



Protective effects of *Cyclea barbata* Miers leaves against aspirin-induced gastric ulcer in mice

Iskandar Muda Siregar* and Isnatin Miladiyah*

ABSTRACT

*Department of Pharmacology,
Medical Faculty,
Islam Indonesia University
Yogyakarta

Correspondence

dr. Isnatin Miladiyah, M.Kes.
Department of Pharmacology,
Medical Faculty,
Islam Indonesia University
Jl. Kaliurang Km 14.5 Sleman
Yogyakarta 55564
Phone: 0274-898444 ext. 2002
Email: isnatin@fk.uui.ac.id

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One side effect of non-steroidal anti-inflammatory drugs is gastric mucosal irritation, possibly causing gastric ulcers. The aim of this study was to evaluate the protective effect of cincau leaves (*Cyclea barbata* Miers) on aspirin-induced gastric ulcer in Balb/c mice. Twenty five Balb/c mice (20-30 g, 2-3 months old) were randomly divided into 5 groups. Group I-III were given cincau leaf infusion at dosages of 2.5 mg/kg BW, 5 mg/kg BW, and 10 mg/kg BW, respectively, while group IV (positive control) received antacid at a dosage of 20 mg/kg BW, and group V (negative control) one milliliter of distilled water. All interventions were given by the oral route, once daily for seven days. On day 7, the mice were given aspirin (600 mg/kg BW) to induce gastric ulcer. After 30 minutes, all mice were sacrificed, and their stomachs examined macroscopically for gastric ulcer, characterized by the presence of ulcer(s) and bleeding. Total ulcer scores were analyzed by one-way Anova to compare between-group protective effect of interventions against aspirin-induced gastric ulcer. Results showed that groups treated with cincau leaf infusion at all dosages experienced a gastric ulcer protective effect. There were significant differences ($p=0.002$) between treatments, compared to the negative control, but no significant differences ($p>0.05$) when compared to the positive control. Thus cincau leaves (*Cyclea barbata* Miers) at dosages of 2.5 mg/kg BW, 5 mg/kg BW, and 10 mg/kg BW, had a protective effect against aspirin-induced gastric ulcer in mice. Higher dosages of cincau leaf infusion have a correspondingly higher gastric ulcer protective power.

Keywords : *Cyclea barbata* Miers, aspirin-induced gastric ulcer, mice

INTRODUCTION

Gastric ulcer is one of the intestinal disorders that occur frequently due to an imbalance between offensive factors (gastric

acid secretion) and defensive factors (gastric mucosal defense).⁽¹⁾ Aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) may induce these ulcers through inhibition of

prostaglandin biosynthesis, reduced gastric blood flow, local irritation, and disruption of local tissue restitution and repair.⁽²⁾

The cincau plant (*Cyclea barbata* Miers) belongs to the *Menispermaceae* family and is traditionally utilized by many Indonesian communities as a refreshing beverage in the form of a gel or infusion. Some communities in Indonesia also use the infusion as medication against cold. Cold is frequently felt to be a digestive disorder, with the complaints of sprue, diarrhea, epigastric fullness, and reflux of gastric acid into the pharynx.⁽³⁾

It is the tannin content of cincau leaves that is believed to be beneficial for abdominal pain. In addition, this plant contains various bis-benzyl-isoquinoline alkaloids, i.e. berbamine, chondocurine, alpha and beta cyclanoline, fangchinoline, homoaromoline, isochondocurine, isotetrandrine, lemacine, and tetrandrine,⁽⁴⁾ as well as the minerals calcium and phosphorus, and vitamins A and B.⁽⁵⁾

Cincau leaves contain mucin, thus cincau leaf extract readily turns into a gel, resembling agar.⁽⁵⁾ The major component of cincau gel is the polysaccharide pectin, consisting of water-soluble vegetable fibers that are readily fermented by the colonic microflora.⁽⁶⁾ Because of the pectin content, cincau leaf extract is considered to be a good source of vegetable fiber, the more so as its caloric content is sufficiently low. The high fiber content of cincau leaves leads to ease of defecation in those consuming cincau gel.⁽⁵⁾ The effect of the pectin fibers in cincau leaves may be enhanced by the addition of citric acid, which also increases their antioxidant effect.⁽⁷⁾

Several studies have been conducted on the medicinal properties of cincau leaves, such as antitumor,⁽³⁾ antipyretic, antimalarial⁽⁸⁾ and antihypertensive.⁽⁹⁾ The pharmacologic activities of cincau leaves are thought to be caused by the bis-benzyl-isoquinoline alkaloids.⁽⁸⁻⁹⁾ Thus far, the effect of cincau leaves on gastric functions has been investigated in a study on gastric hydrochloric acid (HCl)

concentration and gastric histopathology after simultaneous administration of an aqueous extract of cincau leaves and aspirin to Wistar rats. The study results indicated that cincau leaves protected the gastric mucosa from the effects of aspirin, as demonstrated by gastric histopathology, but did not affect the gastric HCl concentration.⁽¹⁰⁾ The present study differs from the abovementioned one in the method of preparation of the cincau leaves (infusion vs gel), and in the observed effects (macroscopic examination and measurement of ulcer index vs microscopic examination and measurement of HCl concentration). On the basis of several studies on the content of active substances in cincau leaves, the present study aims to evaluate the protective effect of cincau leaves against gastric ulcer after administration of aspirin in mice.

METHODS

Research design

The study was designed as a purely experimental laboratory investigation using controls to evaluate effect of cincau leaves on gastric ulcer.

Test animals

This study used 25 Balb/c mice aged 2-3 months and weighing 20-30 grams. The experimental animals were healthy mice (active and without any physical defects), whose condition was monitored before and during the study, and who were handled with reasonable care during the experiments. According to the previous study, with 5 intervention groups, each consisting of 5 test animals (minimum size), the size of the sample was 25, selected by simple random sampling.⁽¹¹⁾

Cyclea barbata Miers

Cincau leaves (*Cyclea barbata* Miers) were obtained during the period of February-March 2009 in and around the Sleman district. The leaves selected were clean and shiny ones,

as such leaves have a substantially higher content of active substances. The leaf picking was started from the lower stem upwards. The leaves were identified at the Pharmacognosy-Phytochemistry Laboratory, Pharmacy Program, Faculty of Mathematic and Natural Sciences (FMIPA), UII, Yogyakarta, from where the chemicals used in the preparation of the cincau leaf infusion were also obtained. A liquid antacid preparation (Bayer) was used as positive control, and aspirin (Bayer) as inducer of gastric ulcer in the laboratory animals.

Preparation of cincau leaf infusion

Cincau leaves were washed, after which 50 grams were weighed off, and 300 mL water was added to cover the leaves. The material was then placed in a waterbath at 80-90°C for 30 minutes. The solution was filtered with filter paper, replaced in the waterbath, and left to evaporate, until only 50 mL water was left.

Induction of gastric ulcer

As inducer of gastric ulcer, aspirin was administered orally to the mice. The animals had been fasted previously for 12 hours to empty the stomach of food, and to increase the gastric acid level, thereby facilitating the induction of gastric ulcer upon administration of aspirin. The dosage of aspirin was 600 mg/kg BW, which was modified from previous studies.⁽¹²⁾

Determination of the dosage of cincau leaf infusion

The basic dosage of cincau leaf infusion was taken from previous studies to be 2.5 mg/kgBW, but modified for the higher dosages to two and four times the basic dosage, becoming 5 and 10 mg/kgBW, respectively.

Determination of antacid dosage

The antacid dosage was calculated from the regular dosage in humans of 50 kg, thus obtaining a dosage of 20 mg/kgBW for the animals weighing 20-30 grams.

Table 1. Ulcer score⁽¹³⁾

Lesions		Score
Ulcer	Length > 10 cm	4
	Length 2-10 cm	2
	Length 1-2 cm	1
	Length < 1 cm	0.5
Bleeding		2

Calculation of gastric ulcer protective power

On macroscopic examination, the signs of gastric ulcer are the presence of an ulcer (or ulcers) and the occurrence of gastric hemorrhage, which were scored as shown in Table 1.⁽¹³⁾

Data analysis

The total scores of ulcer(s) and hemorrhage in the various treatment groups were statistically analyzed using one-way Anova (SPSS version 15 for Windows) for significant differences, with $p < 0.05$ being considered significant.

RESULTS

Among the 25 mice ($n=25$) used in the study, one mouse in group I did not live to completion of the study, as it died through an error in using the stomach tube, thereby leaving only 24 animals for analysis. Mean total ulcer scores of the experimental animals are shown in Table 2 and Figure 1.

DISCUSSION

Aspirin is an anti-inflammatory drug known for its gastric toxicity, which is frequently characterized by gastric ulcers and hemorrhage.⁽¹⁴⁾ Therefore this drug is frequently used as a model in studies on in vivo cytoprotective activity of new substances or compounds.^(1-2,15-18) Administration of NSAIDs, including aspirin and indomethacine, inhibits the biosynthesis of prostaglandins, particularly of PGE2 and PGI2, which are protective factors

Table 2. Mean total ulcer scores based on treatment groups in mice

Treatment Group	Total ulcer score of mice no.					Mean ± SD	p
	1	2	3	4	5		
Infusion of cincau leaves at dose 2,5 mg/kgBW	3	4	2	4	-	3.25 ± 0.96	0.002
Infusion of cincau leaves at dose 5 mg/kgBW	3	1	4	2	2.5	2.5 ± 1.12	
Infusion of cincau leaves at dose 10 mg/kgBW	0.5	0	1	1	1	0.7 ± 0.45	
Antacid at dose 20 mg/kgBW	0	1	0.5	1	0.5	0.6 ± 0.42	
Aquadest	25	6	9	8	6.5	10.9 ± 7.97	

(mucosal resistance factors) against irritation of the stomach by gastric acid.^(18, 19) Inhibition of prostaglandins results in early damage to the mucosal, parietal, and endothelial cells,⁽¹⁵⁾ thus leading to the formation of an ulcer. Moreover, the occurrence of an ulcer is also mediated by free radicals from the conversion of hydroxyperoxyl into hydroxyl fatty acids, causing destruction of the cells. These hydroxyperoxyl compounds in turn are produced by the degranulation of mast cells and the complete lipid peroxidation accompanying cellular damage.⁽²⁰⁾

Figure 1 shows that the mice in the groups receiving cincau leaf infusion and antacid had lower total ulcer scores, in comparison with the negative controls receiving distilled water. This indicates that cincau leaf infusion at the three dosage levels administered simultaneously with antacids, was capable of reducing the occurrence of gastric ulcer due to oral aspirin administration. From these data it is also apparaent that with increasing dosage of cincau leaf infusion, there is a proportional increase in gastric ulcer protective effect, with the highest dosage of 10 mg/kg BW giving the highest protective power.

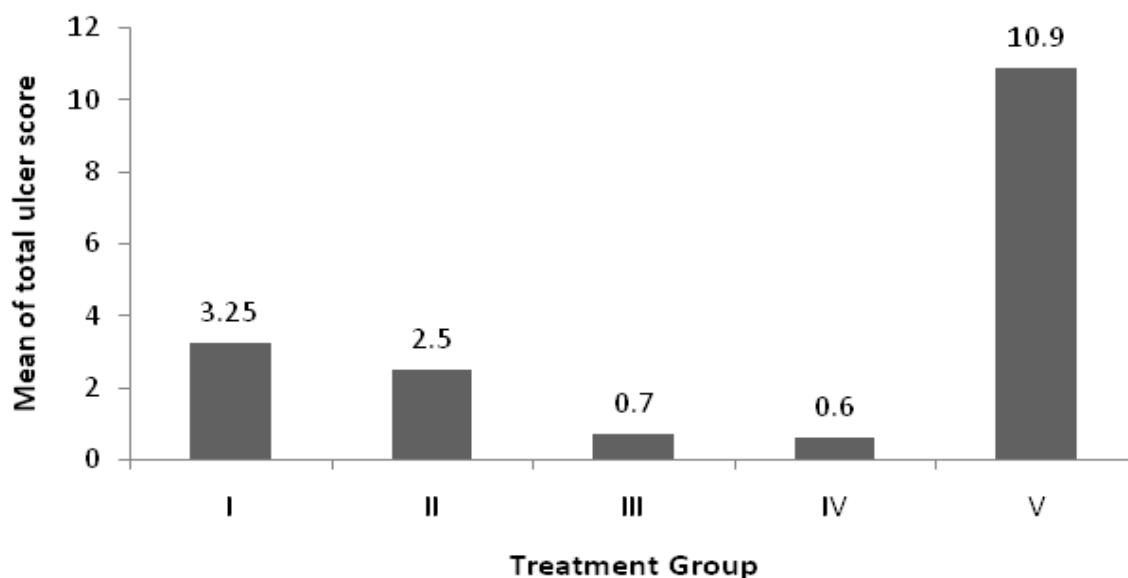


Figure 1. Mean of total ulcer score by treatment groups in mice.

Group I: Infusion of cincau leaves at dose 2,5 mg/kgBW; Group II: Infusion of cincau leaves at dose 5 mg/kgBW; Group III: Infusion of cincau leaves at dose 10 mg/kgBW; Group IV: Antacid at dose 20 mg/kgBW; Group V: Aquadest

The results of one-way Anova indicated that there were significant differences in ulcer protection at all dosage levels of cincau leaf infusion, compared with negative controls ($p=0.002$). However, the three dosages of cincau leaf infusion did not show significant differences with antacid ($p>0.05$). From the lack of significant differences with antacid it may be concluded that cincau leaf infusion at the dosages of 2.5 mg/kg BW; 5 mg/kg BW; and 10 mg/kg BW, has a gastric ulcer protective power equivalent to an antacid dosage of 20 mg/kg BW.

The results of the present study supports those of a previous study, in which cincau leaf press liquid was protective against gastric ulcer, being capable of improving the histopathological picture of aspirin-induced gastric ulcer.⁽¹⁰⁾ The present study supports the previous study results, as with a different method of preparation, cincau leaves are still capable of exerting gastroprotective activity against ulcers.

Although this study was able to provide evidence that the cincau plant (*Cyclea barbata* Miers) possesses protective activity against gastric ulcer, the active compounds involved in exerting this protective effect are not known with certainty. One of the suggestions relate to the antioxidant effects of the flavonoids, saponins, and tannins, that are known to be present in the cincau leaves.^(3,5) Antioxidant compounds have been demonstrated to have cytoprotective effects, thus being capable of protecting cells from various kinds of damage and possibly of functioning as anti-ulcer drugs.⁽²¹⁻²³⁾ Antioxidants act by inhibiting lipid peroxidation and by scavenging free radicals, thereby preventing the occurrence of gastric ulcer.⁽²⁴⁾

Several studies demonstrated that flavonoids from various plants are reportedly capable of preventing the occurrence of gastric ulcer. This may take place through an increase in the amounts of neutral glycoproteins and in prostaglandin concentrations, and inhibition of

histamine secretion from mast cells by inhibition of histidine decarboxylase,⁽²⁵⁾ thus reducing stimulation of H₂ receptors,⁽²⁶⁾ or by secretion of prostaglandin-like compounds.⁽²⁷⁾ Another possible mechanism of action for inhibiting ulcer occurrences is by decreasing pepsin secretion and activity,⁽²⁸⁾ in view of the fact that one of the major pathogenetic mechanisms of ulcer formation is by hypersecretion of gastric juice and by pepsin hyperactivity.⁽²⁹⁾ However, elucidation of the exact mechanism underlying the protective effect of cincau leaves awaits further studies.

One of the flavonoids in the cincau plant are the bisbenzylisoquinolines (including tetrandrine), which is thought to play a role in the mechanism of ulcer prevention. Bisbenzylisoquinoline compounds from various plants possess anti-inflammatory, anti-allergic, antioxidant, antifibrogenetic, and immunomodulating effects. The alkaloid tetrandrine from various plants also has anti-inflammatory properties against a number of lung infections.⁽³⁰⁾ However, whether or not the bisbenzylisoquinolines (including tetrandrine) from the cincau plant possess these effects, is a subject for further study.

In addition to flavonoids, other compounds playing a role in ulcer protection are the saponins, which have hemolytic, expectorant, immunostimulant, and anti-inflammatory properties.⁽³¹⁾ Another member of the *Menispermaceae* is *Cyclea peltata*, which also has a high saponin content.⁽³²⁾ It is thought that the anti-inflammatory effects of saponins reduces the risk of ulcers, by increasing defensive factors of gastric mucosa and stopping the inflammatory process resulting from induction by aspirin (indicated by absence of edema in the gastric mucosa of mice receiving cincau leaf infusion). The protective effect of saponins against gastric ulcer may also be mediated by the formation of a protective mucus layer on the gastric mucosa and by selective inhibition of PGF₂ α .⁽²⁵⁾

One study reported that cincau leaves are safe for consumption by the community, as it was demonstrated that cincau leaf ethanolic extract at a dosage of up to 30,000 mg/kg BW did not induce significant toxic effects, in other words, the LD₅₀ of cincau leaf extract (*Cyclea barbata* Miers) in mice is greater than 30,000 mg/kg BW.⁽³³⁾ There are several limitations to this study, as there were no observations on the effect of cincau leaf infusion in ulcer healing, active compounds playing a role in prevention of gastric ulcer were not isolated, and the specific mechanism of action of the gastroprotective effect was not studied.

CONCLUSIONS

Infusions of cincau leaves (*Cyclea barbata* Miers) have protective activity against the occurrence of gastric ulcers in mice induced by aspirin at a dosage of 20 mg/kg BW, from an initial dosage of 2.5 mg/kg BW. Higher dosages of cincau leaf infusion are correlated with a correspondingly higher gastric ulcer protective power. Further studies are needed on the active compounds in cincau leaves responsible for protective activity against gastric ulcer.

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DAFTAR PUSTAKA

1. Nilesh M, Dinesh S, Dharendra S. Evaluation of anti-ulcer potential of leaves of *Jasminum grandiflorum* L. Int J Ph Sci 2009;1:247-9.
2. Al-Howiriny T, Al-Sohaibani, El-Tahir K, Rafatullah S. Prevention of experimentally-induced gastric ulcers in rats by an ethanolic extract of "Parsley" *Petroselinum crispum*. Am J Chinese Med 2003;31:699-711.
3. Chalid SY. Effect of green cincau leaves (*Cyclea barbata* Miers and *Premna oblongifolia* Merr) extracts on antioxidant activity and tumor growth of mammary gland of transplantable mice (thesis). Bogor:Institut Pertanian Bogor;2003.
4. Tantisewie B, Ruchirawat S. Alkaloids from the plants of Thailand. In: Brossie A, Cordell GA, editors. The alkaloids. Vol. 41st. California: Academic Press, Inc.;1992.p.1-7.
5. Ekasaputra B, Setyawan H, Eko IP, Humar R, Bayu S. Application of cincau leaves for controlling viscosity and gel strength of drilling mud (skripsi). (*Pemanfaatan daun cincau dalam mengontrol viskositas dan gel strength lumpur pemboran*) Yogyakarta: Teknik Perminyakan UPN;2007.
6. Gallagher D. Dietary fiber and its physiological effect in essential of functional food. Maryland: Aspen Publication;2000.
7. Nurdin SU, Zuidar AS, Suharyono. Dried extract from green cincau leaves as potential fibre sources for food enrichment. Afr Crop Sci Conf Proc 2005;7:655-8.
8. Saxena S, Pant N, Jain DC, Bhakuni RS. Antimalarial agents from plant sources. Curr Sci 2003;85:1314-29.
9. Jia-Qing Q. Cardiovascular pharmacological effects of bisbenzylisoquinoline alkaloid derivatives. Acta Pharmacol Sin 2002;23:1086-92.
10. Djam'an Q. Effect of press liquid from leaves of *Cyclea barbata* Miers on gastric acid concentration and histopathological picture of gastric ulcers induced by acetylsalicylic acid (thesis). (*Pengaruh air perasan daun Cyclea barbata* Miers (*Cincau Hijau*) terhadap konsentrasi HCl lambung dan gambaran histopatologik lambung tikus galur Wistar yang diinduksi acetylsalicylic acid.) Semarang: FK Universitas Diponegoro;2008.
11. Festing MFW, Altman DG. Guidelines for the design and statistical analysis of experiments using laboratory animals. ILAR J 2002;43:244-58.
12. Farah AJ, Aslam ASM, Javed K, Jafri MA. Antiulcerogenic activity of *Elettaria cardamomum* Maton and *Amomum subulatum* Roxb seeds. Indian J Trad Knowledge 2005;4: 298-302.
13. Best R, Lewis DA, Nasser N. The antiulcerogenic activity of the unripe plantain banana (*Musa species*). British J Pharm 1984;82:41-5.

14. Toruner M. Aspirin and gastrointestinal toxicity. *Anatol J Cardiol* 2007;(7Suppl 2):27-30.
15. Raj Kapoor B, Anandan R, Jayakar B. Anti-ulcer effect of *Nigella sativa* Linn against gastric ulcer in rats. *Curr Sci* 2002;82:177-9.
16. Khalil J, Akhter S, Bhatti SA, Bukhari MH. Gastric ulcer healing effects of *Nigella sativa*: a comparative experimental study with cimetidine. *Biomedica* 2010;26:61-6.
17. Vinothapooshan G, Sundar K. Anti-ulcer activity of *Imosa pudica* leaves against gastric ulcer in rats. *RJPBCS* 2010;1:606-14.
18. Wang Z, Hasegawa J, Wang X, Matsuda A, Tokuda T, Miura N, et al. Protective effects of ginger against aspirin-Induced gastric ulcer in rats. *Yonago Acta Medica* 2011;54:11-9.
19. Sharma RK, Mandal S, Rajani GP, Gupta N, Srivastava DP. Antiulcer and antiinflammatory activity of fresh leave extracts of *Polyalthia Longifolia* in rats. *Int J Drug Dev Res* 2011;3:351-9.
20. Umamaheswari M, Asokkumar K, Rathidevi R, Sivashanmugam AT, Subhadradevi V, Ravi TK. Antiulcer and in vitro antioxidant activities of *Jasminum grandiflorum* L. *J Ethnopharmacol* 2007;110:464-70.
21. Okokon JE, Nwafor PA. Antiulcer and anticonvulsant activity of *Croton Zambesicus*. *Pak J Pharm Sci* 2009;22:384-90.
22. Dekanski D, Janicijevic-Hudomal S, Tadic V, Markovic G, Arsic I, Mitrovic DM. Phytochemical analysis and gastroprotective activity of an olive leaf extract. *J Serb Chem Soc* 2009;74:367-77.
23. Gregory M, Vithalrao KP, Franklin G, Kalaichelavan V. Anti-ulcer (ulcer-preventive) activity of *Ficus arnottiana* Miq. (Moraceae) leaf methanolic extract. *Am J Pharmacol Toxicol* 2009;4:89-93.
24. Naseri MKG, Mard SA. Gastroprotective effect of *Alhagi Maurorum* on experimental gastric ulcer in rats. *Pak J Med Sci* 2007;23:570-3.
25. Okokon JE, Antia BS, Umoh EE. Antiulcerogenic activity of ethanolic leaf extract of *Lasianthera africana*. *Afr J Trad CAM* 2009;6:150-4.
26. Kishore DV, Jennifer P, Mini KV. Anti ulcer activity of methanolic and aqueous extracts of leaves of *Sapindus Trifoliatus*. Linn. *Int J Pharmaceutic Sci Rev Res* 2011;6:25-7.
27. Nguelefack TB, Watcho P, Wansi S, Mbonuh N, Ngamga D, Tane P, et al. The antiulcer effects of the methanolic extract of the leaves of *Aspilia africana* (Asteraceae) in rats. *Afr J Trad CAM* 2005;2:233-7.
28. Borikar VI, Jangde CR, Philip P, Rekhe DS. Study of antiulcer activity of *Bauhinia racemosa* Lam in rats. *Vet World* 2009;2:215-6.
29. Khandare RA, Gulechal VS, Mahajani MS, Mundadal AS, Gangurdel HH. Evaluation of antiulcer activity polyherbal formulation. *IJPRD* 2006;1:1-6. Available at: [http:// www.ijprd.com](http://www.ijprd.com). Accessed June 16, 2011.
30. Qiang-Min X, Hui-Fang T, Ji-Qiang C, Ru-Lian B. Pharmacological actions of tetrandrine in inflammatory pulmonary diseases. *Acta Pharmacol Sin* 2002;23:1107-13.
31. Sahelian RMD. Antispasmodic saponins from bulbs of red onion (*Allium cepa* L. var tropea). *J Agric Food Chem* 2005;23:935-40.
32. Hullatti KK, Sharada MS. Comparative phytochemical investigation of the sources of ayurvedic drug Patha: chromatographic fingerprinting analysis. *Indian J Pharm Sci* 2010; 72:39-45.
33. Angelina M, Hartati S, Dewijanti ID, Banjarnahor SDS, Meilawati L. Determination of cincau leaf (*Cyclea barbata* Miers) LD50 in mice (*Penentuan LD50 daun cincau (Cyclea barbata Miers) pada mencit*). *Makara Sains* 2008;12:23-6.