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Anti-Gastric Ulcer Mechanism of *Anredera scandens* (L.) Moq Leaves Extract

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Abstract

Anredera scandens (L.) Moq. is one of the plants that have gastric ulcer activity. This study aimed to determine the anti-ulcer mechanism of Anandera scandens (L.). Moq leaves ethanol extract (AEE). Ulcer modeling was made using aspirin-induced Wistar strain male rats. The mechanism investigated by its ability to inhibit gastric acid secretion, reduce the diameter of the wound, and increase gastric volume after being treated for 14 days. Based on the test results, the AEE. At a dose of 250 mg/kg BW, 300 mg/kg BW, and 350 mg/kg BW, has been able to increase the pH of the gastric to become more alkaline, reduce the diameter of peptic ulcers, and increase mucous cell proliferation ($p < 0.05$).

Keywords: *Anredera scandens* (L.) Moq, gastric ulcer, mechanism of action, AEE

INTRODUCTION

Gastric ulcer disease is the most common gastrointestinal disorder. Indonesia ranks third in the prevalence of this disease after the United States (47%), India (43%) and Indonesia (40.85%) (Akmal et al., 2010). The mechanism of stomach ulcers is as a result of an imbalance between aggressive factors and protective factors. Endogenous aggressive factors are HCl, pepsinogen, and pepsin. Defensive factors include protection of mucus bicarbonate and prostaglandin (Prabhu and Shivani, 2014).

Anredera scandens leaf extract has been investigated to have anti-ulcer activity in male white Sprague Dawley rats with ED_{50} values of 356 mg/kg BW (Samirana et al., 2014) as well as deep wounds such as gastric and postoperative wounds (Miladiyah and Bayu, 2012). The purpose of this study was to determine the anti-gastric ulcer mechanism of *Anredera scandens* (L.). Moq leaves ethanol extract (AEE). The benefits of this study will provide information about the mechanism of AEE as one of the anti-gastric ulcers from herbs.

MATERIALS AND METHODS

A. Material

Anredera scandens (L.). Moq leaves powder, 70 % ethanol technically, KLT silica gel GF 254 plate, toluene pa, ethyl acetate pa, diethylamine pa, ammonia pa, chloroform pa, methanol pa, aspirin, sucralfate, Omeprazole, pH meter, spuit 10 cc, male Wistar strain white rat.

B. Methods

Anredera scandens Ethanol Extract (AEE)

A. scandens leaves powder were extracted using the maceration method for one day. The ratio between the leaves and ethanol 70% was 1: 10. The macerate separated from the residue. Repeat the residue maceration two times with the same solvents. All macerates were collected and evaporated at 68 ° C (Ministry of Health RI, 2004).

Phytochemical screening

The phytochemical test was carried out to identify the presence of compounds in AEE. Identification of compounds includes an examination of polyphenols, alkaloids, terpenoids, flavonoids, saponins, and tannins. The phytochemical test method used is TLC (Harbone, 1984; Reich and Blatter, 2004).

Anti-Gastric Ulcer Mechanism

Thirty-five male Wistar white rats (150-200 gram) were divided into seven groups. Normal control, negative control, two positive controls (sucralfate, omeprazole), three groups of AEE. Acclimatization was performed for one week before the experiment. All groups, expect normal control were fasted for two days and given aspirin 18 mg/kg BW induction on the first and second day. Next 14 days, oral treatment has been given per each group:

1. Negative Control: Aquadest 2 ml
2. Positive Control 1: Sulcralfate 3.6 mg/Kg BW 4 times a day
3. Positive Control 2: Omeprazole 0.15 mg/kg BW once daily
4. AEE 250 mg/Kg BW once daily
5. AEE 300 mg/Kg BW once daily
6. AEE 350 mg/Kg BW once daily

The gastric ulcer diameter measurement

Dissecting and removing gastric fluid, the diameter of lesion was performed using a capillary.

Examination of stomach acid levels

Remove the stomach by cutting the upper duodenum and esophagus et cardia. Inject 2 mL of NaCl 0.9 % solution into the stomach. Removed the gastric fluid by dissecting the significant curvature section, holding and centrifuging at 3000 rpm for 10 minutes. Clear fluids are used to determine the pH of gastric fluid.

Gastric Volume measurement

Whole rat stomach put into a measuring cup that has been filled with 10 mL aquadest. Note the increased height in the measuring cup (a). Gastric then surgery to remove the contents, do the same thing as above (b). Gastric volume is calculated from the deviation between a and b.

Data Analysis

The mechanism of anti-gastric ulcer (ulcer diameter, pH, and volume gastric) displayed in the mean ± SD. Data obtained in all groups were compared with each other using the parametric ANOVA test with a confidence level of 95% and continued with *Bonferroni's post hoc test for multiple comparisons*. The statistical program used in 20.0 SPSS software.

RESULT

The results of screening and profiling on the AEE contain active compounds of flavonoid, saponin, triterpenoid (table 2). The third class of chemical compounds will be identified by looking at the color spots and Rf on TLC plates under visible light, UV light 254 nm and 366 nm, and spectrum profiles by TLC-Densitometer at λmax 210 nm (Fig. 1).

The administration of aspirin induces gastric ulceration, decrease gastric fluid pH and gastric volume in animals model (figure 2, 3, and 4). Aspirin loading increased the diameter of the gastric ulcer (negative control). Giving AEE of doses of 250, 300, 350 mg/kg BW, sucralfate, and omeprazole, causes a decrease in gastric ulcer diameter, where there are significant differences with negative controls (Figure 2).

AEE intake doses of 250, 300, and 350 mg/kg BW was able to increase gastric fluid pH, which was significantly different from negative control (Figure 3).

Table 1. Determination chemical compounds of AEE by the TLC method

No	Group	Mobile phase	Substance	Identification
1	Polyphenol	Toluene: Ethyl Acetate (93:7)	Folin	Blackish blue spots (on visible light)
			FeCl3 2%	Blackish spots (on visible light)
2	Alkaloid	Toluene: Ethyl Acetate: Diethylamine (70:20:10)	Dragendrorrf	Orange-brown spots with a yellow background, yellow on UV 254 nm and light green at UV 366 nm
			Wagner	Light brown to yellow spots
3	Flavonoid	Ethyl acetate: Formic acid: Acetic acid: Water (100:11:11:26)	Ammonia vapor	Greenish spots on visible light
4	Saponins	Chloroform: Methanol: Aquades (70:30:4)	Liberman Burcard	Spots are green to blue by heating 90^{o}C for 10 minutes
			Vanillin sulfuric acid	The spots are gray by heating 120^{o}C for 10-20 minutes
5	Tannin	Toluen: Ethyl Acetate (93:7)	Ammonia vapor	Purple patches under UV 366 nm
			FeCl3	Brownish blue spots at visible light
6	Triterpenoid	Chloroform: Methanol (10:1)	Anisaldehyde-sulfuric acid	Spots are red to purple by heating 100^{o}C for 5-10 minutes at UV 366 nm
			Vanillin 1% sulfuric acid	Purple spots by heating 110^{o}C for 5-10 minutes at visible light or UV 366 nm

Table 2. The phytochemical compound of AEE

No	Group	Substance	Result
1	Polyphenol	Folin	Negative
		FeCl3 2%	Negative
2	Alkaloid	Dragendrorrf	Negative
		Wagner	Negative
3	Flavonoid	Ammonia vapor	Positive
4	Saponins	Liberman Burcard	Positive
		Vanillin sulfuric acid	Positive
5	Tannin	Ammonia vapor	Negative
		FeCl3	Negative
6	Triterpenoid	Anisaldehyde-sulfuric acid	Positive
		Vanillin 1% sulfuric acid	Positive

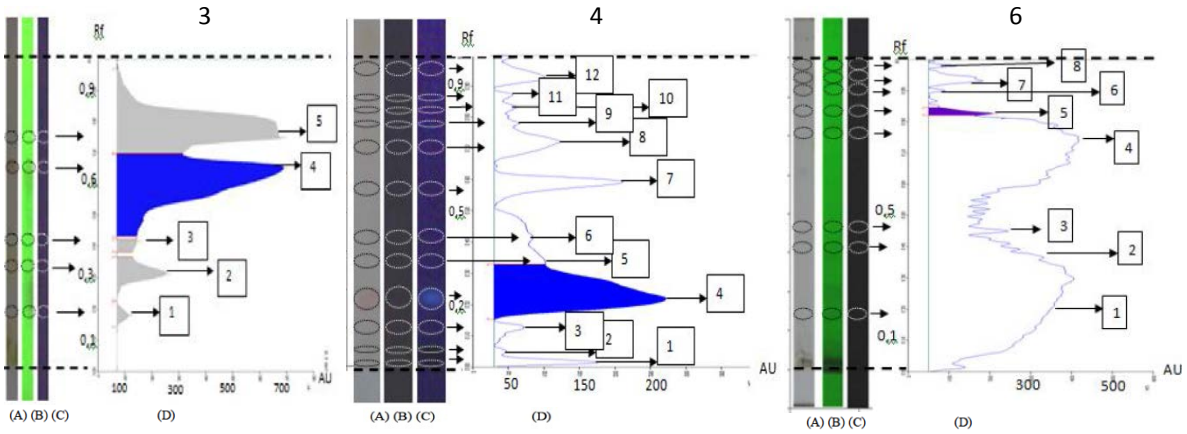

Figure 1. AEE Chromatogram.
Identification of Groups of Flavonoids (3), Saponins (4) and Triterpenoids (6) Observed in Visible Rays (A), Under UV Light 254 nm (B), UV 366 nm (C), and 210 (3) Wavelength Densitometer , 6), 206 (4) nm (D)

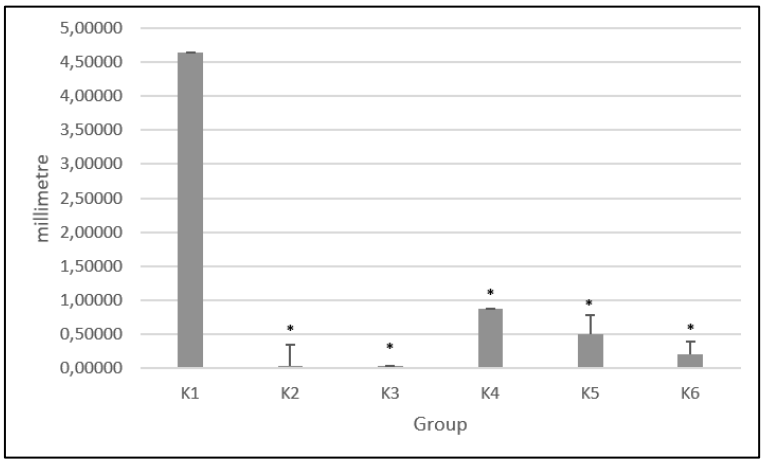

Figure 2. Gastric ulcer diameter
K1 = Negative Control; K2 = Positive Control (Sulcralfate); K3 = Positive Control (Omeprazole); K4 = AEE 250; K5 = AEE 300; K6 = AEE 350; * = significantly different with negative control

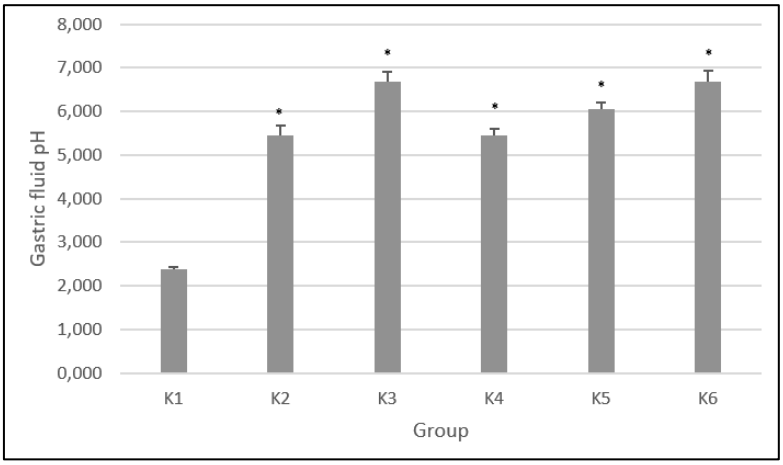

Figure 3. Gastric Acid Levels
K1 = Negative Control; K2 = Positive Control (Sulcralfate); K3 = Positive Control (Omeprazole); K4 = AEE 250; K5 = AEE 300; K6 = AEE 350; * = significantly different with negative control

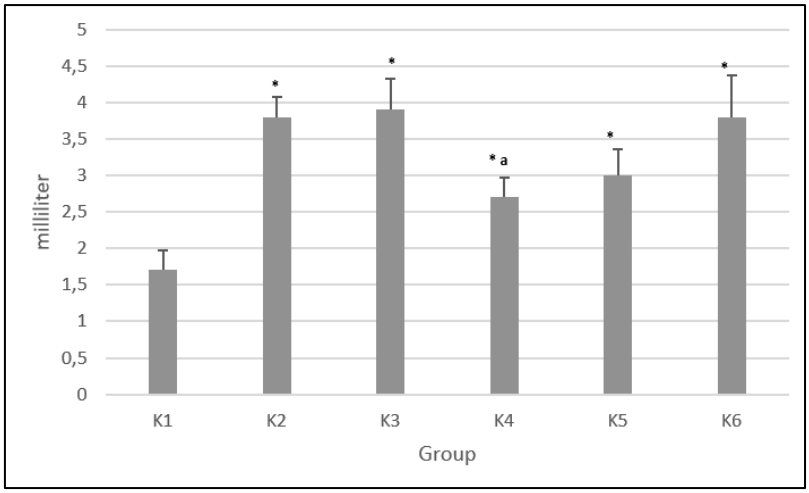

Figure 4. Gastric volume
K1 = Negative Control; K2 = Positive Control (Sulcralfate); K3 = Positive Control (Omeprazole); K4 = AEE 250; K5 = AEE 300; K6 = AEE 350; * = significantly different with negative control; a= significantly different with positive control

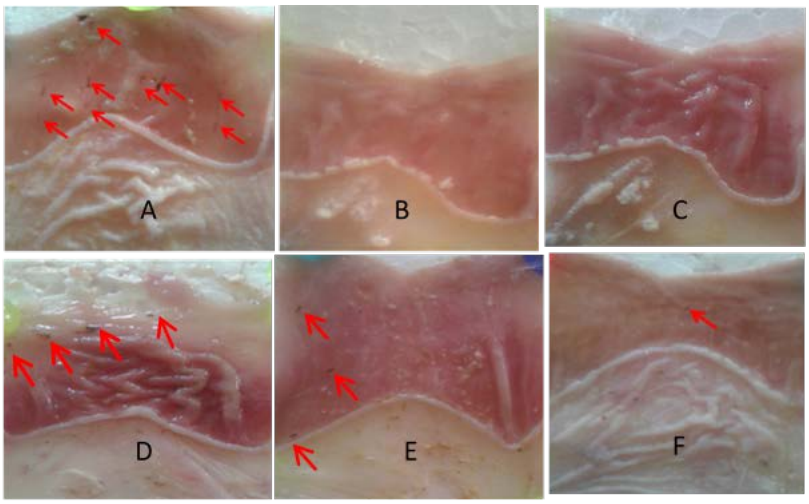

Figure 5. Gastric Ulcer Macroscopy
Ulcer remark by red arrow. Negative Control (A), Sulcrafat (B), Omeprazole (C), AEE 250 mg/kg BW (D), AEE 300 mg/kg BW (E), AEE 350 mg/kg BW (F)

AEE caused an increase in gastric volume, where there were significant differences with negative controls. Giving AEE at doses of 250, 300, and 350 mg/kg BW was able to increase the gastric volume that was significantly different from the negative control. The administration of extracts of doses of 300 and 350 mg/kg BW, which was able to increase gastric volume was the same as the positive control (Figure 4).

The imposition of aspirin causes stomach ulcers where the histopathology of the rat's gastric is shown in Figure 5.A. Rats that had been aspirated and then given sucralfate and omeprazole did not appear to have ulcers (figures 5.B and C). The rat that were charged with aspirin and then given extracts of 250, 300, and 350 mg/kg BW showed that there were diminishing amounts of ulcers. The higher the extract dose, the reduced number of ulcers (figure 5.D, E, and F).

DISCUSSION

The beneficial effect of AEE in treating gastric ulcer is demonstrated by the animal model using rats induced by aspirin. Induction of gastric ulcer is a major effect caused by NSAIDs; in this research, we used aspirin (Saputri, 2008). Aspirin causes damage to the digestive tract through several mechanisms, including lowering the number of mucosal prostaglandins, reducing blood flow to the mucosa, and stimulating neutrophil activation and apoptosis (Spechler, 2004).

Anredera scandens (L.) Moq is Indonesian medicinal plants that empirically used to treat wound and ulcer (Yuniati and Lukiswanto, 2017). Phytochemical screening showed that the AEE contains flavonoids, saponin, and triterpenoids. These results are following previous studies (Samirana, et. al., 2016).

There are several healing mechanisms in gastric ulcers, including inhibition of acid production and secretion, increased mucus production, stimulation of mucosa proliferation, antioxidant activity and inhibiting inflammation (Wei et al., 2014). Sucralfate and Omeprazole are two classes of drugs that are often used to treat stomach ulcers. Sucralfate in an acidic atmosphere will form a thick paste that selectively attaches to the base of the ulcer and protects the ulcer against acid diffusion, and has cytoprotective properties (Neal MJ, 2006). Whereas Omeprazole has a mechanism to prevent gastric acid secretion from canalicular cells so that the activity of driving factors of pepsin is reduced (Finkel R, 2009).

AEE in this study has high potential as an anti-gastric ulcer with three mutually supporting and interrelated mechanisms, by reducing the diameter of peptic ulcers, increasing the pH and gastric volume of rats. AEE at a dose of 250 mg/kg BW in this study was able to increase gastric pH, increase gastric volume, and significantly reduce the diameter of the ulcer compared to the negative control. The increase in dose is directly proportional to the increase in anti-gastric ulcer activity, where a dose of 300 mg/kg body weight, has the equivalent activity with sucralfate and omeprazole, to increase gastric volume within 14 days.

The content of chemical compounds triterpenoid, saponin, and flavonoid groups have their respective roles in the healing of gastric ulcers. Combined saponin and triterpenoid compounds have been investigated to increase prostaglandin levels in the protection of gastric mucosa, decrease histamine secretion from mast cells so that excessive stomach acid production can be prevented (Deoda et al., 2011; Borrelli and Izzo, 2000). Saponin stimulates collagen type I, which is vital in increased cell epithelization and wound closure (MacKay and Miller, 2003). Flavonoid act as an antioxidant by inhibiting free radical scavenging (Nayak et al., 2006), thus preventing cell necrosis, increasing vascularization, and collagen fibers (Astuti et al., 2011). This combined wound healing mechanism stimulates the growth of epithelial cells as well as cell surface repair in the gastric mucosa, thereby maximizing gastric function and increasing gastric volume (Spechler, 2004) in both the positive control and treatment groups in this study.

CONCLUSIONS

AEE potential as an anti-gastric ulcer by reducing the diameter of the gastric ulcer, increasing the pH and volume of the gastric.

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