In Vitro Evaluation of Antacid and Anti-flatulent Activity in Ethanol Extract Syrup of Clove Leaves (*Eugenia Caryophyllata* Thunberg)

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Abstract: Objective: Peptic ulcer has high prevalence in the world that caused by high level of gastric acid. The sample of this study was ethanol extract syrup of clove leaves (*Eugenia caryophyllata* Thunberg) that had gastro-protective and anti-ulcer activities. Methods: The pH of ethanol extract syrup of clove leaves and their neutralizing effects on artificial gastric acids were determined and compared with negative (aluminium hydroxide) and positive controls (magnesium hydroxide). A modified model of Vatier's artificial stomach was used to determine the duration of consistent neutralization effect on artificial gastric acids. The neutralization capacity in vitro was determined with the titration method of Wu model, while Rezak method was used to test the anti-flatulent activity by creating foam from sodium lauryl sulphate. The syrup was subsequently added and the remaining height of foam formed was calculated. Results: The results showed that ethanol extract syrup of clove leaves had antacid and anti-flatulent in vitro. Compared to the water group, ethanol extract syrup of clove leaves was found to possess significant gastric acid neutralizing effects. The duration for consistent neutralization of ethanol extract syrup of clove leaves was significantly longer than that of water group. Also, there are anti-flatulent effects by decrease of foaming in vitro model. Conclusion: Ethanol extract syrup of clove leaves was consistently active in the artificial stomach model and suggested to have antacid and anti-flatulent effects similar to the positive control.

1 INTRODUCTION

Peptic ulcer (PU) is one of the most common gastrointestinal disorders. The goals of treatment for PU are to relieve pain, enhance ulcer healing, prevent complications, and prevent ulcer recurrence. Current drug therapy for PU is oriented primarily toward neutralizing (e.g. antacids) or reducing the amount of acid secreted (e.g. H2 receptor antagonists, proton pump inhibitors) or protecting the gastric mucosa from the effects of acid (e.g. sucralfate). As the role of *Helicobacter pylori* is becoming better understood, treatment with antibiotics is becoming an important part of PU therapy and recurrence prevention (Holle, 2010). Although effectiveness can be obtained with these clinical drugs, their potential side effects and drug interactions represent a major problem in therapy. Moreover, newer drugs introduced for the treatment of PU are expensive and the cost-effectiveness is also an important consideration. Clinically, many people cannot use chemosynthetic drugs because of the potential side effects. Therefore, traditional herbal drugs possessing fewer side effects should be investigated as an ideal alternative for the treatment of PU (Blume *et al.*, 2006)

Eugenia caryophyllata Thunb. is known as clove which belongs to the family Myrtaceae and esteemed as a flavouring agent, used as a spice for scenting, chewing tobacco, an ingredient of betel chew and control nausea, vomiting, cough, diarrhoea, dyspepsia, flatulence, stomach distension and gastro intestinal spasm, relieve pain, cause uterine contractions and stimulate the nerves (Okasha *et al.*, 2007). The pharmacological activities of the plant reported as anti-microbial, anti-viral, anti-oxidant, anti-diabetic, anti-inflammatory, anti-platelet, anti-stress, anti-pyretic, chemopreventive, hepatoprotective, anaesthetic, aphrodisiac, and insecticidal (Khanna & Parle, 2011).

Cloves leaves were used as a sample in this study because clove leaves contain eugenol that has ability to neutralize gastric acid and reduce bloating stomach. The syrup dosage form was chosen because syrup is suitable to cover the bitterness of extract. In addition, the syrup preparation was faster than any

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other dosage forms (Oliveira *et al*., 2014; Oliveira, 2014).

The secretion of gastric acid (HCl) is intimately related to PU disease. Antacids heal ulcers through elimination of gastric acid by neutralization and have been used in the treatment of PU for many years. Common antacid preparations include sodium bicarbonate (SB), calcium carbonate, and salts of aluminium and magnesium. Since some people cannot use chemosynthetic drugs because of their side effects, ethanol extract syrup of clove leaves should be considered as an alternative for the treatment of PU. To reveal the anti-ulcer effects of ethanol extract syrup of clove leaves, therefore, the present study aimed to assess their antacid effects on gastric acid neutralization compared to water and positive controls (aluminium and magnesium). The antacid effects were assessed *in vitro* using the titration method Wu modified model of Vatier's artificial stomach. It was utilized to determine the effects on gastric acid secretion and emptying (Wu *et al*., 2010).

2 MATERIALS AND METHODS

2.1. The Preparation of Cloves Leaves Ethanol Extract Syrup

Clove leaves ethanol extract at 1, 2 and 3% was mixed with 15% propylene glycol. Then, 65% sucrose was added into the mixture and stirred until it was dissolved. Water was subsequently added into that mixture.

2.2. Chemicals and Reagents

Sodium chloride and pepsin were purchased from Sigma (St. Louis, MO, USA) and 1 mol/L hydrochloric acid was obtained from Merck (Darmstadt, Germany), propylene glycol, sucrose, aluminium hydroxide and magnesium hydroxide as positive control drugs of antacid were purchased from PT First Medipharma (Indonesia), dimethylpolysiloxane as positive control of anti-flatulent was purchased from Pharos (Indonesia), and sodium lauryl sulphate (Germany).

2.3. Instruments

The experimental instruments consisted of an adjustable electrode stand, a series flatbed recorder, a micro tubing pump, a standard pH meter, a stirrer/hot plate, and a series multi-functional temperature controller.

2.4. Preparation of Artificial Gastric Acid

Two grams of sodium chloride and 3.2 mg of pepsin enzymes were dissolved in 500 mL water, then 7.0 mL hydrochloric acid and adequate water were added to make a 1000 mL solution of the artificial gastric acid at pH 1.2.

2.5.Determination of the Neutralizing Effects on Artificial Gastric Acids

Ninety mL of each test solution was added to 100 mL artificial gastric juices at pH 1.2. The pH values were determined to examine the neutralizing effect.

2.6. Determination of the Duration of Consistent Neutralization Effect on Artificial Gastric Acids using the Modified Model of Vatier's Artificial Stomach

Ninety mL of each test sample was added to 100 mL of artificial gastric juice at pH 1.2 in the beaker glass of the artificial stomach at 37℃ and continuously stirred (30 rpm) with magnetic stirring apparatus. Artificial gastric juice at pH 1.2 was pumped at 3 mL/min into the container of the artificial stomach, and it was pumped out at 3 mL/min at the same time. A pH meter was connected to continuously monitor of pH meter in the container of artificial stomach. The duration of neutralization effect was determined when the pH value was returned to its initial value (pH 1.2).

2.7. Anti-flatulent Test in Removing Foam

One hundred mL of gastric acid was put into the artificial hull and 0.625 g sodium lauryl sulphate was subsequently added into it. The mixture was heated at 37 ℃ with stirrer speed of 30 rpm to form 150 mL foam. The height of foam formed was measured. Then, 10 mL of clove leaves ethanol extract syrup and water was added until 90 mL. The mixture was observed within 20 minutes and the remaining height foam was measured.

2.8. Statistical Analysis

The statistical calculations were performed using the software IBM SPSS Statistic 23. The experimental data were expressed as mean ± SEM where SEM is Standard error of Mean; Comparison between the groups were analyzed by One-way Analysis of Variance (ANOVA) using Dunnett Multiple Comparisons Test by considering Test Vs control. The differences were considered to be statistically significant when **P<0.05.

3 RESULTS

3.1. Antacid Activity Test

The durations for consistent neutralizing effects of clove leaves ethanol extract syrup at 1, 2 and 3 % solutions were 80.14 ± 3.63, 104.51 ± 6.08 and 140.78 ± 5.33 minutes, respectively. Those of positive control and negative control solutions were 268.31 ± 10.68 and 0.02 ± 0.00 minutes, respectively. The action duration of positive control was the longest, followed by clove leaves ethanol extract syrup at 3% (Table 1 and Figure 1).

Table 1. Duration of antacid effect for consistent neutralization of gastric acid

Drug	Time (minute)
Positive control	268.31 ± 10.68
Negative control	0.02 ± 0.00
Formula 1	80.14 ± 3.63
Formula 2	104.51 ± 6.08
Formula 3	140.78 ± 5.33

Data were presented as mean ± SEM *$P < 0.05$ when compared to water group

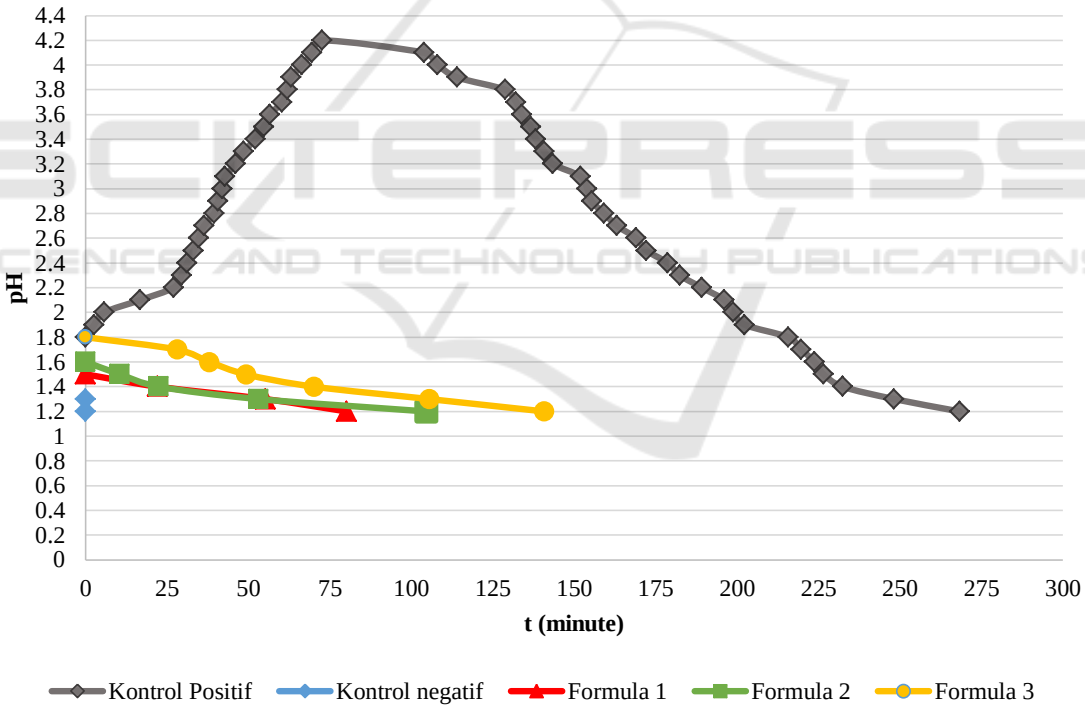

Figure 1. The duration of antacid effect for consistent neutralization of gastric acids

Ninety mL of test sample was added to 100 mL of artificial gastric juices at pH 1.2 in an artificial stomach. The duration was determined as the pH value was returned to 1.2.

3.2. Anti-flatulent Activity Test

Based on the results of anti-flatulent activity test, the data analysis showed that positive control treated with dimethylpiloxane was able to remove foam with 0.2 ± 0.0 cm. While the height foam in negative control treated with syrup containing only carrier

without clove leaves ethanol extract was 1.8 ± 0.1 cm. The height foam formed in the treatment group of clove leaves ethanol extract syrup of 1, 2 and 3% were 0.90 ± 0.0 cm, 1.2 ± 0.3 cm and 1.2 ± 0.3 cm, respectively. The results of clove leaves ethanol extract syrup were significantly different compared to water (table 2 and figure 2).

Table 2. Remaining high foam within 20 minutes

Drug	Height foam (cm)
Positive control	0.2 ± 0.0
Negative control	1.8 ± 0.1
Formula 1	0.9 ± 0.0
Formula 2	1.2 ± 0.3
Formula 3	1.2 ± 0.3

Data were presented as mean ± SEM *P < 0.05 when compared with water

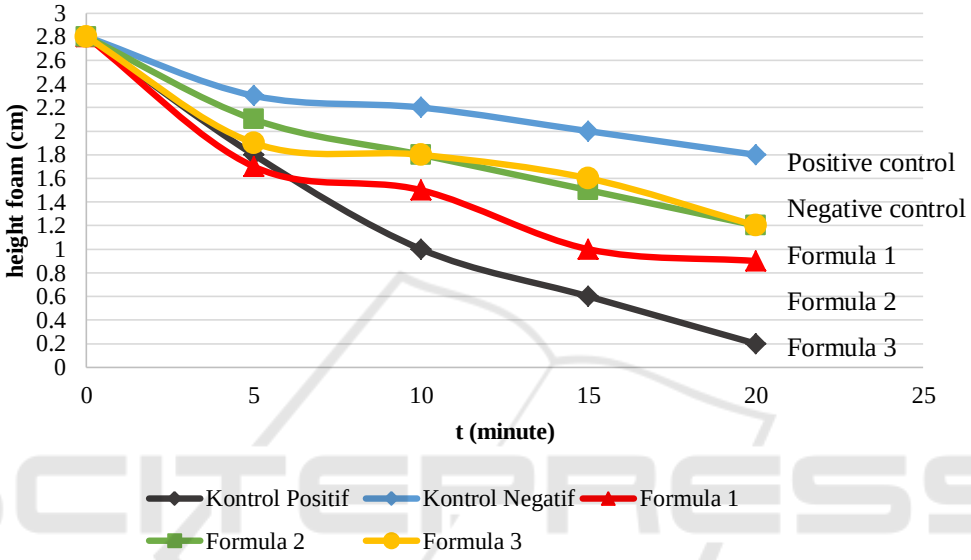

Figure 2 Remaining high foam within 20 minutes

Ninety mililiters of test sample was added to 100 mL of artificial gastric juices at pH 1.2 in an artificial stomach. Remaining high foam within 20 minutes.

3 DISCUSSION

Stomach is an organ that undergoes propulsion, mixing of food, digestion and absorption of food along with secretary functions. The parietal cells of stomach secrete about 2500 mL of gastric juice daily. The acid in this gastric juice kills many bacteria and provide a low pH for pepsin to start protein digestion. Mucosal erosions or ulcerations take place when aggressive factors overwhelm the defensive factors of the gastrointestinal mucosa. It leads to the arrival of gastritis, peptic ulcer and gastroesophageal reflux disease (Katharinne *et al*., 2010). The aggressive factors well established for several decades are acid and pepsin. Hence peptic ulcer diseases are mostly treated with antacids, H2 receptor antagonists and proton pump inhibitors. Among them, antacids have been widely used in the treatment of ulcer.

Antacids are generally inorganic salts which dissolve in acid gastric secretions which release anions that partially neutralize gastric hydrochloric acid. They generally react chemically to neutralize or buffer existing quantities of stomach acid but do not have direct effect on its output. This action results in increased pH value of stomach contents and thus provide relief of hyperacidity symptoms. These medications also reduce acid concentration within the lumen of the esophagus which causes an increase in intra-esophageal pH and a decrease in pepsin activity (Katzung, 2010). These medicaments do not decrease the volume of gastric secretions. Most of the antacids available in the market are efficient but is often unacceptable because of the common side effects, especially altered bowel functions.

Antacids that contain aluminium contribute aluminium to the diet but may cause constipation or lead to phosphorous deficiency where as on long term or inappropriate use can lead to aluminium toxicity. Calcium containing antacids contribute calcium to diet and may produce constipation. Magnesium containing antacids contribute magnesium to diet and produce a side effect of diarrhea on prolonged use may even lead to magnesium toxicity (Gunawan *et al*., 2009). It is reported that SB should be avoided even though it is a potent neutralizer of acid as it contains significant amounts of sodium and may alter the systemic pH. One major fact that should be considered while selecting an antacid for the treatment is its drug interactions. Significant interactions occur with quinolone antibiotics, tetracycline and iron sulphate (Tsung-Hsiu *et al*., 2010). Hence considering the side effects and drug interactions of antacids, the herbal drugs having fewer side effects should be identified as an alternative for the treatment of peptic ulcers. It is widely understood that herbal medicines have recently generated an increased interest in the treatment of gastritis. Hence in our present study we had applied the titration method of Wu the modified model of Vatier's artificial stomach, which mimic the regular physiological functioning of a human stomach, to explore the antacid effects of clove leaves that were well known for its potency to cure all types of wounds.

Clove leaves ethanol extract syrup has antacid and anti-flatulent activity. Eugenol inside this formula was expected to have antacid and anti-flatulent. It included in polyphenol group that has pKa of 10.19, thus it was considered as a weak base. So, it had the same mechanism of action as antacid drugs which are also considered as weak base by reacting with hydrochloric acid to form salts and neutralize gastric acid. In comparison to negative controls, Remington stated that if the pH has reached 2.3 then it has been able to neutralize gastric acid by 90% (Hoover & John, 2005).

Anti-flatulent test was also performed in this study due to the ability of high gastric acid to cause flatulence. Thus, it can be concluded that good antacids are the one that has high efficacy, few side effects, and able to reduce bloating stomach.

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