**Antilipoxygenase and Anti-inflammatory Activities of *Streblus asper* Leaf Extract on Xylene-induced Ear Edema in Mice**

**Abstract**

*Ethnopharmacological relevance*

*Streblus asper* (SA) belonging to the Moraceae family is well known as a folk medicinal plant in Asian countries such as India, Sri Lanka, Malaysia, the Philippines, Southern China, and Thailand. There is limited scientific evidence on the anti-inflammatory effects of SA and no confirmed evidence of an anti-inflammatory mechanism involving the lipoxygenase enzyme.

*Aim of the study*

In this study, we aimed to investigate the antilipoxygenase activity and anti-inflammatory effects of an aqueous SA leaf extract by performing a lipoxygenase assay *in vitro* and animal experiments *in vivo* in a xylene-induced ear edema mouse model.

*Materials and Methods*

The antilipoxygenase activity was measured *in vitro* using a lipoxygenase assay, and the oxidation of linoleic acid into 13-hydroperoxy linoleic acid was detected using a UV spectrophotometer at a wavelength of 234 nm. Ear edema was induced using topical xylene in 25 male ICR mice that were randomly divided into five groups: Group 1 (control group), administered distilled water; Group 2 (positive control group), administered 10 mg/kg diclofenac sodium; and groups 3–5, 125, 250, and 500 mg/kg SA extract; the ear thickness of the mice was then measured.

*Results*

The lipoxygenase assay results showed that the half maximal inhibitory concentrations of diclofenac sodium and SA were 0.0015 and 37.96 μg/mL, respectively. The mice that received diclofenac sodium exhibited significantly reduced ear edema from 30 min after xylene induction, whereas the mice that received 250 and 500 mg/kg SA exhibited significantly reduced ear edema compared with the control group 45 min after xylene induction.

*Conclusions*

These results suggest that SA leaf extract exerts anti-inflammatory activity. However, further studies are warranted to evaluate these effects and the potential of SA in the development of pharmaceutical products that can prevent and treat inflammation.

**Keywords: edema, anti-inflammatory, *Streblus asper***, **phytochemical**

**1. Introduction**

Inflammation is a defense mechanism of living tissues triggered by trauma, pathogens, stress, toxic substances, and cell damage. It is a complex reaction involved in resolving stimuli and initiating the healing process (Chen et al., 2018). If left uncontrolled, acute inflammation might progress to chronic inflammation and contribute to chronic inflammatory diseases (Chen et al., 2018; Landskron et al., 2014). Nonsteroidal anti-inflammatory drugs, steroids, and opioids are accepted and widely used for managing inflammatory symptoms and treating diseases associated with inflammation (Slater et al., 2010). However, they are still used with awareness and are under consideration by physicians owing to their side effects. In the past century, plants and their extracts have gained increasing attention as sources of alternative anti-inflammatory therapeutics (Pountos et al., 2011; Otimenyin, 2018) and their pharmacological activities and phytochemical constituents have been increasingly investigated.

*Streblus asper* (SA), belonging to the Moraceae family, is well known as a folk medicinal plant in Asian countries such as India, Sri Lanka, Malaysia, the Philippines, Southern China, and Thailand. It is a rich source of cardiac glycosides, phenolic compounds, and volatile oils (Rastogi et al., 2006). Several studies have reported its pharmacological activities, including its antibacterial, antiseptic, antidiarrheal, antidiabetic, antioxidant, and anti-Parkinson’s effects (Rastogi et al., 2006; Singsai et al., 2015; Shahed-Al-Mahmud et al., 2020). In addition, SA leaf extract demonstrated an inhibitory effect on carrageenan-induced paw edema in rats. The possible mechanism of this effect is related to the suppression of lipopolysaccharide-induced expression of cyclooxygenase (COX)-2 and inducible nitric oxide synthase mRNA (Sripanidkulchai et al., 2009). Scientific evidence on the anti-inflammatory effects of SA extracts has rarely been reported, and there is no confirmed evidence of an anti-inflammatory mechanism involving the lipoxygenase enzyme. Therefore, the purpose of this study was to evaluate the anti-inflammatory activity of an aqueous SA leaf extract by performing a lipoxygenase assay *in vitro* and animal experiments *in vivo* in a xylene-induced ear edema mouse model. The results of this study will provide potential information for the treatment of neurogenerative disorders caused by neuroinflammation (Glass et al. 2010).

**2. Materials and Methods**

**2.1. Animals**

In this study, adult male ICR mice (40–60 g) were used. The mice were housed in a room maintained at 25 ± 2°C on a 12 h light/dark cycle. All experiments were carried out according to the guidelines of the Institute of Animal for Scientific Purposes Development and approved by the Lab Animal Research Center, University of Phayao ().

**2.2. Preparation of the Extract**

Fresh SA leaves were collected from the botanical garden of the School of Pharmaceutical Sciences, University of Phayao (Phayao, Thailand). The fresh leaves were desiccated, crushed, and weighed. For aqueous extract preparation, the dried mashed powder was soaked in deionized water at 60°C for 6 h, filtered, and lyophilized. The percentage yield of the SA extract was 14.59% of the dried leaves. The dry powdered extract was kept in airtight, light-protected containers at 2–4°C and dissolved in distilled water before use (Singsai et al., 2015).

**2.3. Phytochemical Screening**

Phytochemical screening of the SA extract was performed following the method of Farnsworth (1996). Flavonoids and triterpenoids were detected using Shinoda’s test and the Liebermann–Burchard test. Cardiac glycosides, including the steroid nucleus, unsaturated lactone ring, and 2-deoxy sugar, were detected using the Liebermann–Burchard test, Kedde’s test, and the Keller–Kiliani test, respectively. The forth test was used to detect saponins.

**2.4. Antilipoxygenase Activity of the SA Extract**

An *in vitro* anti-inflammatory experiment was performed using a lipoxygenase assay adapted from that performed by Leelaprakash et al. (2012) and Chung et al. (2009). Briefly, the oxidation of linoleic acid into 13-hydroperoxy linoleic acid was detected by UV spectrophotometry at a wavelength of 234 nm. Diclofenac sodium as a standard was prepared at concentrations of 0.001, 0.01, 0.1, and 1 μg/mL, and the SA extract was prepared at concentrations of 5, 10, 25, 50, and 100 μg/mL. The percentage inhibition was calculated and expressed as the mean ± standard error of the mean of three replicates.

**2.5. Anti-inflammatory Effects of the SA Extract on Xylene-induced Ear Edema**

The anti-inflammatory effects of SA on xylene-induced ear edema were determined using the experimental method of Sadeghi et al. (2014) and Anyasor and Ijituyi (2018). Twenty-five male ICR mice were randomly divided into five groups: Group 1 (control group) mice were administered distilled water; Group 2 (positive control group) mice were administered 10 mg/kg diclofenac sodium; and the mice in groups 3–5 were administered 125, 250, and 500 mg/kg SA extract, respectively. All mice received the respective treatment once daily for 7 days. On day 8, inflammation was induced in the animals as ear edema using topical xylene; the mice were administered distilled water, diclofenac sodium, or SA extract 15 min later, after which they were induced with xylene. The right ear thickness of the mice was measured with a digital thickness gauge after 15, 30, 45, and 60 min of the induction. The thickness of the ears was used to calculate the percentage edema using the following equation:



where *Tt* represents the ear thickness at *t* min and *T*0 represents the ear thickness at 0 min.

**2.6. Statistical Analysis**

Statistical analysis was performed using SigmaPlot (version 14.0). The data were analyzed using one-way analysis of variance followed by Tukey’s multiple comparisons test. The criterion for statistical significance was set at.

**3. Results and Discussion**

**3.1. Phytochemical Analysis**

The phytochemical screening of the SA extract afforded flavonoids, triterpenoids, cardiac glycosides, and saponins (Table [1](https://www.hindawi.com/journals/aps/2020/3176391/tab1/)). The phytochemicals in SA include cardiac glycosides (Neekhra et al., 2017), flavonoids, triterpenoids, and saponins, which might be responsible for the distinct anti-inflammatory activities of the extract (Ahmadiani et al., 2000). Flavonoids have therapeutic potential in acute inflammation (Middleton et al., 2000) and act by inhibiting arachidonic acid release, which plays a central role in prostaglandin synthesis (Tordera et al., 1994; Owolabi et al., 2018). Triterpenoids may exert their anti-inflammatory actions by decreasing inducible nitric oxide synthase expression (Lucetti et al., 2010; Schmid et al., 2009).

**3.2. Effects of the SA Extract on Antilipoxygenase Activity**

The percentage inhibition of lipoxygenase activity by the SA extract was determined from the results of the lipoxygenase assay (Table [2](https://www.hindawi.com/journals/aps/2020/3176391/tab2/)). The half maximal inhibitory concentrations of diclofenac sodium (as a standard) and the SA extract were 0.0015 and 37.96 μg/mL, respectively.

Lipoxygenase is the enzyme involved in the arachidonic acid pathway that produces leukotrienes (Zhang et al., 2002). The present study was performed to investigate the antilipoxygenase activity of an SA leaf extract *in vitro*. The results showed that the SA extract has lower antilipoxygenase activity than diclofenac sodium. It is possible that SA exerts anti-inflammatory activity via inhibition of COX as the main pathway; lipoxygenase is involved in a minor pathway that slightly involves leukotrienes; therefore, the mechanism underlying the anti-inflammatory action of SA might involve prostaglandins, which are the products of the COX pathway.

Many studies have reported that phenolic compounds inhibit inflammation via inhibition of the lipoxygenase enzyme, which is involved in the transformation of arachidonic acid to inflammatory mediators and in free radical scavenging in arachidonic acid metabolism (Middleton et al., 2000; Arts and Hollman, 2005). Flavonoids exert antioxidant activity by decreasing capillary permeability, disturbing the arachidonic acid pathway, and inhibiting COX and lipoxygenase enzymes, resulting in decreased prostaglandin and leukotriene levels (Tordera et al., 1994). In our previous study, polyphenolic compounds such as gallic acid, isoquercetin, quercetin, rutin, catechin, and tannic acid were found in SA aqueous extracts. Thus, it can be inferred that the antilipoxygenase activity of SA extract might be affected by the antioxidant action of phenolic compounds (Khalaf, 2008).

**3.3. Effects of the SA Extract on Xylene-induced Ear Edema in Mice**

The mouse ear thickness values (expressed as the mean ±  standard error of the mean) are shown in Table [3](https://www.hindawi.com/journals/aps/2020/3176391/tab3/).

The results showed that the administration of diclofenac sodium (10 mg/kg) significantly reduced ear edema induced by xylene from 30 min onwards, while the mice that received 250  and 500 mg/kg SA showed significantly reduced ear edema compared with the control group 45 min after induction with xylene (Figure [1](https://www.hindawi.com/journals/aps/2020/3176391/fig1/)).

Animal experiments and studies on the anti-inflammatory effects of SA on xylene-induced ear edema in mice indicated that SA exerts anti-inflammatory activity by reducing ear edema in a dose-dependent manner; however, the onset of action of SA is slower than that of diclofenac sodium. This study shows the acute anti-inflammatory effect of SA extract—innate immune cells form the first line of immune defense and regulate the activation of adaptive immune responses. Most features of acute inflammation persist as inflammation becomes chronic, including the expansion of blood vessels (vasodilation), the increase in blood flow and capillary permeability, and migration of neutrophils into the infected tissue through the capillary wall (diapedesis) (Pahwa et al., 2020). Xylene-induced edema has been shown to partially involve substance *P* as a common inflammation model for increasing capillary permeability and leukocyte infiltration (Eidi et al., 2016). Thus, through the proposed mechanism, SA might reduce the release of substance *P* or antagonize its action in the inflammation process. During the initial phase, also called the neurogenic phase, substance *P* and bradykinin are released. Substance *P*, a neurotransmitter in the central nervous system, induces nitric oxide release causing vasodilation and plasma exudation (Sadeghi et al. 2014; Anyasor and Ijituyi, 2018). Therefore, the anti-inflammatory activity of SA might be focused on neurogenic inflammation.

**4. Conclusions**

The present study indicated that SA leaf extract has anti-inflammatory effects, including antilipoxygenase activity, and reduces mouse ear edema. However, further studies are warranted to evaluate the chronic inflammatory activities and potential of SA in the development of pharmaceutical products to prevent and treat inflammation.

**Glossary**

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**Author contributions**

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**Table captions**

**Table 1:** Phytochemical screening of the SA extract

**Table 2:** Percentage inhibition of lipoxygenase activity by the SA extract

**Table 3:** Ear thickness (mm) expressed as mean ± standard error of the mean

**Figure captions**

**Figure 1:** Percentage ear edema induced by xylene at 15, 30, 45, and 60 min.

The figure shows that the administration of diclofenac sodium (10 mg/kg) significantly reduced xylene-induced ear edema from 30 min onwards, while the mice that received 250  and 500 mg/kg SA showed significantly reduced ear edema compared with the control group 45 min after induction with xylene: (i) different from the control group and (ii)# different from the diclofenac group.