

PHYTOCHEMICAL SCREENING, ANTIOXIDANT ACTIVITY, ANTI-INFLAMMATORY, TOXICITY USING BRINE SHRIMP LETHALITY TEST OF VARIATION EXTRACTS OF *Senna alata* L. LEAF EXTRACT

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ABSTRACT

This study determined phytochemical screenings, antioxidant activity, anti-inflammatory, and toxicity of *Senna alata* L. leaf extract by using several method extractions that as maceration (A), reflux (B), and Ultrasonic Assist Extraction (UAE) (C). Phytochemical screening was obtained that leaves extract of *Senna alata* L. variations A, B, and C contained alkaloids, flavonoids, phenolics, terpenoids, and saponins. FRAP test was used to determine antioxidant activity and IC 50. It was obtained IC50 < 50 ppm and classed as a potent antioxidant. The anti-inflammatory activity of each *Senna alata* L leaf extract was obtained with IC50 < 50 ppm. The Bhrine Shrimp Lethality Test (BSLT) was utilized to test toxicity by calculating the LC50 value. It was obtained that the LC50 value of *Senna alata* L. leaf extracts <1000 ppm showed that *Senna alata* L. leaf extracts are toxic to *Artemia salina* Leach larvae so it is estimated to have anticancer potential. The highest toxicity (LC50) was *Senna alata* L. leaf extracts by variation C 235.79 mg/L. Variation C is *Senna alata* L. leaf extract using the Ultrasonic Assist Extraction (UAE) method.

Keywords: *Senna alata* L., Antioxidant, Anti-Inflammatory, Ultrasonic Assist Extraction, Brine Shrimp Lethality Test, Maceration, Reflux.

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INTRODUCTION

According to the 2007 Basic Health Research (Riskesdas) report, with a prevalence of 4.3% per 1,000 people. The World Health Organization (WHO) and the World Cancer Prevention Organization (UICC) anticipate a 300 percent global increase in donations to cancer patients by 2030. This number is 70 percent in developing countries such as Indonesia.¹ In its development in the health sector, anticancer drugs have been found and chemotherapy has been carried out, but the high-cost factor is an obstacle. This encourages people to do treatment using natural medicines or traditional medicines.² Traditional medicines or natural medicines have been known by the people of Indonesia since ancient times. In addition to its properties that have been used for generations by the community, this drug is cheaper and easier to obtain, but further research is needed because there are many plants whose toxicity levels are unknown.³ The Brine Shrimp Lethality Test (BSLT) method is one approach that can be used to calculate plant toxicity. The BSLT method is a simple preliminary test that uses *Artemia salina* Leach as a test animal to detect the amount of acute toxicity of a chemical or extract.⁴ *Senna alata* L. is one of the herbs used in traditional medicine. It comes from the tropics of America and usually lives in the lowlands to the mountains with a height of 1,400 meters above sea level.⁵ Due to its chemical constituents, *Senna alata* L. leaves can be used as traditional medicine. These constituents include rein aloe emodina, rein aloe emodina diantron, rein aloe emodina chrisophanic acid (dehydroxy methyl antroquinone), and tannins. In addition, the chemical compounds contained in *Senna alata* L. leaves extract are flavonoids, saponins, tannins, alkaloids, and anthraquinone compounds.⁶ Flavonoids and anthraquinones from *Senna alata* L. leave function as an antidote to free radicals in the body and as an anti-cancer.⁷

The technique most frequently used for the isolation of active substances in plants is solvent extraction, namely the separation of components from a mixture using a solvent that aims to withdraw the active

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substance in the sample.⁸ Common methods used for extraction are maceration, soxhletation, percolation, and reflux. This conventional method has various weaknesses including a long extraction time, a lot of solvent needed, and if it is carried out at high temperatures it can damage the active compound itself.⁹ One of the developments in the extraction method is using Ultrasonic Assist Extraction (UAE). The principle of this extraction is to increase the permeability of the cell wall with cavitation as a dynamic stress so that the interphase phase arises.¹⁰ UAE can increase the extraction yield in a short time and at low temperatures. Another advantage of the UAE method is that the volume of solvent needed is small so it can minimize the use of solvents.¹¹ Researchers were interested in determining the anti-inflammatory, antioxidant, and toxicological properties of *Senna alata* L leaf extracts utilizing BSLT with *Artemia salina* shrimp larvae based on the above description. The methods used to produce the extract are maceration, reflux, and Ultrasonic Assist Extraction (UAE) to show the effectiveness of the three methods as antioxidants, anti-inflammatories, and toxicity.

EXPERIMENTAL

Materials

Senna alata L., a plant native to the tropics of South and Central America, was used in this study and typically grows at a height of 1,400 meters above sea level. In this study, *Senna alata* L. leaf methanol extract was prepared under three different conditions: A (maceration), B (reflux), and C (reflux). (Ultrasonic Assist Extraction).

Phytochemical Analysis

Using the Ciulei method, a phytochemical analysis of crude methanol extract was conducted. It was as part of the phytochemical screening.¹²

Antioxidant Activity by FRAP Method¹³

In a 5 mL measuring flask, A sample solution with a concentration of 1,000 mg/L was produced by dissolving 5 mg of the extract in methanol pa. In five 5 mL measuring flasks, 40 μ L; 80 μ L; 160 μ L; 320 μ L; and 640 μ L of solution were pipetted, followed by the addition of 0.4 mL of 0.001 M citric acid; 0.2 mL of Fe^{3+} 0.002 M solution; 0.4 mL o-phenanthroline 0.2%, filtration with purified water, and homogenization (sample concentrations 8, 16, After 35 minutes of 37°C incubation, the absorbance of the solution at 510 nm was measured using a visible light spectrophotometer; the process was repeated twice. Using a gallic acid comparator with concentrations of 0.5, 1.0, and 1.5 mg/L, the same method was carried out. Activity reduction can be calculated using the formula below:

The percentage of reduction can be calculated by the equation:

$$\% \text{ reduction} = \frac{(A_{\text{sample}} - A_{\text{blank}})}{A_{\text{blank}}} \times 100\%$$

A_{blank} = Absorptivity without sample

A_{sample} = Sample absorbance

The determined value is entered into a linear equation ($Y = aX + b$) with the concentration in ppm (mg/L) serving as the basis (X-axis) and the reduction percentage value serving as the ordinate (Y-axis). The calculation yields the IC50 value when the % capacity is 50%.

Anti-inflammatory Activity

500 L of each concentration of the test solution and diclofenac sodium are diluted until the final volume reaches 5 mL with 0.2% BSA. Each concentration will produce 12.5, 25, 50, 100, and 200 parts per million (ppm). The sample was then incubated for 15 minutes at 37 °C. After warming for 5 minutes at 70 °C, the liquid is permitted to cool to room temperature. The solution is vigorously agitated. Shake the agar in the tube so that no clustering occurs; this facilitates reading spectrophotometry and absorbance measurements with a wavelength of 660 nm.^{14,15}

Brine Shrimp Lethality Test (BSLT)

100 μ L of seawater containing 10 shrimp larvae was put into the openings on the test vial, and then 100 μ L of the extract solution was also added so that the concentration of the extract in each vial hole was respectively: 1000; 100; and 10 μ g/mL. For each concentration, three repetitions were carried out. 100 L

of seawater containing 10 shrimp larvae was utilized as a control, along with 100 L of seawater. After 24 hours, the quantity of living and deceased shrimp was determined. Using the Sam approach based on the calculation of the number of dead and living larvae, the BSLT test data were examined. The death or mortality rate (%) is obtained by comparing the number of dead divided by the total number of larvae. The LC50 value is obtained by determining the probit value, namely converting the percent death value to the probit table. Plotting the data between the probit value and the log concentration will obtain the regression line equation given below¹⁶:

$$y = a + bx$$

y = 50 (stated that 50% of shrimp larvae died after an incubation period of 24 hours)

b = slope

a = intercept x = specifies the solution concentration at which fifty percent of the larvae perish

RESULTS AND DISCUSSION

Phytochemical Screening

Phytochemical screening of *Senna alata* L. leaf extracts was carried out as an initial procedure to determine the certain compounds contained in the simplicia studied. One of the important things that main role in the phytochemical screening procedure is solvent for extraction. It is due to the presence of other groups of compounds that affect the solubility of the compounds studied.¹⁷ The outcomes of the phytochemical analysis of *Senna alata* L. leaf extracts by maceration (A), reflux (B), and UAE (C) techniques are shown in Table-1.

Table-1: Phytochemical Screening of *Senna alata* L. Leaf Extracts

Test	<i>Senna alata</i> L. leaf extracts		
	A	B	C
Alkaloid (Mayer)	(-)No precipitation	(-)No precipitation	(-) No precipitation
Alkaloid (Dragendurf)	(+++) Orange	(+) Orange	(-) Yellow
Alkaloid (Wagner)	(++) greenish brown	(-) Orange	(+) Orange
Flavonoid	(+++) orange	(+) orange	(+) orange
Phenolic	(++) brown	(+) greenish yellow	(++) greenish brown
Terpenoid	(++) red	(++)red at the bottom of the tube	(++) red at the bottom of the tube
Saponin	(+)foam as 1 cm (15 minutes)	(-) no foam	(++) foam as 1 cm (15 minutes)

The leaf extracts of *Senna alata* L. were discovered to include alkaloids, flavonoids, phenolics, terpenoids, and saponins, according to the research. Several biological effects of phenolic compounds, including antioxidant, anti-carcinogenic, inhibitor of alpha-glucosidase activity, anti-inflammatory, and ability to scavenge free radicals, have been examined.^{18,19} Many studies have proven that flavonoid compounds have activity as antioxidants, inhibitors of alpha-glucosidase activity, anti-carcinogenic, cardiovascular, hyperglycemic, anti-inflammatory, antiallergic, analgesic, antibacterial, and antidepressant. Saponins derived from green tea are reportedly anticancer anti-inflammatory, and antioxidants.²⁰

Antioxidant Activity by FRAP Method

The FRAP method is an antioxidant test based on the ability of antioxidants to decrease the Fe^{3+} -2,4,6-tripyridyl-s-triazine complex to Fe^{2+} -2,4,6-tripyridyl-s-triazine, resulting in a color change from colorless to blue.²¹ Antioxidant activity of *Senna alata* L. leaf extracts variants A, B, and C may be assessed with a UV-Vis spectrophotometer at 510 nm and a linear line equation of % reduction is obtained for each concentration of *Senna alata* L. leaf extracts, as depicted in Fig.-1.

Through the linear equation in Fig.-1, the IC 50 value of *Senna alata* L leaf extracts was obtained. Variations A, B, and C respectively were (72.44 ± 1.20) mg/L, (90.34 ± 1.70) mg/L, and (95.33 ± 1.10) mg/L. The IC 50 value quantifies an antioxidant's capacity to neutralize fifty percent of free radicals. Molyneaux (2004) divides antioxidants into five categories: 50 ppm (extremely strong), 50-100 ppm (strong), 100-150 ppm (moderate), 150-200 ppm (weak), and > 200 ppm. (very feeble). The lower the IC50

value of a sample, the higher its antioxidant activity.^{22,23} *Senna alata* L. leaf extracts are classified as powerful antioxidants with antioxidant activity, according to the study.

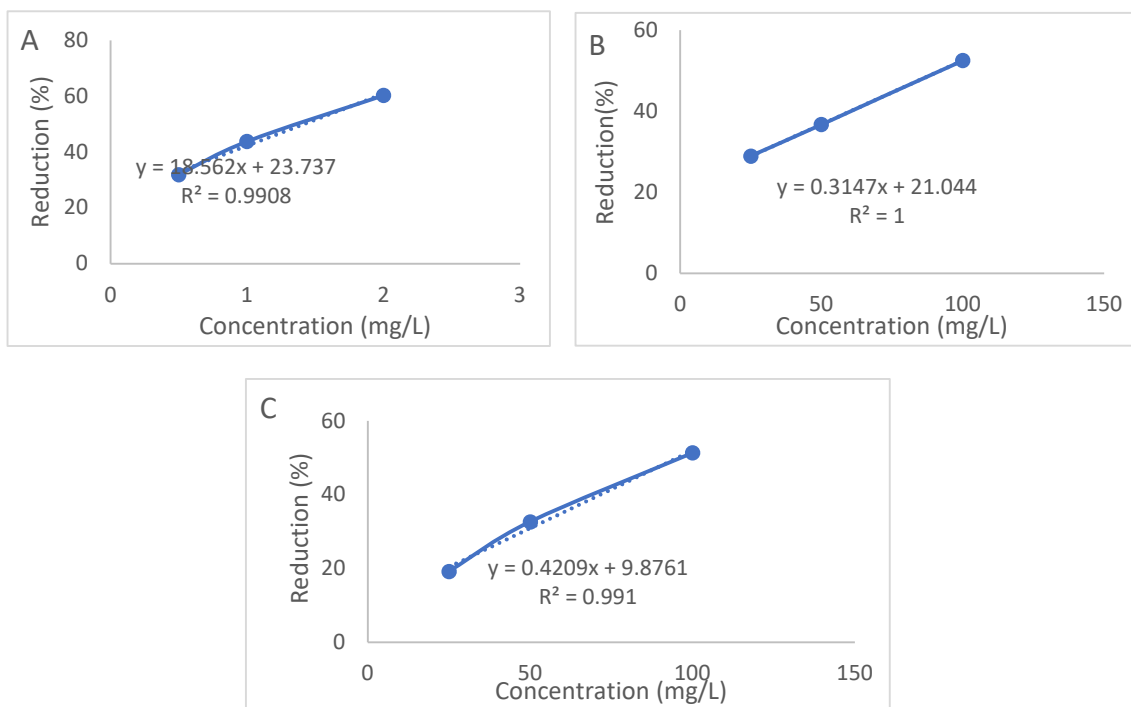


Fig-1: Antioxidant Activity of *Senna alata* L. Leaf Extracts as Determined by the FRAP Method

Anti-inflammatory Activity

Inflammation is a response to damaged or infected tissue. Due to the presence of outside agents or substances, inflammation is a normal process that maintains the body's equilibrium.²⁴ Histamine, prostaglandins, eicosanoids, leukotrienes, cytokines, nitric oxide, and others mediate the inflammatory process. Anti-inflammatory activity testing results are reported as IC₅₀ values. Figure-2 depicts the linear regression equation of percent inhibition to concentration from which the IC₅₀ value can be derived.

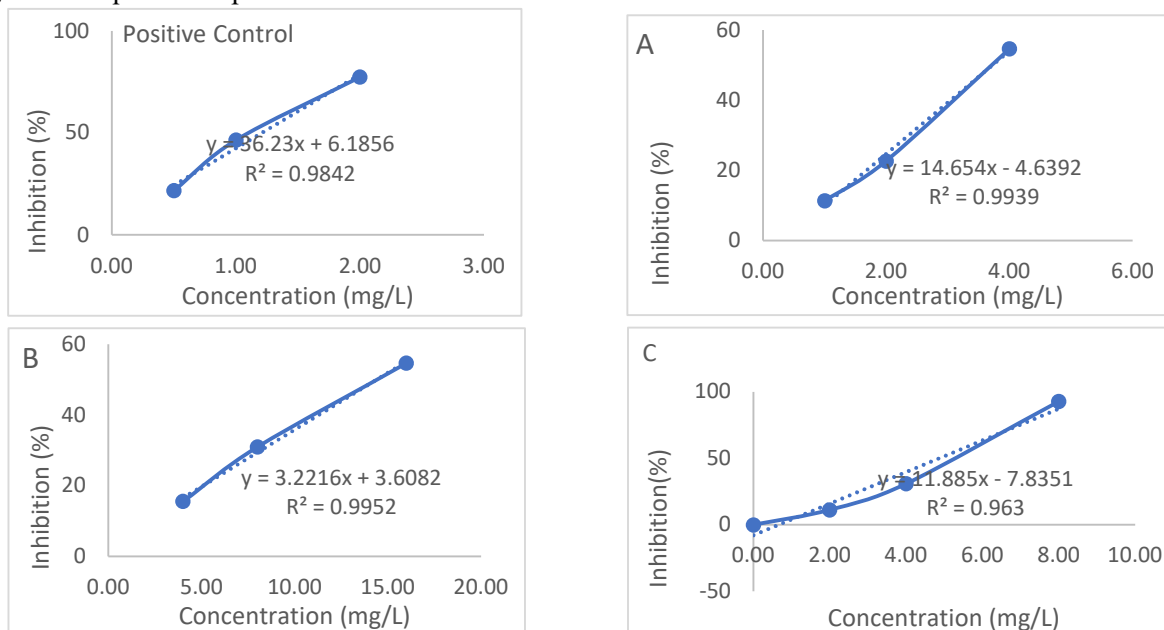


Fig-2: Anti-inflammatory Activity of *Senna alata* L. Leaf Extracts

From the linear regression equation, it was discovered that the IC 50 value of *Senna alata* L. leaf extracts. Variations A, B, and C and the positive control were as shown in Table-2.

Table-2: IC 50 of *Senna alata* L. leaf extracts

Sample	IC 50 (mg/L)
Positive Control	(1.21 ± 0.03)
A	(3.73 ± 0.05)
B	(14.40 ± 0.31)
C	(4.87 ± 0.12)

Diclofenac sodium was employed as the positive control because it is an anti-inflammatory medication.²⁵ The purpose of determining the IC50 value of diclofenac sodium is to quantify its anti-inflammatory activity. According to the research, *Senna alata* L. leaf extracts have an IC 50 value of <50 ppm, indicating a highly potent anti-inflammatory effect.

Brine Shrimp Lethality Test (BSLT)

By calculating the LC50 value of the active chemical, the BSLT method is used to detect the existence of toxic compounds in the process of isolating compounds from natural sources that have a cytotoxic effect.²⁶ LC50 is the concentration of a chemical compound in air or water that can cause 50% death in a population of test animals or certain living things. Meyer (1982) utilized *Artemia salina* Leach to test biological activity in general. This ability to kill shrimp larvae can be used as a quick and simple preliminary test to determine the bioactivity of a compound in vivo.²⁷ The Cancer Institute in the United States was the first to use this method as a preliminary test for anticancer activity.²⁸ BSLT test with *Artemia salina* Leach shrimp larvae was carried out on *Senna alata* L. leaf extracts variations A, B, and C. 10 shrimp larvae were used for each test extract, and the experiment was carried out simply at concentrations of 10, 100, 250, 500, and 1000 ppm. The LC 50 value was derived using the concentration-probit value linear log regression equation. Table-3 obtained the LC 50 value for *Senna alata* L. leaf extracts.

Table-3: LC 50 *Senna alata* L. Leaf Extracts

Sample	LC 50 (mg/L)
A	248.26
B	249.77
C	235.79

According to Meyer *et al.* (1982), the toxicity level of plant extracts can be determined by looking at their LC50 values. If the LC50 value is less than 1000 mg/L it is said to be toxic, otherwise, if the LC50 value is greater than 1000 mg/L it is said to be non-toxic. It shows that *Senna alata* L. leaves extract has an LC50 value of <1000 ppm and is toxic to *Artemia salina* Leach larvae so it is estimated to have anticancer potential.²⁹

CONCLUSION

The leaf extracts of *Senna alata* L. variants A, B, and C were found to contain alkaloids, flavonoids, phenolics, terpenoids, and saponins, according to the research. Antioxidant activity was carried out using the FRAP test with IC 50 values of *Senna alata* L. leaf extract variations A, B, and C were (72.44 ± 1.20) mg/L, (90.34 ± 1.70) mg/L and (95.33 ± 1.10) mg/L, respectively. The anti-inflammatory activity of each *Senna alata* L leaf extract was obtained with an IC 50 value were A(3.73 ± 0.05) mg/L, B (14.40 ± 0.31), and C (4.87 ± 0.12). BSLT was used to establish toxicity by the value of LC50. It was obtained that LC50 value <1000 ppm showed that *Senna alata* L. leaf extracts are toxic to *Artemia salina* Leach larvae so it is estimated to have anticancer potential. The highest toxicity (LC50) was *Senna alata* L. leaf extract by variation C 235.79 mg/L. Variation C is *Senna alata* L. leaf extract using the Ultrasonic Assist Extraction (UAE) method.

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CONFLICT OF INTERESTS

No conflicts of interest have influenced this study, as declared by the authors.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to this manuscript, participated in its review and revising, and gave their approval for publication of the final draught. The ORCID identifiers of the authors can be used to verify their research profiles:

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