





Structural Assessment of Chemical Constituent of Sidaguri (*Sida rhombifolia* Linn) and Its Ability to Inhibit Cyclooxygenase

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Abstract

Objective: To elicit the structure of isolated compounds from roots of sidaguri (*Sida rhombifolia* Linn). **Material and Methods:** Several organic standard protocols were involved, including extraction, fractionation, and phytochemical testing. Further spectroscopy methods, FTIR and ¹HNMR, were used to determine the predicted structure of molecules, while their ability to inhibit cyclooxygenase (COX 1 and 2) were tested using in vitro method. **Results:** Overall assessments showed that the structure of the sidaguri is a long chain aliphatic carboxylic acid and identified as Z-3, 6, 6 trimethylhept-2-en-1-ol (T12) and nonanoic (T13). Both isolates significantly inhibit COX-1 and COX-2 non-selectively (the COX-1/COX-2 ratio for T12 was 0.91 and 0.82; while COX-1/COX-2 ratio for T13 was 0.89 and 0.87 at concentrations of 0.05 and 0.025 µg/mL respectively). **Conclusion:** The active compounds of Sidaguri have anti-inflammatory effect by inhibiting COX non-selectively.

Keywords: Sida Plant; Anti-Inflammatory Agents; Cyclooxygenase 1; Cyclooxygenase 2.

Introduction

Sidaguri or *Sida rhombifolia* Linn from family of Malvaceae is found widely used as anti-inflammatory agents for peoples in some area [1]. That extract of water-alcoholic of the leaf of sidaguri with a dosage of 400 mg/kg body-weight could inhibit edema in rats, which induced by 1% of carrageenan [2]. The longest duration for analgesic activities of root extract of sidaguri was found at concentration 2.4 g/kg/bw in 13.02 minutes [3].

Mostly all parts of sidaguri can be used for medical treatment such as treatment the stings and bites of scorpions, snakes, and wasps; skin diseases, and sores; fever, gum infections; and swelling. Studies of ethanol and water extract of the whole part of sidaguri demonstrated highly potent anti-inflammatory activities [4]. Besides anti-inflammatory potency, extract of sidaguri also exhibits other pharmacological and biological activities by inhibiting cyclooxygenase enzyme, especially prostaglandin biosynthesis and bacterial activity [5,6].

Recent study using Wistar rats as a model of periapical lesion, the efficacy of an ethanolic extract of the root bark of sidaguri and confirmed its potency as an anti-inflammatory agent. However no study has been carried out to evaluate the pharmacological potency of root bark extract of sidaguri in inhibiting cyclooxygenase enzyme [7,8].

In the previous studies, the presence of some ecdysteroid and their glycosides in sidaguri extract has been reported. They consist of four known compounds — ecdysone; 20-hydroxyecdysone; 2-deoxy-20-hydroxyecdysone-3-O-beta-D-glucopyranoside; 20-hydroxyecdysone-3-O-beta-D-glucopyranoside which are reported for the first time. Sterols (β -sitosterol, stigmasterol, campesterol, stigmasterol, spinasterol, and cholesterol), n-alkanes (e.g., nonacosane and hentriacontane) and n-alcohols were also identified from the whole dried and aerial parts of *S. rhombifolia*. β -phenethylamine, ψ -ephedrine, quinazoline like vasicine, vasicinol, vasicinone, carboxylated tryptamines such as S-(+)-Nb-methyltryptophan methyl ester, choline, and betaine were isolated from aerial part [9]. However lack of studies examine the chemically active constituent of the root bark extract of sidaguri.

Based on the above finding, the aim of this research is to isolate, identify, characterize and elucidate the active constituent of root bark extract of sidaguri in its polar and non-polar fraction, and to examine its inhibition activity to cyclooxygenase enzyme.

Material and Methods

Collection and Preparation of Plant Material

Root-bark of sidaguri were taken from Bone Regency, South Sulawesi. The material was washed with clean water few times and openly air-dried at room temperature before cutting to small pieces and grinding to form soft particles, approximately 100 mesh in size, and kept in a dry place before using.

Extraction, Fractionation, and Isolation

Approximately 1 kg of softly grinding dried samples of sidaguri root-bark was consecutively extracted under a reflux extraction using methanol at analytical grades to resulted in 4.33 g of viscous dark brownish color crude extract. This crude was then fractionated following *Kupcha's* fractionation and its modified methods [10].

The results were five major factions with a decreased of polarity from H₂O, n-butanol, methanol, dichloromethane, and n-hexane. All resulted fractions were then tested for its inhibitory properties on cyclooxygenase enzyme. Prior to the inhibitory test, the fraction was taken for a series of phytochemical analysis in order to check the class of secondary metabolites that contained in the fraction as well as the crude extract.

Identification and characterization of the active fractions were carried out using various chromatography analysis, including thin layer, flash, and vacuum column chromatography. Prior to the major chromatography works, a series of the single and double dimension of preparative thin layer chromatography was done to find suitable and appropriate eluent systems for the chromatography works.

Predicted Structure of Molecules

The results of pure compounds were then subjected to an instrumental analysis of UV, FTIR, and ¹H-NMR spectroscopy to get the presumably correct structure of the active constituent sidaguri namely T12 and T13. The T12 and T13 further process was phytochemistry assessment using the protocol proposed by Harborne involving a test of alkaloids, steroids, flavonoids, saponins, and tannins [11].

COX-1 and COX-2 Inhibitory Activity

The COX-1 and 2 assay were performed based on methods described by standard kit.

Selectivity on COX-1 was calculated by
$$\text{Selectivity} = \frac{\text{Inhibition on COX-2}}{\text{Inhibition on COX-1}} \times 100\%.$$

Results

Approximately 500 g of the soft grinding root bark of sidaguri were divided into 5 portions of 100 g. First portion of the materials was extracted under soxhlet with ethanol. This treatment was repeated for four remain portions. The results were then combined and evaporated under vacuum to provide brown viscous liquid crude extract about 4.33 g.

Those fraction of n-hexane, approximately 0.77 g were subjected to liquid pressure column chromatography by impregnated the sample into a silica gel and flash gradually with solvent-based the solvent polarity from n-hexane 100% (few times as required) ; n-hexane-chloroform 8:2, 7:3, and 5:5 chloroform-n-hexane 7:3; chloroform 100%; chloroform 100%; ethyl acetate-chloroform 2:8, 5:5; and 7:3, ethyl acetate; acetone, and methanol each 100% and overall resulted in 17 fractions. Two

fractions of T12 and T13 were then taken into further steps for purification by crystallization and recrystallization eluent of methanol-ethyl acetate for T12 and methanol-chloroform for T13 to resulted in 62.2 mg brownish color crystals and 4.2 g yellowish crystals of T12 and T13, respectively. Both compounds were then subjected to further one and two-dimensional thin layer chromatography using a series of eluents, which gave a bright spot under UV lamp (Table 1).

Table 1. Organoleptic characterization of fractionation.

Fraction	Weight (g)	Color	Form
n-hexane	0.77	Brown	Paste
Dichloromethane	0.49	Darkest brown	Paste
2-butanol	0.95	Brown	Paste
Chloroform	0.85	Light brown	Paste
H ₂ O	1.27	Yellow	Liquid

Table 2 shows the chemical compound group of isolates. Terpenoid group were identified in both T12 and T13 and continued to structure elucidation using spectroscopy analysis including FTIR and ¹H-NMR to determine the structure. The analysis of FTIR spectra of T12 and T13 were mentioned in (Table 3).

Table 2. Phytochemistry assignment of T12 and T13.

Secondary Metabolites	Test	Compounds	
		T12	T13
Alkaloids	Meyer	-	-
	Wagner	-	-
Flavonoids		-	-
Saponin		-	-
Steroid		-	-
Terpenoid	Lieberman-Baurchard	++	+

+++ Strong Intensity Reaction; ++ Medium Intensity Reaction; + Weak Intensity Reaction; - No Detected.

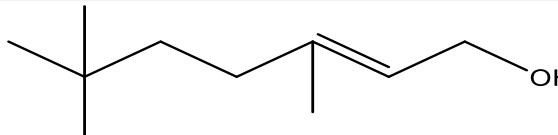
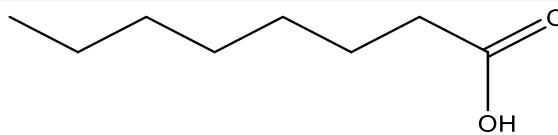
Table 3. FTIR spectrum of T12 and T13.

T12		T13	
Wavelength abs (in cm ⁻¹)	Functional Group	Wavelength abs (in cm ⁻¹)	Functional Group
3446.79	-OH strains	3452.58	-OH strains
2922.16 and 2852.72	-CH ₂ -bending	2920.23; 2850.79; 1465.90	Aliphatic -CH
1377.17	-CH ₃ bending	1377.17	-CH ₃ bending
1465.04 and 1413.62	-CH ₂ and -CH ₃	1653.71	C=C aliphatic stretching
1737.86 and 1710.86		1739.79; 1710.86	C=O strains
1172.72	-C-O strains and stretch	1028.09	-C-O stretching and straining

Further confirmation was done using ¹H-NMR to give the assumptive structure of compounds T12 and T13. The ¹H-NMR spectral data of both compounds were given in (Table 4 and Figure 1).

Two long-chain aliphatic carboxylic acids isolated from root bark of sidaguri were found (Table 4). T12 refers to Z-3, 6, 6 trimethylhept-2-en-1-ol, while T13 was nonanoic.

Table 4. ¹H-NMR spectrum of T12 and T13 and the proposed structure of compounds (T12 and T13) isolated from roots of *S. rhombifolia*.

T12		T13	
δH (in ppm)	Multiplicity	δH (in ppm)	Multiplicity
0.88 and 1.27	Multiplets	0.89	Singlet
1.63	Singlet	1.32 and 1.62	Doublets and singlet
5.36	Singlet	5.37	Singlet
2.04 and 2.34	Doublet and singlet	3.32	Singlet
4.17	Singlet	2.82	Doublet
		2.29	Doublets of doublet
Z-3,6,6 trimethylhept-2-en-1-ol		Nonanoic	
			

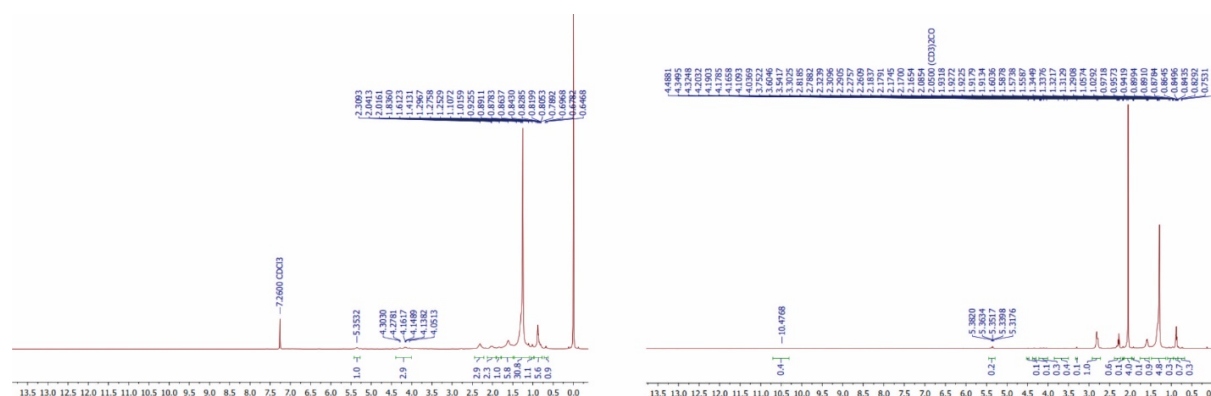


Figure 1. NMR spectrum of (A) T12 and (B) T13.

Both T12 and T13 showed higher inhibitory activity to COX-1 than COX-2 without depending on the concentration. T12 and T13 demonstrated no selectivity as COX-2 inhibitor (Table 5).

Table 5. COX-1, COX-2 and the ratio inhibitor of two isolates by ELISA method.

Isolate	Concentration (µg/mL)	Inhibition (%)		Selectivity
		COX-1	COX-2	
T12	0.025	98.18	80.78	0.91
	0.050	97.67	89.36	0.82
T13	0.025	95.91	83.97	0.86
	0.050	96.72	83.56	0.87

Discussion

The active compounds isolated from the root bark of sidaguri identified the presence of terpenoid groups, which is in accordance with the earlier reported studies although it was extracted from the whole part of sidaguri [5]. This means that different part of sidaguri contain the same terpenoid group. However further study should be carried out to examine the concentration of

terpenoid group and other active groups. The isolated compounds refer to Z-3,6,6 trimethylhept-2-en-1-ol and nonanoic. Nonanoic was also isolated from *Piper nigrum* at 60 µg/mL. The active compound of sidaguri extract from acetone fraction is n-hexacos-11-enoic acid. These differences may be due to different solvent used. These compounds were reported to show antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella Typhimurium* [6], while root bark of sidaguri only showed antibacterial activity against facultative anaerob Gram + bacteria, *Enteroc faecalis* but no antibacterial activity against obligate anaerob bacteria; *Actinomyces spp* [3]. Another investigation on the leaves of *S. rhombifolia* found aliphatics group cyclopropenoid fatty acid, myristic acid, palmitic acid, stearic acid, oleic acid, and linoleic acid [12]. More studies should be carried out to examine other functional groups contained in the root bark of sidaguri and its biological activities against other bacteria.

In this study, the active compounds isolated from the root bark of sidaguri (T12 and T13) showed anti-inflammatory effect by inhibiting cyclooxygenase enzymes. Unfortunately, the isolated compounds not only inhibit COX-2 but also inhibit COX-1. The human COX enzyme is known to exist in two forms; COX-1 and COX-2. COX-1 is constitutively expressed to maintain housekeeping functions such as cytoprotection in the stomach, regulation of blood flow in the kidneys, and the formation of thromboxane which involves in platelet aggregation. In contrast to COX-1, COX-2 is inducible and synthesized in inflamed tissues. COX-2 is important in the production of proinflammatory mediator, the prostaglandins (PGs) which are known to sensitize peripheral nociceptors and increase neuronal excitability by regulating the activity of certain ion channels [13].

This may partly explain the root bark extracts of sidaguri can reduce the inflammation on rat periapical lesion model [8]. Extracts isolated from *Piper nigrum* at 60 µg/mL was also reported to be able to inhibit COX. [14] A low COX-2/COX-1 ratio is preferred which indicates a preferential COX-2 inhibitor that has a valuable pharmacological effect. Selective COX-2 inhibitor has been shown to have no severe side effects that can cause acute injury on tissue, and no prostaglandin synthesis on gastric mucosal that can develop to chronic ulceration [15,16].

In summary, two isolates have been identified from root bark extract of sidaguri which showed anti-inflammatory effect with no selective inhibitory activity on COX enzymes. Elucidation of molecular docking of the binding mode of inhibitors to COX should be carried out.

Conclusion

The structure of T12 and T13 are similar to the formation of fatty acid Z (-3, 6, 6 trimethylhept-2-en-1-ol and nonanoic, respectively, and are able to non-selectively inhibit COX enzymes.

Authors' Contributions: Each author made significant individual contributions to this manuscript. MT, IKM, NN, and SS conceived and designed the experiment. MT, FM, and LM performed the experiments. IKM, NN and SS analyzed the data. All authors wrote the paper, read and confirmed publication of the paper.

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Conflict of Interest: The authors declare no conflicts of interest.

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