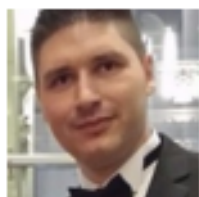


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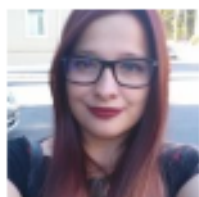
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## Antidiarrheal activity of ethanolic extract of leaves of Sangkareho (*Callicarpa longifolia* Lam) from Central Kalimantan

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### ABSTRACT

Diarrhea is one of the leading causes of preventable death in developing countries including in Indonesia and Malaysia. It has been reported that the leaves of sangkareho (*Callicarpa longifolia* Lam) is used traditionally as an antidiarrheal treatment in Central Kalimantan. The present study was aimed to evaluate the pharmacological activity against diarrhea using the ethanolic extract of sangkareho leaves. The extract was used for castor oil-induced diarrhea in mice. The extracts were given orally to the mice at 200, 300, 400 mg/kg bodyweight and loperamide was used as a standard drug for diarrhea. The results showed % inhibition of diarrhea with values of no detected, 42.62%, 81.97%, 70.49% at 200, 300, 400 mg/kg bodyweight dose levels of the extract and loperamide, respectively as compared to the negative control. It can be considered at a dose level of 400 mg/kg bodyweight the extract has a greater anti-diarrheal effect than loperamide. In conclusion, ethanolic extract of sangkareho leaves (*Callicarpa longifolia* Lam) has anti-diarrheal activity and this supports the use of this plant as antidiarrhea in traditional treatment.

**Keywords:** Antidiarrheal; *Callicarpa longifolia*; Central Kalimantan; Loperamide; Sangkareho.

### 1. INTRODUCTION

According to the WHO and UNICEF reports, 1.9 million children below 5 years of age die because of diarrhea each year, of who most are from developing countries [1]. Diarrhea is one of the public health problems in Indonesia and Malaysia, based on data from the Indonesia health profile in 2010, the number of diarrhea cases found was around 213.435 patients with 1.289 deaths and 70-80% of these occurred in children especially those under the age of 5 years [2], Indonesia has the second highest number of people (54 million) in the world that practice open defaecation, this increase health problems especially diarrhea because of poor quality of water and sanitation [3]. Although in Malaysia enjoys a high coverage of treated water, diarrhea episodes among children are still reported, based on the 2006 National Health and Morbidity Survey (NHMS), the overall prevalence of diarrhea in children below 5 years old in Malaysia was 4.5%, detailed analysis of the NHMS done in 2011 found that children below 5 years old were 33% higher compared to 5-9 years old aged group [4], from these data it can be estimated that over the next 20-30 years diarrhea will be the center of attention in Indonesia and Malaysia. Infection like diarrhea is primarily associated with enteric bacteria most common of which is *Escherichia coli* [5, 6, 7].

### 2. MATERIALS AND METHODS

**Drug and Chemicals.** Loperamide HCl 2 mg, Castor oil and CMC Na 1%.

**Preparation of Plant Extract.** The fresh leaves of Sangkareho were used as plant materials. The leaves were dried under the sun

During the 20<sup>th</sup> century, due to increasing resistance to antibacterial agents, the side effects and the cost of treatments, the interest in traditional medicine and herb has been progressively increased [8-9]. WHO has encouraged studies for treatment of diarrheal conditions, therefore, as alternative medicinal plants were needed in discovering and developing for new anti-diarrhea agents [10]. Central Kalimantan is one of the islands in Indonesia that have varieties of traditional plants. Sangkareho (*Callicarpa longifolia* Lam) is one medicinal plants in Central Kalimantan (Fig. 1), that locals used against diarrhea, dengue, and gastric disorders. Therefore, the present study was aimed to evaluate the antidiarrheal activity of Sangkareho (*Callicarpa longifolia* Lam) in experimentally induced acute diarrhea in mice.



Figure 1. Sangkareho leaves (*Callicarpa longifolia* Lam).

for 5-7 days. The leaves were crushed by a grinder. A total of 500 g of powder was extracted with 96% ethanol by using a percolator and the extract was concentrated in a rotary evaporator.



**Experimental Animals.** Adult male swiss albino mice with age of 2-3 months, weighing between 20-50 g were used for experiment. The mice were obtained from the Animal Unit of Faculty of Mathematics and Science Lambung Mangkurat University. The animals were housed in a proper plastic cages at room temperature and acclimatized for a week before the commencement of dosing. During the entire period of the study, the animals were supplied with standard pellet diet and water *ad libitum* [11].

**Animal grouping and dosing.** Animals were randomly divided into five groups (negative control, positive control and three treatment groups) comprising of three animals in each group. Negative control group received the CMC Na 1% and positive control group received the loperamide (2 mg usual dosage for human) at 0.0052 mg/20 g of bodyweight. The treatment groups received different doses levels of sangkareho leaves (200, 300 and 400 mg/kg respectively).

**Preliminary Phytochemical Screening.** Qualitative phytochemical screening test was carried to determine the presence or absence of alkaloid, tannins, flavonoid, saponin, steroid in the 96% ethanolic leaf extract of (*Callicarpa longifolia* Lam) [12,13,14].

### 3. RESULTS

Sangkareho leaves (*Callicarpa longifolia* Lam) according to Purukcahu (Kabupaten Murung Raya, Central Kalimantan) community is effective as an anti-diarrheal however research on sangkareho as anti-diarrheal has not been widely studied. Based on some conducted phytochemical studies in Indonesia sangkareho (*Callicarpa longifolia* Lam) contains alkaloid, flavonoid, tanin, saponin and steroid [20-22]. Our preliminary phytochemical screening on *Callicarpa longifolia* has confirmed the presence of alkaloid, flavonoid and steroid (Table 1). Flavonoids that exist in the extracts could play an important role, and the phenolic compounds have several pharmacological properties such as anti-diarrheal activities and spasmolytic. The alkaloid and steroid have been reported to have antibacterial and antifungal which can help in treating diarrhea [23-24]. Flavonoid, tannin and alkaloid are the major constituents that are primarily responsible for antidiarrheal activity with a specific mechanism [25].

Isolation and identification of flavonoid compounds from sangkareho leaves have been studied by Pasaribu *et al* the group of flavonoids derived from the ethyl acetate fraction of sangkareho leaves (*Callicarpa longifolia* Lam) was thought to be a flavonol group, flavonoid compounds have a very high level of toxicity against *Artemia salina* leach indicated by the LC50 value of 26.8824 ppm and indicate that the isolates have potential for a high bioactive compounds [26].

This study aims to evaluate anti-diarrheal activity of sangkareho leaves by inducing diarrhea using castor oil. Recent study claims that nitric oxide in castor oil is responsible for the diarrheal effect, there are several mechanisms proposed to explain the diarrheal effect of castor oil one of which is inhibition of

**Castor Oil Induced Diarrhea.** Adult male swiss albino mice were fasted for 18 h with free access to water grouped and treated as described under the grouping and dosing section. One hour after treatment, diarrhea was induced by giving oral administration of 1 ml castor oil to each mouse. The animals were housed in a separate transparent plastic cage in which the floor is lined with white paper. The paper was changed hourly every hour for a total of four hours. During the observational period, the onset of diarrhea, number and weight of wet stools, total number and the total weight of faeces were recorded for 4 hours after the administration of castor oil. The percentage of diarrhea inhibition was calculated with wet faeces using the published formula [11, 15-17].

$$\% \text{ Inhibition} = (\text{control} - \text{test}) / \text{control} \times 100\%$$

**Statistical analysis.** The experimental results were analyzed using the Statistical Package for the Social Sciences (SPSS) version 20.0 software. All the experimental data are expressed as mean  $\pm$  standard error of means. Statistical significance of differences between groups was assessed by one-way ANOVA followed by post-hoc Tukey's multiple comparison test. The results were considered significant at  $p$ -value  $< 0.05$  [18-19].

intestinal  $\text{Na}^+ \text{K}^+$  ATPase activity, consequently reducing normal fluid absorption. Usually, castor oil is metabolized into ricinoleic acid in the gut, which causes irritation and inflammation in the intestinal mucosa, resulting in the release of inflammatory mediators (prostaglandins). The released prostaglandins initiate vasodilation, smooth muscle contraction and mucus secretion in the small intestines, in experimental it can be considered to be good diarrheagenic agents [27].

In this study compared to the negative controls, the three dose levels of ethanolic extract *Callicarpa longifolia* leaves significantly inhibited castor-oil induced enteropooling in mice at dose levels of 200, 300, 400 mg/kg bodyweight of extract, respectively. The percent inhibition of diarrhea calculated for the positive control (loperamide) was 70.49% (Table 2.). At 200, 300, 400 mg/kg bodyweight dose levels of the ethanolic extract, the means weight of faeces were  $5.16 \text{ g} \pm 0.025$ ,  $1.05 \text{ g} \pm 0.015$  and  $0.33 \text{ g} \pm 0.031$ , respectively observed for 4 hours. At dose level of 200 mg/kg bodyweight of the ethanolic extract did not show inhibitory effects as compared to negative control ( $1.83 \pm 0.021$ ) and has a higher number of faeces weight ( $5.16 \pm 0.025$ ). The highest dose level of the plant extract (400 mg/kg bodyweight) showed a more significant reduction of faeces weight and has a percent inhibition of diarrhea 81.97, higher than loperamide (70.49%). The leaves of sangkareho (*Callicarpa longifolia* Lam) contain alkaloid and flavonoids which may be responsible for its pharmacological antidiarrheal activity. Based on the study by Russo *et al* oral administration of tannins flavonoids seems safe and effective treatment in shortening the duration of acute gastroenteritis [28].

**Table 1.** Preliminary phytochemical composition of ethanolic leaf extract of *Callicarpa longifolia* Lam.

Test	Alkaloid	Flavonoid	Saponin	Tanin	Steroid	Tanin
Inference	+	+	-	-	+	-

+ = Presence; - = Absence

Table 2. Effect of ethanol extract *Callicarpa longifolia* Lam leaves on castor oil induced diarrhea in mice.

Groups	Onset (minute)	Weight of faeces (g) ± SD	% inhibition of diarrhea
Negative control (CMC na 1%)	10.44 ± 0.663	1.83 ± 0.021	-
Positive control (loperamid)	7.49 ± 0.465*	0.54 ± 0.025*	70.49
ES 200 mg/Kg	11.42 ± 0.526	5.16 ± 0.025*	-
ES 300 mg/Kg	8.90 ± 0.344*	1.05 ± 0.015*	42.62
ES 400 mg/Kg	10.50 ± 0.600	0.33 ± 0.031*	81.97

\**P* < 0.05 vs control negative

#### 4. CONCLUSIONS

In conclusion, the ethanolic extract of sangkareho leaves (*Callicarpa longifolia* Lam) has an antidiarrheal activity as revealed by reductions weight of faeces. Hence, this study supports the use of the plant in the treatment of diarrhea in the

traditional settings, but further studies are also required to identify the phytoconstituents responsible for these pharmacological activity and to establish the mechanism of action of antidiarrheal activity.

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