

The Neutralizing Effect of Squeezed Juice, Infusion and Ethanol Extract of Miana Leaves

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ABSTRACT: The traditional use of miana leaves as a remedy for various ailments, including stomach acid, is well-documented. The utilization of traditional treatments is prevalent due to the absence of side effects associated with synthetic chemical treatments. The consumption of miana leaves by communities is typically achieved through the ingestion of the juice extracted from fresh leaves or by boiling the leaves in water. The purpose of this study is to investigate the mechanism of action of miana leaves as a stomach acid remedy, specifically, its neutralizing effect on stomach acid. The study was conducted on water extracts using squeezed juice and infusion methods, as well as ethanol extracts using the maceration method. The dosage used was obtained by converting the commonly used dosage by the community, which is 3 medium-sized leaves. The average weight of 1 fresh leaf is 1g and the volume of medicine consumed is 1 tablespoon or 15 mL. The concentration for squeezed juice is 20%, infusion 2.08% and ethanol extract 0.28%. The results obtained from maceration, infusion, and miana leaf extract demonstrate a neutralization capacity of 26.617 ± 0.236 ; 26.7 ± 0.05 and 26.633 ± 0.07 respectively. In conclusion, this study demonstrates that miana leaves possess a neutralizing effect on stomach acid, and may be used as a remedy for stomach acid.

KEYWORDS: Miana leaves; neutralizing effect; titration; stomach acid; dyspepsia.

1. INTRODUCTION

Indonesia is a country that has diverse plants that can be used as medicine for various diseases. Currently, most people prefer to use plants as traditional medicines because they have minimal side effects compared to chemical drugs. One of the plants that can be used as a medicine for digestive disorders such as dyspepsia is miana leaves (*Coleus scutellarioides* (L.) (Pusat Studi Biofarmaka LPPM IPB, 2014).

Miana is a plant with many benefits, one of which is a medicine for digestive disorders. miana leaves contain saponins, flavonoids, alkaloids, essential oils, phenolic compounds, phytosterols, rosmarinic acid, and phytol which are volatile diterpene compounds. Flavonoids contained in miana leaves are quercetin (Artini, 2018). A phytochemical screening test is a test that aims to provide an overview of the group of compounds contained in the plant. The method used in the phytochemical screening test is done by looking at the color change reaction using a reagent (Kristanti, 2008).

Dyspepsia, according to Rome IV, is a collection of several symptoms, such as bloating, early fullness, and epigastric pain. These symptoms greatly interfere with activities and occur at least three days per week for the last three months, with onset at least six months earlier (Stanghellini, 2017). Dyspepsia is a word that comes from the Greek where *dys* means bad and *pepsi* or digestion. So it can be interpreted that dyspepsia is poor digestion or discomfort around the epigastric such as pain (Madisch et al., 2018).

Dyspeptic symptoms, as described by Rome IV criteria, are the feeling of a full stomach when you are about to start eating and the size of the food that is not suitable so that the food does not run out. These symptoms are symptoms of dysmotility or motility disorders in the stomach when there is a decrease in the flow of blood to the stomach caused by an increase in stomach acid that it can inhibit the release of the stomach after eating. In addition, delayed gastric emptying can cause symptoms such as nausea even though the stomach is empty, but symptoms of nausea when the stomach is empty are rare symptoms. Excessive increase in stomach acid can occur due to irregular eating patterns and stress factors (Putri & Widyatuti, 2019). The process of gastric acid secretion must be regulated properly so as not to cause damage to the gastric mucosa. The acidic condition of the stomach aims to kill pathogenic microorganisms such as *Helicobacter pylori*. Gastric pH is maintained below pH 4 to maintain the bactericidal effect of gastric acid. A decrease in gastric acid pH can cause bacterial infections in the gastrointestinal tract. However, excessive gastric acid secretion can cause inflammation of the gastric mucosa, so the gastric mucosa must maintain a balance and physiological conditions between gastric acid secretion and mucosal protection mechanisms (Miftahussurur, 2021). Antacids are one of the chemical drugs that can be used as a therapy for dyspepsia. Antacids or *anti* means opposite and *acidic means* sour. Antacids are weak base compounds that can neutralize stomach acid. Antacids can increase the pH, thereby reducing the optimal proteolytic action of pepsin at pH 2. Pepsin activity decreases above pH 4 (Tjay, 2015). Antacids work to neutralize stomach acid secretions. However, giving antacids cannot be done continuously because they are only symptomatic to reduce pain (Zakiyah et al., 2021).

Testing the neutralizing effect of miana leaves can be done by determining the acid capacity using the direct titration method. Direct titration, namely, the sample solution, is directly titrated with a standard solution. One plant that is thought to have a neutralizing effect is the miana leaf plant. This is the background for researchers to research the neutralization effect of freshly squeezed extract, infusion, and ethanol extract of miana leaves.

2. EXPERIMENTAL SECTION

2.1. Time and place

The research was conducted at the Pharmaceutical Laboratory of the Faculty of Pharmacy, Indonesian Muslim University. The time of research was conducted from October to December 2022.

2.2. Instrument

The tools used are spray bottle, burette (Pyrex), funnel, Erlenmeyer glass (Pyrex), measuring cup (Pyrex), clamp and stative, magnetic stirrer (Ika® c-mag hs 10), oven (Mettler), pH meter (Inolab), pycnometer (Pyrex), test tube (Pyrex), balance (Acids ad-600i), tissue, maceration jar, and rotary vacuum evaporator.

2.3. Materials

The materials used are distilled water, Miana leaves, 96% ethanol, FeCl₃, HCl 1.0 N, NaOH 0.5 N, bouchardat reagent, dragendroff reagent, meyer reagent.

2.4. Sample preparation

2.4.1. Preparation of squeezed water

Weigh 3 grams of fresh Miana leaves, then grind them and add 15 mL of distilled water. Next, the filtering process is carried out using filter paper.

2.4.2. Preparation of infusions

Infusions of miana leaves is made by boiling miana leaves in 200 mL of water for 15 minutes starting when the water temperature has reached 90°C. Next, the filtering process is carried out using filter paper. Screening results in adequacy volume with aquades up to 200 mL (BPOM, 2013).

2.4.3. Preparation of ethanol extract

As much as 231.24 grams of dried miana leaves that had been powdered were put into the maceration container, and 750 mL of 96% ethanol solvent was added. The maceration process was carried out for 5 days, and stirring was done daily. After 5 days, it is filtered and followed by the re-maceration process. Furthermore, the filtering results from the maceration process were mixed and then evaporated using tools rotary vacuum evaporator. Then the resulting extract was evaporated again to obtain a viscous extract (Anita *et al.*, 2019).

2.4.4. Phytochemical screening

Alkaloid tests can be detected using several reagents such as Meyer (potassium tetraiodomercurate (II)), Wagner (iodine in potassium iodide), and Dragendroff (bismuth nitrate in potassium iodide). An orange-brown precipitate characterizes samples containing alkaloids, and a precipitate is formed when reacted with the three reagents. A flavonoid compound identification test can be done using magnesium powder reagent (Mg) and concentrated hydrochloric acid (HCl). Magnesium powder is added to form bonds with carbonyl groups in flavonoid compounds. HCl is added to form flavilium salts marked by a color change to orange-red.

The identification test of saponin compounds was carried out by dissolving the sample in distilled water and heating for 15 minutes and shaking it for 10 seconds. Positive contains saponins if the froth persists after adding 2 N HCl. Tanin can be identified as polyphenol compounds, the sample is added with 5% FeCl₃ solution. Positive samples contain tannin/polyphenols if a color changes to blue-black or brownish-green (Harbone, 1987).

2.4.5. Determination of specific water

The pycnometer is cleaned using alcohol and then dried. Weigh the clean and dry empty pycnometer (W1). Fill the pycnometer with distilled water and wipe the outside of the pycnometer dry and weigh (W2). Remove the aquadest and dry the pycnometer, then fill it with the liquid to be measured by its specific gravity, clean and dry the outside of the pycnometer again, then weigh it (W3) (Fickri, 2019). Calculate the specific gravity of the liquid with the formula:

$$\text{Specific Gravity} = \frac{W_3 - W_1}{W_2 - W_1}$$

Description:

W1 = Weight of empty pycnometer

W2 = Weight of pycnometer and distilled water

W3 = Weight of pycnometer and test sample

2.4.6. Preparation of 1.0 N HCl solution

A pipette of as much as 5 mL of 1.0 N HCl was placed into a volumetric flask filled with a few aquadest. HCl was added through a 500 mL volumetric flask's wall and then aquadest up to mark. The volumetric flask was closed and shaken until homogeneous.

2.4.7. Preparation of 0.5 N NaOH solution

Weigh 10 grams of sodium hydroxide, put it in a beaker and dissolve it with sufficient distilled water until it dissolves. Once dissolved, transfer to a 500 mL volumetric flask and add aquadest up to mark. Then shakes until homogeneous.

2.4.8. Determination of the neutralization effect

Weigh the sample according to the concentration and put it in a 250 mL beaker, add it aquadest up to 70 mL. Mixed and stirred using a magnetic stirrer for 1 minute. Measure the pH of the solution using a pH meter. Then add 30 mL of 1.0 N HCl LV and stir with a magnetic stirrer and then measure the pH of the solution. Fill the burette with 0.5 N NaOH solution. After that, titrate with 0.5 N NaOH to obtain pH 3.5, which is stable for 10-15 seconds. Then calculate the amount of mEq (Depkes RI, 2020).

3. RESULTS AND DISCUSSION

Determination of the specific gravity of samples of fresh juice, infusa, and ethanol extract of miana leaves was carried out using a pycnometer. Determination of specific gravity aims to determine the specifications of a sample. The results of testing the specific gravity of miana leaf samples can be seen in **Table 1** below.

Table 1. Results of determination of the specific weight of squeezed juice, infusions and ethanol extract of miana leaves

Sample	Specific gravity (g/mL)
Squeezed juice	0.999
Infusions	1.005
Ethanol extract	1.002

Phytochemical screening of Miana leaves showed that the ethanol extract positively contained secondary metabolite compounds namely alkaloids, phenolics and saponins. Secondary metabolites such as tannin showed negative results. The results of the phytochemical screening test of ethanol extracts of miana leaves can be seen in **Table 2** below.

Table 2. Phytochemical screening results of miana leaf ethanol extract

Phytochemical Screening	Reagent	Parameters	Statement
Alkaloids	Bouchardat	Forms brown to black precipitate	Negative (-)
	Dragendroff	Formed orange brown precipitate	Positive (+)
	Meyer	Forms a white to yellow precipitate	Negative (-)
Phenol	FeCl ₃	Forms a blackish-green color	Positive (+)
Saponins	HCl	Froth formed	Positive (+)
Tannins	FeCl ₃	Red in color	Negative (-)

Identification of alkaloid group compounds in ethanol extracts of miana leaves using dragendroff reagent showed positive results characterized by the formation of orange-brown precipitates. The precipitate formed is a potassium alkaloid (Marliana & Suryanti, 2005). Testing of alkaloid compounds using bouchardat and meyer reagents showed negative results. Phenolic compounds in ethanol extracts of miana leaves showed positive results marked by the formation of a blackish-green color. Testing of phenolic and tannin compounds is done with the addition of FeCl₃. FeCl₃ reagent is widely used to identify phenol compounds, including tannin. The presence of phenol groups is characterized by the occurrence of a blackish-green color. The ethanol extract of miana leaves also contains saponins, which at the time of testing, formed foam after shaking, and the foam remained stable when adding HCl. Saponin has a characteristic of bubbling, so if the extract containing saponin is reacted and shaken with water, the foam will form, which can last a long time. The formation of foam on saponins is caused by the combination of hydrophobic saponins with hydrophilic sugar chains (Batubara & Wahyuni Tri, 2022). Foam is formed due to the content of glycosides that have the ability to form foam in water that is hydrolyzed into glucose and other compounds. The hydrolysis process occurs due to the addition of HCl, which aims to break the glycoside group (Mien *et al.*, 2015). Alkaloid and saponin group compounds contained in the ethanol extract of miana leaves are alkaline (Sumardjo, 2006). These secondary metabolites indicate that miana leaves have a pharmacological effect that neutralizes the increase in stomach acid.

Acid neutralizing capacity (KPA) is one of the factors important in determining the effectiveness of preparation such as antacids. Every preparation of antacids has different strengths depending on the value of each KPA (Deny & Sundani, 2010). The principle of determining the acid-neutralizing capacity is by reacting between excess acid and alkaline solution until it reaches a pH of 3.5, where the pH represents the normal condition of the human stomach acid (Kusumaningtyas, 2012). Basically, the determination of the acid-neutralizing capacity uses acid-base titrations or neutralization reactions using the direct titration method, where the sample solution is directly titrated using a standard solution, namely 0.5 N NaOH.

Determination of acid neutralizing capacity begins with sample preparation, weighing the sample according to concentration, 15 mL of fresh extract, 5 mL of infusion, and 15 mL of ethanol extract. A predetermined number of samples were then added to water to a total volume of approximately 70 mL and mixed using a magnetic stirrer for 1 minute. Furthermore, titrated using the back titration method in which 1.0 N HCl, which had been prepared, was pipetted as much as 30 mL and added to the sample, and then stirred with a magnetic stirrer. Then titrate with 0.5 N NaOH to a stable pH of 3.5 for 10-15 seconds. Record the pH of the sample solution before and after titrated (Pakadang et al., 2015). Then calculate the amount of mEq with the formula:

$$\text{Totals mEq: } (30 \times N \text{ HCl}) - (V \text{ NaOH} \times N \text{ NaOH})$$

This in vitro research procedure represents the dynamic nature of the stomach, especially the stomach in the human body. The solution was prepared by making a standard solution of 0.5 N NaOH and preparation of 1.0 N HCl. This sodium hydroxide (NaOH) solution serves as a secondary standard solution. Hydrochloric acid (HCl) serves as a sample titration solution, namely fresh juice, infusion, and ethanol extract of miana leaves. Determination of the acid neutralizing capacity was carried out using the titration method of 1.0 N HCl excess with 0.5 N NaOH. The titration was stopped when it reached a stable pH of 3.5 for 10-15 seconds, measured using the PH meter. A pH of 3.5 is used because this pH is similar to the pH of stomach acid, which ranges from 2-3.5. Then a calculation of the amount of acid mEq was carried out (Adi et al., 2021). The results of the research that has been done to test the neutralization effect of miana leaves can be seen in **Table 3** below:

Table 3. Acid neutralizing capacity values of squeezed juice, infusions and ethanol extract of miana leaf

Sample	Value (mEq)
Squeezed juice	26.61
Infusions	26.7
Ethanol extract	26.63

According to (Pegu, 2020) and (Salisbury & Terrell, 2022), acid neutralizing capacity (ANC) or gastric acid neutralizing capacity of antacid preparations with standard doses can be measured in mEq. The commonly used antacid drug is aluminum hydroxide $\text{Al}(\text{OH})_3$. In Tables 1, 2, and 3, it can be seen that freshly squeezed miana leaves have an mEq value of 26.616 ± 0.236 , infusa as much as 26.7 ± 0.05 , while the ethanol extract of miana leaves is 26.633 ± 0.076 . According to the book Pharmacology and Therapy 5th edition, one gram of $\text{Al}(\text{OH})_3$ can neutralize 25 mEq of acid. Aluminum hydroxide $\text{Al}(\text{OH})_3$ is an essential compound found in antacid drugs (Gunawan, 2007). Therefore, it can be said that the neutralization effect of infusa and ethanol extract of miana leaves has the same effect as antacids.

4. CONCLUSION

Based on the research that has been done, it can be concluded that the total mEq value of fresh juice, infusion, and ethanol extract of miana leaves are 26.616 ± 0.236 ; 26.7 ± 0.05 ; 26.633 ± 0.076 , respectively. So, it can be concluded that fresh juice, infusion, and ethanol extract of miana leaves neutralize stomach acid.

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