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Research Article

Free Radical Scavenging and Analgesic Activities of 70% Ethanol Extract of *Luvunga sarmentosa* (BI.) Kurz from Central Kalimantan

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Abstract

Luvunga sarmentosa, commonly known as saluang belum, is widely used in Kalimantan to relieve pains, rheumatism, boost the immune system, and fever. The research on the free radical scavenging and analgesic effect of the *L. sarmentosa* stem extract has not been reported. This study aimed to evaluate the free radical scavenging and analgesic activity of the ethanol extract of *L. sarmentosa*. The *L. sarmentosa* stem was extracted using 70% ethanol and tested for free radical scavenging using the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) and analgesic activity, acetic acid-induced writhing test, and hot plate test in an animal model. The results showed that the 70% ethanol extract of the *L. sarmentosa* had an anti-free radical scavenging and analgesic activity. The extract has weak free radical scavenging with an IC_{50} value of 293.45 µg/mL. Analgesic activity using the writhing test indicated that the extract significantly reduced the writhes count after oral administration in a dose-dependent manner compared to the negative control. Extract at a dose of 550 mg/kg BW can reduce the writhing test by 67.60% compared to others. In contrast, the diclofenac sodium reduced the number of writhes by 74.74%. While in a hot plate, the extract at a dose of 550 mg/kg BW produced a maximum possible analgesia (MPA) of 17.64%, lower than the MPA of diclofenac sodium (51.01%). Analgesic activity of the extract has higher inhibition on the writhing test than on the hot plate. The extract could be responsible for the peripheral mechanism by inhibiting the prostaglandin biosynthesis.

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INTRODUCTION

Pain is a body's defense mechanism that reacts to stimuli to avoid further tissue damage[1]. Pain can also be defined as pathological conditions that arise due to free radicals and oxidative stress in body cells[2]. The body produces free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) in endogenous systems when exposed to various physiochemical or pathological conditions. Excessive ROS production causes oxidative stress, a process that can damage cell structures, including lipids, proteins, and DNA[3]. Pain is an unpleasant sensory and emotional experience associated

with actual or potential tissue damage[4]. However, pain is beneficial to the immune system. Nevertheless, it causes much suffering and discomfort to the victims, lowering the quality of life, disability, or mortality in several cases. Therefore, pain needs to be managed[5].

The onset of pain makes a person look for treatment to reduce pain. Attempts to reduce the pain are with analgesics drug[6]. Analgesics are substances that can reduce or dispel pain without losing consciousness. Evaluation of analgesic drugs in public hospitals in China from 2013 to 2018 showed an increase in analgesic drugs every year. From 2013 to 2018, NSAIDs' annual clinical drug dose increased by about 0.6 times[7]. However, prolonged use of these NSAIDs produces significant side effects and are toxic to the liver, kidney, gastrointestinal linings, and reduced auditory ability[8]. As such, research to discover other alternatives to treat pain is crucial.

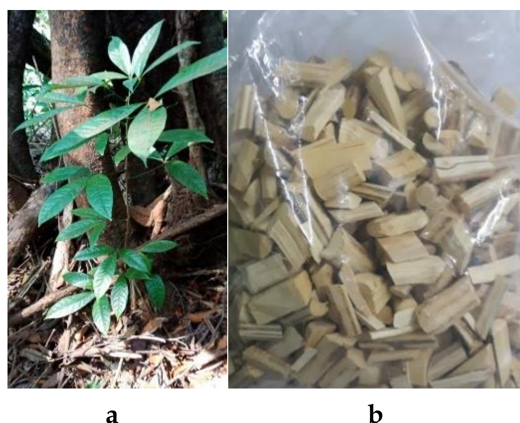
Medicinal herbs have been used for centuries for therapeutic purposes. Many of these herbs with analgesic activity had been used without any side effects and at a lower cost[9]. World Health Organization (WHO) estimates that more than 80% of the world population relies on traditional medicines, and the market is rapidly growing. Saluang belum (*Luvunga sarmentosa* (BI.) Kurz) belongs to the family of Rutaceae, which develops and spreads in the tropical forests of Kalimantan[10]. Several studies have been carried out to identify the phytochemicals of *L. sarmentosa*. Its leaves contain apotirucallane triterpenoids named luvungins A-G, 1α-acetoxyluvungin A, coumarins ostruthin, and 8-geranyl-7-hydroxycoumarin, and triterpenes friedelin, flindissone, melianone, niloticin, and limonin have been isolated[11].

In Central Kalimantan, this plant was prescribed traditional medication to increase stamina and antioxidants[10]. The part of the stem, root, and leaves of *L. sarmentosa* is used in traditional herbal recipes. The stem and root were consumed three times a day by boiling or brewed with hot water and consumed once a day while warm[12,13]. The traditional healers prescribed the stem more to treat soreness, fatigue, or pains. Thus far, research on the analgesic activity of *L. sarmentosa* stem has not been widely reported. Therefore, this study was conducted to determine the analgesic and free radical scavenging activity of 70% ethanol extract of *L. sarmentosa* stem. The results study will provide scientific-based evidence on the use of stems in traditional medicine.

MATERIALS AND METHODS

Materials

The stems of *L. sarmentosa* was collected in September 2019 from traditional healers in Rakumpit District, Palangka Raya, Central Kalimantan, Indonesia (**Figure 1**). A licensed botanist at Purwodadi Botanical Garden, East Java, Indonesia, conducted authentification and identification of the plant with voucher specimen 1048/IPH.06/HM/IX/2019. Diclofenac sodium was used as a standard drug for analgesics and vitamin C for antioxidants. Both of them were obtained from PT. Kimia Farma Tbk, Indonesia. Other materials used were distilled water, acetic acid, carboxymethyl cellulose sodium (CMC-Na 0.5%), 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), and 70% ethanol.



a **b**

Figure 1. *Luvunga sarmentosa* at the forest (**a**) and the stem simplicia package from the traditional healer (**b**)

Methods

Plant extraction

The stem of *L. sarmentosa* was shade dried and powdered mechanically. The powdered (400 g) was macerated in ethanol 70% three times every 24 hours. The extract was then filtered and concentrated with a vacuum evaporator, then dried with a freeze dryer to obtain a dry extract.

DPPH radical scavenging assay

The free radical scavenging activity of the extracts was tested by DPPH radical scavenging assay. The extract was diluted with methanol at 1000; 800; 600; 400; 200; 100; 50; 25; and 12.5 µg/mL. Meanwhile, vitamin C as standard was diluted at 100; 50; 25; 12.5; 6.25; 3.12; 1.56; 0.78; 0.39; 0.19; 0.095; and 0.0475 µg/mL. A solution of 0.25 mM DPPH in methanol was prepared, and 100 µL of this solution was mixed with 100 µL of extract/standard in methanol at different concentrations. The reaction mixture was incubated in the dark at room temperature (26°C) for 30 minutes. The experiment was carried out with three replications, and the absorbance was observed at a wavelength of 517 nm. DPPH radical scavenging activity was calculated using equation [**1**], in which A_0 was the absorbance of the control and A_1 was the absorbance of the extract/standard.

$$\%\ DPPH\ radical\ scavenging\ activity = \frac{A0-A1}{A0} \times 100\% \qquad \ldots [1]$$

Experimental animal

Male mice (Deutschland, Denken, and Yoken strains) weighing 25-30 g and aged 4-8 weeks were obtained from Farma Veterinary Center, Surabaya, Indonesia. All animals were maintained on a standard animal pellets diet and water ad libitum at the Animal Laboratory of the Institute of Tropical Disease, Universitas Airlangga, Surabaya. All the animals were acclimatized for seven days to the laboratory conditions before the experiment. All animal protocols were critically reviewed and approved by the Faculty of Veterinary Medicine, Universitas Airlangga, with approval number 2.KE.117.03.202.

Analgesic activity in animal model with acetic acid-induced writhing test

Thirty male mice were randomly divided into five groups, and each group consisted of six mice. Group 1 was treated with carboxymethyl cellulose (CMC-Na 0.5%) as a negative control, group 2 was treated with diclofenac sodium as a positive control at a 40 mg/kg BW, and groups 3, 4, and 5 were treated with ethanol extract of *L. sarmentosa* at a dose of 50, 300, and 550 mg/kg BW, equal to the dose of 10, 40, and 80 g of simplicia, respectively. All treatments were administered orally. The extract and the standard drug were treated 30 minutes before 1% acetic acid injection at a dose of 10 mL/kg BW intraperitoneally. After five minutes, each group of mice was observed for the number of writhes for 45 minutes. The mean value for each group was calculated and compared with the control. The percentage of analgesic activity was calculated using the equation [**2**], in which W is the number of writhing, c is the negative control, and t is the test[14,15].

$$\%\ Inhibition = \frac{Wc-wt}{Wc} \times 100\% \qquad \ldots [2]$$

Analgesic activity in animal model with hot plate test

The analgesic activity was also evaluated using the hot plate method[15-17]. Mice were given oral therapy according to groups. After 30 minutes of treatment, the experimental animals were placed on a hot plate maintained at 55°C within the restrainer. The reaction time (in seconds) or latency period was determined as the time for the rats to react to the thermal pain by licking their paws or jumping. The reaction time was recorded before treatment (0 minutes), then 30, 60, 90, and 120 minutes after administering the treatments. The maximum reaction time was fixed at 20 seconds to prevent any injury to the tissues of the paws. The maximum possible analgesia (MPA) was calculated using the equation [**3**][18].

$$\%\ MPA = \frac{test\ group\ mean - control\ group\ mean}{cut\ off\ time\ 20\ seconds - control\ group\ mean} \times 100\% \qquad \ldots [3]$$

Data analysis

The results of the study were presented in mean±SEM. Statistical analysis was used one-way ANOVA followed by post hoc Dunnett's test for multiple comparisons (GraphPad Prism 7.0, Co., Ltd., San Diego, US). The difference between groups was considered significant at a p-value <0.05.

RESULTS AND DISCUSSION

This study was conducted to determine the free radical scavenging and analgesic activities of *L. sarmentosa* stem ethanol extract. The *L. sarmentosa* was carried out using 70% ethanol as a solvent by the maceration method. The extraction yielded 3.9% w/w dry matter and was light brown.

DPPH radical scavenging assay

DPPH radical scavenging model is the widely used method to evaluate the anti-free radical activity of natural compounds and plant extracts. The results showed that extract had to scavenge the free radical, with an IC_{50} value of 293.45 µg/mL (**Figure 2**), while vitamin C had an IC_{50} value of 11.39 µg/mL (**Figure 3**). The extract was weak, and vitamin C was highly active as a free radical, based on Marjoni and Zulfisa[19]. The scavenging activity shows that extract and vitamin C could provide a hydrogen atom to the DPPH radical. The DPPH would oxidize and be decolorized. Stable free radical DPPH could accept an electron or hydrogen radical to become stable. Its solution appears a deep violet color. As this electron becomes paired off, the absorption vanishes, resulting in decolorization[20].

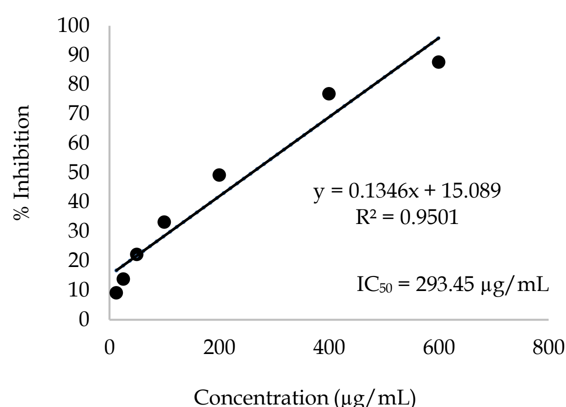


Figure 2. The IC_{50} value for radical scavenging activity of *L. sarmentosa* extract

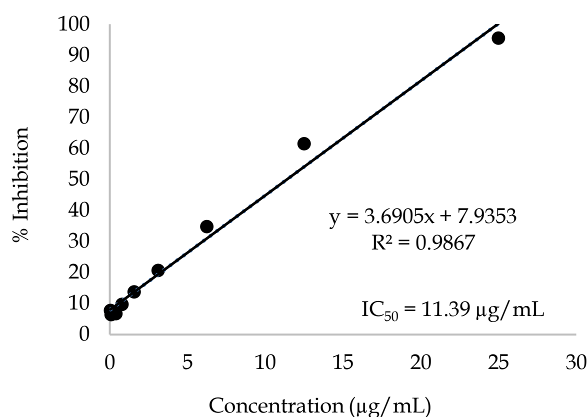


Figure 3. The IC_{50} value for radical scavenging activity of vitamin C

Analgesic activity with acetic acid-induced writhing test

The acetic acid-induced writhing test has widely been used for screening analgesic drugs[14]. The writhing test is used to assess peripheral acting analgesics. In the writhing test, the acetic acid injection causes pain by releasing serotonin, histamine, prostaglandins, and bradykinin from arachidonic acid through cyclooxygenase (COX) enzymes[1,14,16]. The synthesis of endogenous substances induces contraction of the abdominal muscles that touch the floor, pull the legs back, and stretch the body[21-23].

Analgesic activity in this study indicated that the 70% ethanol extract of *L. sarmentosa* stem significantly reduced the writhes count after oral administration in a dose-dependent manner compared to the negative control. After forty-five minutes of the test period, the extract at 550 mg/kg BW demonstrated the highest analgesic activity by reducing the number of writhes by 67.60%, while 300 mg/kg BW reduced the number of writhes by 49.30%. The 50 mg/kg BW dose reduced the writhes by 33.28%. However, diclofenac sodium reduced the writhes by 74.74% — the analgesic activity of 70% ethanol extract of *L. sarmentosa* stem presented in **Table I**.

Table I. Analgesic activity of 70% ethanol extract of *L. sarmentosa* stem in acetic acid-induced writhing test

Group	Dose (mg/kg)	Number of writhes in 45 minutes (mean ± SEM)	Inhibition (%)
Negative control	-	95.67 ± 5.70	-
Positive control	40	24.17 ± 1.58****	74.74
Luvunga sarmentosa extract	50	63.83 ± 1.68****	33.28
	300	48.50 ± 2.57****	49.30
	550	31.00 ± 2.72****	67.60

Data were reported as mean ± SEM and analyzed by ANOVA followed by Dunnett's multiple comparison test. * indicate a statistically significant value from negative control, ****p <0.0001.

Analgesic activity with hot plate test

The hot plate is a standard method for evaluating central analgesic activity in animal models that use thermal stimuli as pain inducers with temperature was maintained at 55°C. The principle of this method is a change in spinal cord level, which effectively describes the centrally mediated anti-nociceptive response[24]. The paw-licking or jumping are defined as pain reflex behavior[25-27]. The analgesic activity of the 70% ethanol extract of *L. sarmentosa* stem using the hot plate was presented in **Table II** and **Figure 4**.

The treatment of 70% ethanol extract of *L. sarmentosa* stem (50–550 mg/kg BW) and diclofenac resulted in a significant dose-dependent increase in the reaction time to thermal stimulation compared with the negative control. The MPA value of extract did not show any analgesic effect 60 minutes after treatment but increased at 90 minutes and declined after that. The highest increase in reaction time was observed with a 550 mg/kg BW dose at 90-minutes post-treatment (17.64%). Diclofenac sodium elicited significant analgesic activity within 30 minutes following administration, as evidenced by the gradual increase throughout the observation period. At the peak of activity (90 minutes), diclofenac sodium showed an MPA of 51.01%.

Table II. Analgesic activity of 70% ethanol extract of *L. sarmentosa* stem in hot plate test

Groups	Dose (mg/kg)	Reaction time (minutes)				
		0	30	60	90	120
Negative control	-	3.56±0.20	4.06±0.25	4.96±0.02	5.42±0.13	5.20±0.30
Positive control	40	5.13±0.52*	7.27±0.06****	9.26±1.29****	12.86±1.59****	11.54±1.29****
Luvunga sarmentosa extract	50	5.32±0.39**	6.25±0.41**	6.94±0.39**	7.40±0.47*	6.52±0.33
	300	5.49±0.21**	6.72±0.23***	7.34±0.30**	7.67±0.28*	7.16±0.26**
	550	5.43±0.52**	6.69±0.61***	7.36±0.56**	8.00±0.76**	7.43±0.55**

Data were reported as mean ± SEM; n=6. One-way ANOVA was carried out using Dunnett's multiple comparison test. *indicatea statistical significant: *p <0.05; **p <0.01; ***p <0.001; ****p <0.0001

Writhing response induced by acetic acid and hot-plate latent pain response in mice are two common and important models for screening analgesics. These two models could cause pain by liberating PGs and many others that excite pain nerve endings. Both methods were used to study ethanol extract's peripheral and central analgesic activities. The results showed that ethanol extract has higher inhibition on the acetic acid test than the hot plate. This observation points out that extract possesses peripherally-mediated antinociceptive properties that may work via reducing the level of prostaglandin synthesis or other inflammatory mediators, which is much like diclofenac. Diclofenac sodium performs its action in peripheral acting by inhibiting the synthesis of prostaglandins (pain mediators) by inhibiting COX-1 and COX-2[15]. The analgesic activities of 70% ethanol extract of *L. sarmentosa* stem could be attributed to one or more phytochemical compounds present in the extract.

The phytochemical screening in this study showed that 70% ethanol extract of *L. sarmentosa* stem contained terpenoid and flavonoid compounds. Their compounds in the extract may contribute to the anti-free radical and analgesic activity. Flavonoids are polyphenolic compounds that can change or reduce free radicals[28]. Triterpenoids or steroids are compounds that have a role as antioxidants. The antioxidant mechanism of triterpenoids is by scavenging reactive species, such as superoxide and metal chelating[29]. Besides that, a flavonoid is also known to have analgetic activity[30]. Flavonoids can also reduce arachidonic acid production by inhibiting neutrophils' degranulation[31].

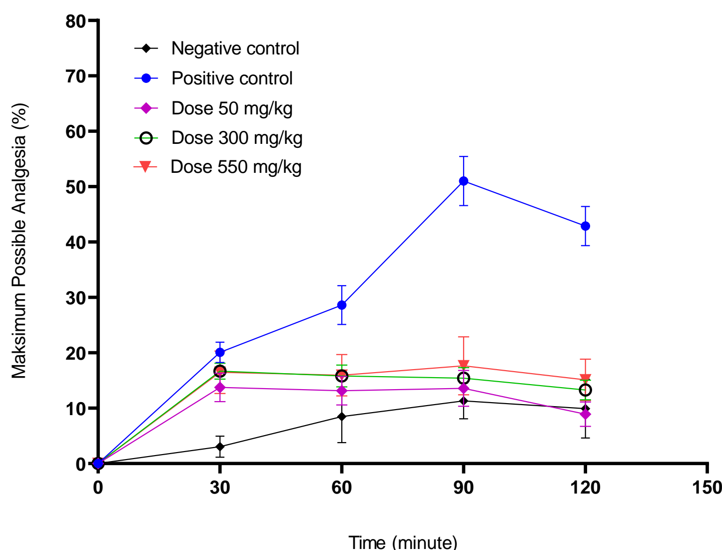


Figure 4. Maximum possible analgesia of *L. sarmentosa* stem extract compared to diclofenac sodium evaluated by hot plate test

CONCLUSION

The 70% ethanol extract of *L. sarmentosa* stems had weak anti-free radical activity. However, it exhibited significant analgesic activity, possibly by a peripheral pain mechanism inhibiting the prostaglandin pathway.

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AUTHORS' CONTRIBUTION

All authors have an equal contribution in carrying out this study.

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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