors have not declared any conflict interests.

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