

Antioxidant activity and analgesic assessment of *Lansium domesticum* stem bark infusion

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Abstract. *Apridamayanti P, Fajriaty I, Etni Hatita E. 2018. Antioxidant activity and analgesic assessment of Lansium domesticum stem bark infusion. Nusantara Bioscience 10: 71-75.* *Langsat (Lansium domesticum* Correa) is empirically used as a medicine for fever and pain. This study intended to determine the effectiveness of stem bark infusion of *langsat* as an antioxidant and analgesic. Antioxidant activity test was performed by DPPH method (2,2-Diphenyl-1-picrylhydrazil), with infusion at concentration of 1000, 2000, 3000, 4000 and 5000 ppm. Absorbance of the sample was measured using a UV-Vis spectrophotometer. The analgesic test was performed on male Swiss mice by Writhing test using acetic acid 0.6%, CMC-Na induced negative control, Paracetamol 65 mg/kg BW induced positive control, dose I (65 mg/kg BW), dose II (130 mg/kg BW) and dose III (195 mg/kg BW). Data was analyzed using One Way ANOVA in SPSS and the percentage of writhing protection at each dose was calculated. The results of phytochemical metabolites screening of stem bark infusion showed the presence of flavonoids, terpenoids/steroids, tannins, phenols and saponins. Antioxidant activity measured by spectrophotometric measurements showed that the antioxidant activity of stem bark infusion was IC_{50} 2820 μ g/ml. The percentage of writhing protection on dose I, II, and III was 57.52%, 42.48% and 24.51% respectively, showing a significant difference with negative control at minute 5, 10 and 15 ($P < 0,05$). There were no significant differences between positive control and dose I, at minute 30 to minute 60 ($P > 0,05$). The effective dose of stem bark infusion was obtained as an analgesic at dose of 65 mg/kg BW from 30 to 60 minutes.

Keywords: Analgesic, antioxidant, *Lansium domesticum*

INTRODUCTION

Langsat plant, especially its stem bark is empirically used by dayaknese ethnic society in West Kalimantan Province as an antipyretic and analgesic medication. Based on Medicinal Plants and Herb Research in 2015, dayaknese society in Sekajang hamlet, West Kalimantan, used *langsat* stem bark as infused water. The residue of the stewing process is grounded and applied to an injured body part (Sadeli et al. 2015).

The dried *langsat* stem bark in the form of ethanolic extract contains secondary metabolites i.e alkaloids, flavonoids, saponins, tannin, and triterpenoids (Semuel 2008). According to Subandrate et al. (2016), the extract of duku seed with dose of 100 mg/kg BW has an optimum potency as an antioxidant. Duku seed extract was able to increase GSH (glutathione) and lowers MDA (malondialdehyde) in alcohol-induced rats. GSH is a tripeptide having sulfhydryl/thiol (-SH) groups that can counteract free radicals while MDA is the final product of lipid peroxidation by free radicals (Subandrate et al. 2016). Based on research by Mokosuli (2008), the ethanolic extract of raw *langsat* stem bark and its dried counterparts with a concentration of 250 ppm possessed the yield inhibition value of 57.72% and 55.78% respectively. This result was better than the inhibition percentage of BHT as the control group (43.38%) at the same concentration. Butylated hydroxytoluene (BHT) is a lipophilic organic compound (fat soluble), chemically a derivative of phenol, which is a synthetic antioxidant.

Free radical is a molecule, atom or groups of atom with one or more unpaired electron in its outermost orbital (Muchtadi 2013). Free radicals were suspected to be the cause of some diseases including cardiovascular disease, cancer, and aging. More than 60% of new cases and about 70% of death caused by cancer occurred in around the world especially in Africa, Asia, Central and South America (Ministry of Health of the Republic of Indonesia 2015). Adverse effects caused by free radical towards body could be prevented using substances called antioxidants. Antioxidant is a chemical substance, which can donate one or more electron to free radical, thus suppressing it (Kurniawan 2011).

A metabolite compound containing antioxidant properties is being able to inhibit the activity of cyclooxygenase enzyme. Cyclooxygenase is an enzyme, which is responsible for the synthesis of pain mediator, namely prostaglandin. Pain is defined as sensory and emotional feeling of uncomfortable, which is tied to (the threat of) tissue damage (Tjay and Rahardja 2007). In England, it is reported that 17.3 million British people have at least experienced back pain in time, and 1.1 million of them were disabled due to back pain. In Indonesia, the prevalence was 7.6% to 37%. (Koesyanto 2013). The compound, which contains polyphenol and flavonoid, is reported to be able to inhibit cyclooxygenase enzyme and has been proven to have the activity for capturing free radicals (Ebadi 2001). The use of antioxidant-based from nature is mainly developed because of its minimum side effects. Therefore, the objective of this research was to

determine the antioxidant potency of Langsat (*Lansium domesticum* Correa) as one of the native Indonesian plants.

MATERIALS AND METHODS

Extraction

The sample used in this research was the stem bark of langsat (*Lansium domesticum* Correa), which were obtained in the forest area of Sekajang hamlet, Suruh Tembawang village, Entikong district, Sanggau Regency, West Kalimantan. 25 grams of langsat stem bark powder was poured into an infusion pot, and 250 ml of aquadest were added. The mixture was heated for 15 minutes, which was started at the temperature of 90°C, and occasionally stirred. The solution was then sieved using flannel cloth while it was still warm, until no sediment was observed (Rina, et al., 2007)

Phytochemical screening

Phytochemical screenings including alkaloid tests (Dragendorff, Meyer and Wagner test), flavonoid test (Schinoda test), terpenoids and steroid test (Liebermann Burchard test), tannin tests (FeCl₃ and gelatin test), Phenolic test (FeCl₃) and saponins test (bubbling test) were performed on langsat stem bark infusion.

Thin Layer Chromatography (TLC)

Identification using TLC utilizes GF₂₅₄ silica plates. Concentrated langsat stem bark infusion was applied onto the TLC plate using capillary tube. The plate developed in a pre-saturated chamber with mobile phase was then developed until designated borderline. Mobile phase used in this research was optimized by varying the mixture of n-butanol: acetic acid: water (BAW) with ration of 4:1:5. The plate was then dried in open air, and spots were observed under UV 254 nm and UV 366 nm light. AlCl₃ was then sprayed onto the plate to visualize spots on the plate (Rivai 2012).

Antioxidant activity preliminary test

The infusion was applied onto GF₂₅₄ plate using capillary tube. Mobile phase consisted of BAW mixtures in a ratio of 4:1:5. The plate was developed inside a chamber until it was saturated. The plates were sprayed with 20 ppm of DPPH in methanol. The plate was left for several minutes, and the remain spot were observed. Free radical binder substance could be seen as pale yellow spot with purple background after spraying.

Preparation of Infusion

This method was chosen based on the empirical uses by Dayaknese ethnic society in Sekajang hamlet by stewing langsat stem bark with boiled water. Dried langsat stem bark powder was stewed with aquadest in a pot using heating plate for 15 minutes, which was then measured after the temperature of the pot reaches 90°C, while it was stirred occasionally. The solution was sieved through flannel clothes, until no residue was observed.

Antioxidant activity test using UV-Vis Spectrophotometry

25 grams of langsat stem bark infusion in 250 ml of aquadest with 10% concentration as stock solutions were made into series of concentration: 1000 ppm; 2000 ppm; 3000 ppm; 4000 ppm; and 5000 ppm. Each solutions was pipetted for 1ml into several test tube. In every tube, 3 ml of 20 ppm DPPH were added, and aquadest was added up to 5 ml, and the test tubes were incubated at 37°C for 30 minutes. Blanks used were 95% methanol. Absorbance was measured at 515.5 nm wave length. IC₅₀ was calculated separately using regression equation.

Analgesic activity assessment of langsat stem bark infusion

Several mice used in this test were fasted for ±12 h, by continuous supply of water, when oral was used as the route of test substance. Mice were weighed, and randomly grouped into several groups consisting of 5 mice in each group, such as negative control group by giving CMC Na 0,5%; positive control group by giving comparison drug (paracetamol suspension); Dose group 1 administered by langsat stem bark infusion of 65 mg/kg BW; Dose group 2 by giving langsat stem bark infusion of 130 mg/kg BW; and Dose group 3 given by langsat stem bark infusion of 195 mg/kg BW. Thirty minutes after the administration of test substances, 0.6% acetic acid was injected via intraperitoneal. Writhing of the mice was observed every 5 minutes for 60 minutes. Observation was conducted after the induction of pain substance. Writhing data of mice every 5 minutes for 60 minutes was tabulated for each mouse. Analgesic assessment data was analyzed using One Way ANOVA. This research has been approved by ethics committee from Faculty of Medicine, University of Tanjungpura, Pontianak, indonesia ethics commission no 6078/UN22.9/DT/2016.

RESULTS AND DISCUSSION

Phytochemical screening

Phytochemical screening is the first step to identify the chemical content contained in plants, because in this stage we can know the chemical compound group contained in the plant we are testing. The phytochemical screening using test tube method showed that langsat stem bark infusion contained flavonoid, terpenoids and steroids, phenolic, tannin and saponins (Table 1). Alkaloids apparently have nonpolar properties, so that the use of aquadest as polar properties in the infusion preparation of langsat stem bark, resulted in alkaloid could not successfully being extracted from the stem bark.

In order to more identify the metabolite compounds of langsat stem bark infusion, the analysis using thin layer chromatography has been also conducted. Results showed that compound separation in the form of chromatogram pattern showed the spot separation after it has been visualized with or without visualization reagent (spray) in visible light, or ultraviolet light in 254 nm and 366 nm wavelength (Ministry of Health of the Republic of Indonesia 1995). These separated compounds were used in

the form of solution, in order to produce a spot or band. According to Harbone, for flavonoid compounds, BAW mobile phase could be used for visualization of the compounds in TLC (Harborne 1973). Result shows that the sample contains flavonoid, can we see at Figure 1.

Antioxidant activity tests

Mobile phase which is used for antioxidant test using TLC is a mixture of n-butanol: acetic acid: water with a ratio of 4:1:5. The use of this mobile phase is based on optimization using several solvents and ratio. The best separation of compounds was achieved using BAW (4:1:5) mobile phase. From figure 2 the separation of concentrated infusion using BAW resulted in 3 separated spots. Visually, it could be seen that two of the spot was brown, and one of the spots was green. Detection using UV 366 nm light was also conducted to visualize fluorescence spot. The obtained spots might be due to the interaction between UV light and the chromophore paired with auxochrome (Pratiwi et al. 2013). TLC plates were then sprayed with 1000 ppm DPPH solvent. Qualitative antioxidant activity test shows brownish yellow color with purple background. This result was also supported by the results of phytochemical screening, where langsung stem bark infusion contains flavonoid.

The langsung stem bark powder was made into infusion with a concentration of 10% as stock solution. In order to confirm the antioxidant activity, the analysis using UV-Vis Spectrophotometry has been also conducted. Based on visual observation, it can be seen that langsung stem bark infusion sample which has been reacted with DPPH and incubated for 30 minutes had changed color from purple to

washed out purple (Figure 2). The formation of golden yellow color is caused by the picril group in DPPH solution (Molyneux 2004).

The result of antioxidant activity is interpreted as IC_{50} . IC_{50} (50% inhibition concentration) is a minimum concentration of a solution that can inhibit free radical activity up to 50%. The lower IC_{50} is, the higher antioxidant activity of the test substances (Molyneux 2004).

The calculation of inhibitory regression is $y = 0.002x + 44.36$ (figure 3) and percentage resulted in IC_{50} of langsung stem bark infusion at 2820 ppm, table of percentage inhibition stem bark infusion can we see at Table 2. Based on Molyneux (2004) classification, where langsung stem bark ethanolic extract has an IC_{50} of 174.19 ppm, and raw langsung stem bark ethanolic extract has an IC_{50} of 205.38 ppm. The difference in IC_{50} can be as a result of different solvent used, and methods of extraction. The temperature and period of stewing in infusion preparation could result in differences in antioxidant activity (Wassalwa 2016).

Table 1. Phytochemical screening results of langsung stem bark infusion

Tests	Results
Alkaloid	(-)
Flavonoid	(+)
Terpenoids and steroid	(+)
Tannin	(+)
Phenol	(+)
Saponins	(+)

Note: (+) Detected; (-) Not Detected



Figure 1. Results of Flavonoid Chromatogram Pattern using TLC. A. Spots observed under UV 254 nm; B. Spots observed under UV 366 nm; C. Spots observed after being sprayed with $AlCl_3$ 5% in methanol reagent

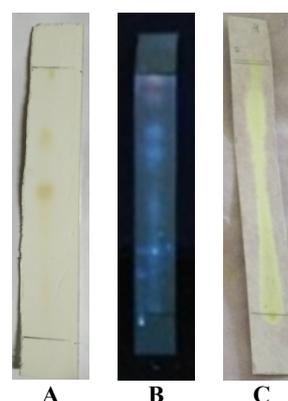


Figure 2. Antioxidant Activity Test using TLC. A. Visual appearances; B. Visualization under UV 366 nm light; C. Visualization after being sprayed with 20 ppm DPPH 20 reagent

Table 2. Inhibition concentration of langsung stem bark infusion resulted from UV spectrophotometer wavelength(λ) 515,1

Concentration (ppm)	Absorbance	% Average inhibition	Linier regression	IC_{50}
1000	0.26339 ± 0.0074	46.82857	$y = 0.002x + 44.36$	2820 $\mu\text{g/mL}$
2000	0.24978 ± 0.0006	49.57607		
3000	0.23710 ± 0.0081	52.13582		
4000	0.22403 ± 0.0151	54.77431		

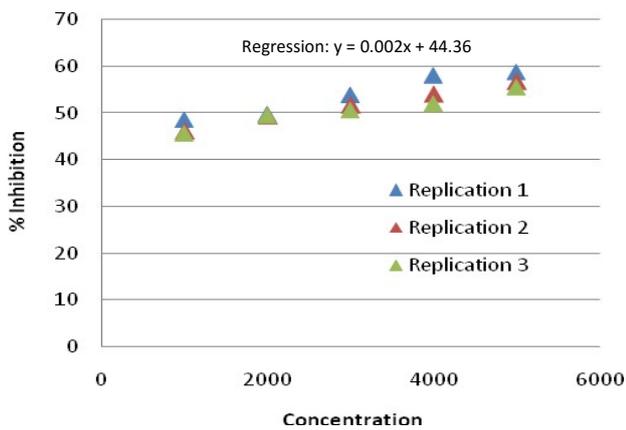


Figure 3. Percentage inhibition langsat stem bark infusion

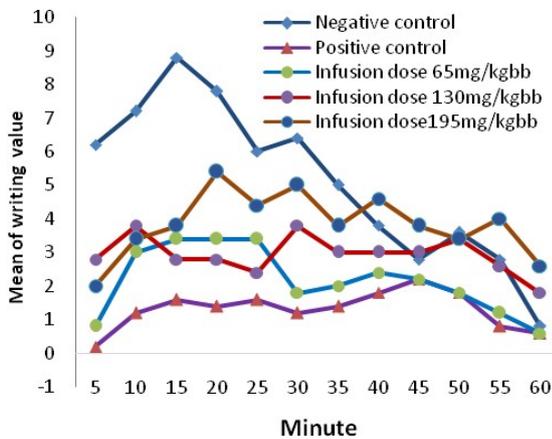


Figure 4. Mean writhing value of mice writhing after induction every 5 minutes over 60 minutes

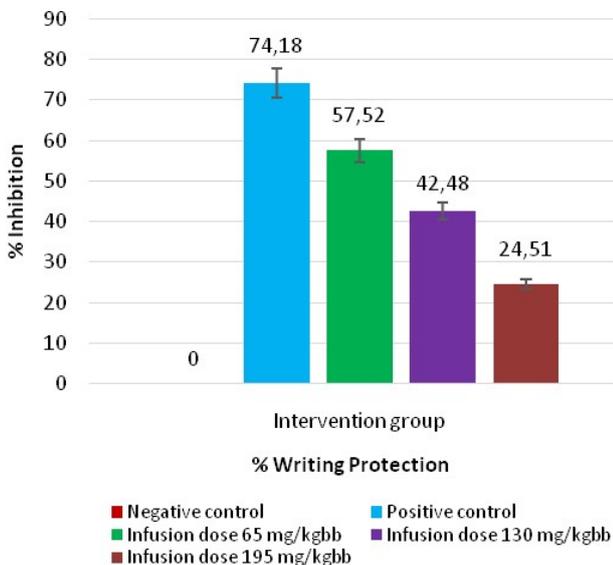


Figure 5. Test substance writhing protection percentage

The compounds suspected of antioxidant activity in plants are phenolic and flavonoids groups. Phenol compounds are antioxidants activity because the compound character has reducing character of hydrogen donors. The antioxidant activity of phenol compounds is determined by the molecular structure, number and position of the hydroxyl groups in the aromatic rings and unpaired electrons in the phenol compound involved in the delocalization of electrons (Lugasi et al. 2003). Flavonoids have antioxidant effects through two mechanisms. The first mechanism is to inhibit the enzymes responsible for the production of superoxide anions, hydroxyl radicals and SORs, such as xanthine oxidase, protein kinase C, cyclooxygenase, lipoxygenase, microsomal mono-oxygenase, glutathione s-transferase, mitochondrial succinoxidase and NADH oxidase. The second mechanism is flavonoid can reduce free radicals such as superoxide radicals, peroxy, alkoxy and hydroxyl by giving hydrogen atoms to a stable quinone structure (Amic et al. 2003).

Analgesic activity assessment of langsat stem bark infusion

Analgesic test in this research used 3 variance dose i.e lower dose 65 mg/kg BW, dose 130 mg/kg BW, and higher dose 195 mg/kg BW. Writhing value in samples treated with variance dose was low than negative control Na CMC 0,5%. Writhing value of sample treated with stem bark infusion at the dose of 65 mg/kg BW had maximal effect compared to those samples treated at the dose of 130 mg/kg BW and dose of 195 mg/kg BW. The dose of 65 mg/kg BW of stem bark infusion showed ineffective performance than that from positive control paracetamol.

Based on the protection percentage graph, it can be seen that the best protection percentage of test substance in reducing mice writhing was achieved from positive control group, followed by dose 1,2 and 3 of langsat stem bark infusion and negative control group. The availability of other secondary metabolites could be synergist or antagonist towards desired effect. Antagonist effect could be potentially disadvantageous due to lowered analgesic effectiveness (Molyneux 2004). Based on the graph, it can be concluded that langsat stem bark infusion had an analgesic effect in dose of 65 mg/kg BW, 130 mg/kg BW and 195 mg/kg BW with writhing protection percentage of 57.52%; 42.48%; and 24.51% respectively. Effective dose of langsat stem bark infusion potential used as analgesic properties was at a dose of 65 mg/kg BW.

Based on data analysis using One Way ANOVA, results showed that inhibition percentage between dose 1 group with the langsat stem bark infusion at dose of 65 mg/kg BW had significant differences compared to that in positive control group (paracetamol, 65 mg/kg BW). This means that dose 1 of langsat stem bark infusion had the lower analgesic effects compared to paracetamol in terms of reducing pain.

In conclusion, based on antioxidant and analgesic assessment of langsat stem bark infusion, it can be concluded that langsat stem bark infusion had a weak

antioxidant properties with IC_{50} of 2820 ppm. Langsung stem bark infusion had analgesic properties with a dose of 65 mg/kg BW, 130 mg/kg BW, and 195 mg/kg BW, with writhing protection at percentage of 57.52%, 42.48%, and 24.51% respectively. Effective dose of langsung stem bark infusion as an analgesic was obtained at the dose of 65 mg/kg BW.

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