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Research Article

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Phytochemical Screening, Antioxidant Activity, and Acute Toxicity Evaluation of *Physalis angulata* L. Leaf Infusion

Arindina Mahendra[1*], Aryani Sismin Satyaningtijas[2], Andriyanto[3], Ronald Tarigan[2]

[1]Graduate School of Veterinary Biomedical Science, School of Veterinary Medicine and Biomedical Science, IPB University, Indonesia
[2]Division of Physiology, School of Veterinary Medicine and Biomedical Sciences, IPB University, Indonesia
[3]Division of Pharmacology and Toxicology, School of Veterinary Medicine and Biomedical Sciences, IPB University, Indonesia

*Corresponding author: arindina.arindina@apps.ipb.ac.id

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ABSTRACT

Background: *Physalis angulata L. (ciplukan)* is an herbal plant rich in secondary metabolites and has antioxidant activity. Antioxidant activity of this plant is commonly the reason why people of many Asian countries use this plant as traditional medicine or a supplement through the infusion/tea brewing method.

Aims: This study aims to be an exploratory study to identify the metabolite molecules that are extracted using the infusion method and their acute toxicity.

Methods: Simple phytochemistry screening tests are conducted to identify the secondary metabolites composition of ciplukan leaf infusion. Antioxidant activity using DPPH radicals was conducted to obtain the inhibitory concentration (IC50) value of the solution. Twenty-five female mice were divided into 5 groups: a control group and ciplukan leaf infusion with doses 1, 5, 10, and 15 g/kg BW. Infusion was administered on day one, while the mortality rate and toxicity symptoms were observed in 14 days.

Results: Ciplukan leaf infusion contains alkaloids, flavonoids, and tannins, which are all successfully extracted by water as a polar solvent. This solution has an IC50 DPPH value of 106.81 µg/ml, which is categorized as a moderately active antioxidant extract. Acute toxicity test showed no mortality and toxicity symptoms in each group, resulting in an LD50 value of more than 15 g/kg BW.

Conclusion: Ciplukan leaf infusion as a polar extract successfully extracted polar molecules alkaloid, flavonoid, and tannin which have moderate antioxidant activity. This infusion showed no mortality up to 15 g/kg BW.

INTRODUCTION

Physalis angulata L., also known as ciplukan, is an herbal plant that has been used as traditional medicine in many Asian countries. Ciplukan was used as an alternative medicine and daily supplement for decades until recently, to treat various symptoms and diseases. This plant is known to have various pharmacological activities, such as anti-inflammatory, antioxidant, antipyretic, and many others (Rohmawaty et al. 2021). The main pharmacological feature of this plant is its strong antioxidant activity. Antioxidants have an important role in the body since it neutralizes pro-oxidant molecules such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), which are produced during metabolic processes (Devitria et al. 2020). Most of the pro-oxidant molecules are very reactive because it has an unpaired electron, that it could react and form a bond with other molecules in the cell. Physiologically, a cell has a defense mechanism against pro-oxidants by producing its own antioxidant (endogenous antioxidant). The production of pro-

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oxidant molecules will be stimulated during most degenerative diseases and inflammatory responses. Those conditions were caused by oxidative stress, which is an imbalance in the production between oxidants and antioxidants that leads to tissue injury and organ dysfunction (Kahveci et al. 2022). Stress oxidative, which is associated to the cause of many diseases, could be resolved by consuming exogenous antioxidants, which could be found in Ciplukan.

Traditional herbal medicine remains an important component of healthcare worldwide, with approximately 80% of the global population relying on herbal remedies for primary healthcare. In Indonesia, traditional herbal medicine is commonly consumed in the form of *jamu*, which consists of various combinations of indigenous medicinal plants, as well as herbal infusions prepared for daily supplementation (Anggun 2023). Their popularity is largely attributed to their affordability, accessibility, and perceived safety.

Medicinal plants produce a wide range of secondary metabolites that exhibit diverse pharmacological activities and serve as valuable sources of bioactive compounds for drug development. These metabolites also play essential roles in plant defense mechanisms against pathogens, insects, herbivores, and extreme environmental conditions (Ciocan et al. 2023, Yang et al. 2018). The composition of these metabolites in an herbal medicine is based on several factors, which are environmental factors before harvesting and how it was extracted after harvesting the plant (Zhang et al. 2021).

Although ethanolic and methanolic extracts of ciplukan have been extensively investigated, studies examining the phytochemical composition, antioxidant potential, and acute oral safety of water-based infusions prepared to mimic traditional consumption remain limited. Aqueous infusions are the most common preparation used in traditional practice, so their phytochemical characteristics, biological activity, and safety profile should be evaluated independently rather than extrapolated from organic solvent extracts. Therefore, this study aimed to characterize the phytochemical profile, evaluate the antioxidant activity, and determine the acute oral toxicity (LD50) of ciplukan leaf infusion prepared according to traditional practices.

MATERIALS AND METHODS

Research Preparation

This research was conducted from December 2025 to January 2026 at the Laboratory Animal Management Unit (UPHL), and Physiology Laboratory, School of Veterinary Medicine and Biomedical Sciences, IPB University. All animal procedures were approved by the Animal Ethics Committee of School of Veterinary Medicine and Biomedical Science under approval number 320/KEH/SKE/IV/2025.

Physalis angulata Extraction

Physalis angulata L. leaves were obtained from Balai Pengujian Standar Instrumen Tanaman Rempah, Obat, dan Aromatik (BPSI TROA) Bogor, Indonesia, which have been identified by Badan Riset dan Inovasi Nasional (BRIN) No. B-1070/II.6.2/IR.01.02/3/2026. The leaves were dried at 50C for 3 days and were blended into a simplicial powder. The infusion method was done by brewing simplicial powder in hot water (± 90C) (1:1 w/v) for 15 to 20 minutes (Ministry of Health of the Republic of Indonesia 1995), and then the solution was filtered using filtered paper, resulting in a 100% w/v ciplukan leaf infusion. The infusion (100% w/v infusion) was stored in a sealed container at 4C in a refrigerator until use. To ensure its stability and quality, the infusion was used within 3 days of preparation.

Phytochemistry and Antioxidant Activity Tests

A screening qualitative phytochemistry test was done to identify the secondary metabolites that were successfully extracted from the ciplukan leaves. Antioxidant activity test was performed using DPPH (2,2 – diphenyl – 1 – picrylhydrazyl) method to obtain the inhibitory concentration (IC50) value, the concentration of the sample required to inhibit 50% of DPPH radicals. Both tests were performed in triplicate. The absorbance of the mixture containing the infusion sample and DPPH solution was measured at a wavelength of 517 nm. The infusion was tested at concentrations of 5, 10, 50, 100, and 200 µg/mL, using ascorbic acid as the positive control. The percentage of inhibition was calculated using the following equation:

$$Percentage\ of\ inhibition\ (\%) = \frac{(Absorbance_{Control} - Absorbance_{sample})}{Absorbance_{control}} \times 100$$

Acute Toxicity Test

An acute toxicity test was performed according to the method by the Organization for Economy and Co-operative Development No. 420 (OECD 2002) and the Indonesian Food and Drug Authority (BPOM 2022) to obtain the LD50 (lethal dose value) of the extract. Twenty-five female 8-week-old ddY mice (25 – 30 g) were divided into 5 groups: control group and ciplukan leaf infusion dosages: 1, 5, 10, and 15 g/kg BW. The dosage used in the toxicity test was according to a previous toxicity test by Ayun et al. (2021) and Kasolo et

al. (2011), referring to Miller & Tainter (1994). The mice were housed under standard laboratory conditions at a temperature of 22–25 °C, relative humidity of 50–70%, and a 12 h light/12 h dark photoperiod, with free access to standard feed and drinking water. Ciplukan leaf infusion was administered on the first day using gastric lavage; doses greater than 1 ml were administered several times within 24 hours. Mice were weighed on days 0, 7, and 14, while the toxicity symptoms and mortality were observed for 14 days.

Data Analysis

The IC50 value of the infusion sample was determined from the linear regression equation obtained by plotting the percentage of inhibition against the sample concentration. The mortality rate data were analyzed using the logit regression method to obtain LD50, while the weights were analyzed using the one-way ANOVA method and followed by Tukey's post-hoc test using IBM SPSS Statistics 26.

RESULTS AND DISCUSSION

Phytochemistry and Antioxidant Activity

Secondary metabolites composition and its antioxidant activity are very important to analyze the quality of an herbal medicine, because its composition will be affected by the selection of solute and its extraction method (Ramphinwa et al. 2023). The infusion method of herbal plants is widely used because it is simple, relatively cheap, and easy to make. On the other side, the methodological process of producing this infusion could cause metabolite losses based on the variety of volume and quality of water, and also the temperature and duration of brewing (Ramphinwa et al. 2023). The phytochemical screening of ciplukan leaf infusion is represented in Table 1.

Alkaloid, flavonoid, and tannin were successfully extracted from the ciplukan leaf infusion, which have similar properties. Those three metabolites and saponin were semi-polar to polar molecules, while steroids and terpenoids are non-polar molecules (Rohmawaty et al. 2021). Water is a polar solvent, which extracts polar molecules, such as alkaloids, flavonoids, and tannins. Previous studies by Pillai et al. (2022) and Donn et al. (2024) reported that ciplukan leaf ethanolic and methanolic extracts (semi-polar solvent) consisted of high flavonoid content, while non-polar solvent extraction, such as the ethyl-acetate fraction and n-hexane fraction of an herbal plant, most likely extracted more of steroids and terpenoids. The ciplukan leaf had gone through a heating process during this infusion method; meanwhile, most of the flavonoids, saponin, tannin, and terpenoids are heat-labile (stable at 60 – 70C), which could be damaged during the brewing process (Rohmawaty et al. 2021). Consequently, the flavonoids, alkaloids, and tannins contents, which were successfully extracted by this infusion, are the heat-stable type of molecules.

Antioxidant activity of the infusion was measured by quantifying the concentration of infusion needed to inhibit 50% (IC50) of DPPH radicals. The IC50 value of ciplukan leaf infusion is presented in Table 2, which, based on Molyneux (2004), was categorized as moderate antioxidant activity. DPPH antioxidant assay aims to analyze the ability of the solution to act as an electron or hydrogen donor against DPPH radicals. The antioxidant activity of this infusion is the result of the radical scavenging activity of flavonoids, alkaloids, and tannins contained in this infusion. Flavonoids (quercetin) in ciplukan have five hydroxyl groups from their phenolic compound, which allows them to be a hydrogen donor (Boo et al. 2025). Alkaloids could also

Table 1. Secondary metabolites and antioxidant activity in ciplukan leaf infusion

Parameters	Method Specification	Result
Alkaloid: Mayer, Wagner, Dragendorf	Qualitative	Positive (+)
Flavonoid	Qualitative	Positive (+)
Steroid and Terpenoid	Qualitative	Negative (-)
Saponin	Qualitative	Negative (-)
Tannin	Qualitative	Positive (+)

act as an electron or hydrogen donor from their amino group or phenolic compound (Wasityastuti et al. 2025). Meanwhile, tannins are polyphenolic compound which contains hydroxyl groups (Rohmawaty et al. 2021).

Previous studies have reported IC50 DPPH of various extracts of ciplukan, which is an ethanolic extract of ciplukan leaf 58,12 µg/ml (Olusola and Adeseye 2020), n-hexane fraction is 793,91 µg/ml, and the ethyl acetate fraction is 407,91 µg/ml (Sari 2018). Ciplukan leaf infusion has weaker antioxidant activity than ethanolic extract, but still has stronger antioxidant activity than n-hexane fraction and ethyl acetate fraction. This correlates to the polarity of the solvents used in the extract, with the ethanolic extract (semi-polar solvent) having the strongest antioxidant activity and non-polar solvent extraction having the weakest antioxidant activity.

This could happen because most of the alkaloids, flavonoids, tannins, and saponins that act as antioxidants were mostly semi-polar and heat-labile molecules, which could only be extracted using semi-polar solvents without going through high-temperature extraction processes. This experimental study only performed a qualitative phytochemical screening, so the contribution of each metabolite class to its antioxidant activity could not be quantitatively determined.

Acute Toxicity Test (LD50) of Ciplukan Leaf Infusion

Table 3 showed that there were no toxicity symptoms or mortality in each group during 14 days of observation. According to Organisation for Economy Co-operative and Development No. 420 (OECD 2002), this result is categorized as a practically non-toxic solution. There was no mortality in any of the experimental groups; therefore, LD50 could not be numerically calculated and was considered to be greater than 15 g/kg BW. Previous studies reported on the acute toxicity test of ciplukan in ethanolic extract (Sunday and Ilesanmi 2023) and methanolic extract (Rathore et al. 2011), were also practically not toxic.

Body weight gain on day-7 and day-14 didn't show any significant differences among different groups (Table 3). Those results indicate that there were no systemic toxicity effect, and all groups showed stable weights. Organ relative weight is used to analyze targeted organ toxicity from administering ciplukan leaf infusion. Table 4 showed no significant differences in the relative weights of the heart, lungs, and kidneys among different groups. Interestingly, 10 g/kg ciplukan leaf infusion showed the highest relative weight of liver and spleen without any macroscopic changes. Several studies have reported that the ethyl acetate fraction of ciplukan (Sylviningrum et al. 2025) and ciplukan root ethanolic extract (Nwankwo and Oluka

Table 2. Antioxidant activity of ciplukan leaf infusion

Linear Regression Analysis	Positive Control	Ciplukan leaf infusion
Linear Equation	$y = 18.6270\,x - 15.5590$	$y = 0.4486\,x + 2.0831$
Coefficient of Determination (R^2)	0.9983	0.9974
IC50 Value	3.52 µg/ml	106.81 µg/ml

Table 3. Toxicity symptoms, mortality, and body weight on day-7 and day-14

Dosage (mg/kg BW)	Toxicity Symptoms	Mortality	Body Weight	
			H-7 (g)	H-14 (g)
Control	- (0/5)	- (0/5)	28 ± 0.3^a	29 ± 0.4^a
1,000	- (0/5)	- (0/5)	24 ± 0.3^a	23 ± 0.2^a
5,000	- (0/5)	- (0/5)	28 ± 0.1^a	29 ± 0.2^a
10,000	- (0/5)	- (0/5)	28 ± 0.2^a	29 ± 0.4^a
15,000	- (0/5)	- (0/5)	31 ± 0.2^b	31 ± 0.5^b

Different superscripted letters indicate significant differences ($p<0.05$)

Table 4. Organ relative weight to body weight

Dosage (mg/kg BW)	Heart (%)	Lungs (%)	Liver (%)	Kidneys (%)	Spleen (%)
Control	0.52 ± 0.23^{a}	0.82 ± 0.05^{a}	5.10 ± 0.58^{a}	1.04 ± 0.17^{a}	0.92 ± 0.30^{ab}
1,000	0.52 ± 0.13^{a}	0.74 ± 0.05^{a}	4.18 ± 0.34^{a}	1.08 ± 0.19^{a}	0.56 ± 0.17^{a}
5,000	0.42 ± 0.13^{a}	0.72 ± 0.01^{a}	5.22 ± 1.23^{a}	1.04 ± 0.15^{a}	0.80 ± 0.19^{ab}
10,000	0.54 ± 0.29^{a}	0.82 ± 0.08^{a}	6.40 ± 0.46^{b}	1.26 ± 0.23^{a}	0.97 ± 0.82^{ab}
15,000	0.48 ± 0.08^{a}	0.92 ± 0.36^{a}	5.32 ± 0.33^{a}	1.36 ± 0.21^{a}	0.80 ± 0.05^{ab}

Different superscripted letters indicate significant differences ($p<0.05$)

2023) has acute subclinical liver, kidney, and spleen toxicity, while ciplukan ethanolic extract has subacute liver toxicity (Lestiariani et al. 2023).

A study of the acute toxicity of the ethyl acetate fraction of ciplukan by Sylviningrum et al. (2025) reported that the earliest damage happened in the spleen, which is reflected by multifocal haemorrhage and necrosis; meanwhile, the damage in the liver showed multifocal lipid degeneration as well as hydropic degeneration and haemorrhage. Both organ damages reported by Sylviningrum et al. (2025) showed an early stage of hepatocellular injury, which is mainly caused by a dose-dependent pro-oxidant effect of physalins and flavonoids. Flavonoids as antioxidant agents, stabilize superoxide (O_2^-, ROS) into hydrogen peroxide (H_2O_2, ROS), but when consumed in high concentrations, they could accumulate hydrogen peroxide, which undergo fenton reaction and produces hydroxyl radical molecules (Sylviningrum et al. 2025). Physalin is a marker bioactive compound found in ciplukan, which has an anti-inflammatory effect by stimulating the vasoprotective mediator nitric oxide. Nitric oxide (NO) as a reactive nitrogen species (RNS) could bind superoxide (ROS) to produce peroxynitrite (RNS), which could cause more serious damage (Febianti et al. 2019). Those pro-oxidant effects could cause oxidative stress in cells and result in mitochondrial, DNA, and membrane cellular damage (Sylviningrum et al. 2025).

Nevertheless, a subclinical toxicity in the liver or the spleen could only be confirmed through further tests such as a complete blood count test, a comprehensive blood biochemistry test, and histological analysis. This research is an explorative study that is limited to analyzing only the LD50 value and systemic acute toxicity of ciplukan leaf infusion without any further tests. Although physically and macroscopically there were no changes in the body and organs, further tests should be done in order to confirm the subclinical organ toxicity at a specific dose. Despite there being no further tests, this infusion showed no acute mortality or overt clinical toxicity up to 15 g/kg BW under the present experimental conditions.

CONCLUSION

Water, as a polar solvent used in the infusion method, is able to extract alkaloids, flavonoids, and tannins of ciplukan leaves. This infusion also has moderate antioxidant activity with an IC50 DPPH value of 106.81 µg/ml. Ciplukan leaf infusion showed no systemic toxicity symptoms and mortality up to 15 g/kg BW.

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AUTHORS CONTRIBUTION

AM: investigation, writing – original draft, formal analysis; ASS: supervision, conceptualization, writing – review & editing; AA: conceptualization, methodology, supervision, validation; RT: supervision, data curation, conceptualization.

"The authors declare that there is no conflict of interest with the parties involved in this research".

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