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QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF MAULI BANANA STEM AND BASIL LEAVES EXTRACT MIXTURE

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ABSTRACT

Background*: Mauli banana stem extract and basil leaves extract are ingredients that has been involved in in vitro and in vivo researches for their pharmacognosy and therapeutic applications. Preliminary phytochemical test of both individual extract revealed the presence of triterpenoid, tannin, phenolic, saponin, steroid, alkaloid, and flavonoid compound. A combination of mauli banana stem and basil leaves extract is believed to work synergistically and may have a more beneficial effect than in single herbal extract.* ***Purpose:*** *To analyze the results of quantitative phytochemical of mauli banana stem and basil leaves extract mixture.* ***Method:*** *Non-experimental research with quantitative laboratory examination to determine sample content.* ***Results:*** *The result showed that mauli banana stem and basil leaves extract mixture contain triterpenoid (30%), saponin (24,75%), flavonoid (15%), alkaloid (22%), phenolic (8%), steroid (4,62%), and tannin (0,03%).* ***Conclusion:*** *Mauli banana stem and basil leaves extract mixture correlated with an increase in majority of the bioactive compound compared to the single extract and would serve as a promising source for phytomedicine. The bioactive compound with the highest content was triterpenoid with 30%, while the lowest was tannin with 0,03%.*

Keywords: *Basil leave extract, Mauli banana stem extract, Quantitative phytochemical test*
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INTRODUCTION

The trend of using herbal medicines is emerging tremendously in the last decade. The increase in the consumption of herbal formulations by the public is due to the belief that herbal formulations are more effective, safe, and affordable.[1] Polyherbal formulations have been used in Ayurveda, Indonesian and Chinese traditional medicine practice for many years. Many commercial and non-commercial polyherbal formulations exist in different parts of the world. The lack of phytochemical research on most of the polyherbal formulations has prompted research on the content of bioactive compounds in a mixture of mauli banana stem extract and basil leaves.[2]

Mauli banana stem has diverse and potential medicinal uses in traditional herbal medicine as a wound-healing in South Kalimantan. Basil leaves have been used in traditional medicine to cure various diseases, including stomatitis.[3] Mauli banana stem extract combined with basil leaf extract can achieve a synergistic and non-toxic effect on fibroblasts of Baby Hamster Kidney 21 (BHK-21).[4] Phytochemical test of the bioactive compound of individual medical plants is well established. Mauli banana stem methanol extract contain alkaloid 0,34%, flavonoid 0,25%, saponin 14,49%, and tannin 67,59%. Mauli banana stem water extract contain flavonoid 0,31%, alkaloid 4,15%, hydrolyzed tannin 1,055%, and condensed tannin 0,42%. Basil leaves methanol extract contain tannin 1,07%, triterpenoid 2%, steroid 1,18%, alkaloid 1,4%, saponin 2,23% and phenol 1,27%.[5,6,7]

Previous studies have shown that when the two plants are combined, the antibacterial effect obtained is greater than the effect of the individual plants.[3,8] The bioactive compound responsible for the therapeutic effect are unknown. Standarization of herbal formulations is very

important to ensure the safety, efficacy and quality of traditional medicines used. It is necessary to know the content of bioactive compounds of an extract to standarize.

However, there are no reports available related to bioactive compound contained in a mixture of mauli banana stem and basil leaves extract. Based on this, the present study aims to determine and analyze the quantitative phytochemical in the extract using UV-Vis Spectrophotometry and gravimetric methods.[9, 10]

MATERIAL AND METHODS

This research was a non-experimental research with quantitative laboratory examination to determine the sample content. This research has been declared ethically feasible by the Health Research Ethics Commission of Universitas Lambung Mangkurat No. 044/KEPKG-FKGULM/EC/III/2021. The determination test was carried out at the Laboratory of Mathematics and Natural Sciences, Universitas Lambung Mangkurat. Extracts and quantitative phytochemical tests were carried out at the Biochemistry Laboratory, Faculty of Medicine, University of Lambung Mangkurat Banjarbaru. Tests for the levels of compounds contained in the mixture of Mauli banana stem and basil leaves extract were carried out on seven compounds, namely alkaloid, flavonoid, phenolics, triterpenoids, saponin, steroids, and tannin. Quantitative phytochemical tests were carried out using the uv-vis spectrophotometric method for triterpenoids, steroids, tannin, flavonoid, and phenolic compounds as well as gravimetric methods for alkaloid and saponin. The repetition was carried out three times for each compound. The results obtained were the average value of the errors made.

Materials

The material for the extraction consists of mauli banana stem, basil leaves, and 70% ethanol. The quantitative test material consists of Aqua bidestilata, denis folin preservative (E. Merck), acetic acid (E. Merck), ammonium hydroxide (E. Merck), sodium acetate (E.Merck), NaNO2 (E.Merck), AlCl3 (E.Merck), NaOH (E.Merck), aquadest, folin ciocelteau reaction (E.Merck), and sodium carbonate (E.Merck).

The research equipment consisted of UV-Vis spectrophotometry (Shimadzu 1800), magnetic stirrer (MSH-3), filter paper (WH40), oven (Memmert), measuring glass (Iwaki), micropipet (Dragon lab), reaction tube (Iwaki), funnel (Iwaki), stirring rod. Tools for extraction consisted of extractor, filter paper (WH40), analytical balance (Precisa), erlenmeyer pumpkin (Iwaki), blender (Phillips), oven (Memmert), and waterbath (Memmert).

Extraction Procedure of Mauli Banana Stem

Mauli banana stem as much as 5 kg were taken and cut into small pieces, cleaned, then dried using an oven at a temperature of 40-50℃ for 5x24 hours. Mauli banana stem that have been dried were then mashed using a blender, soaked in 70% ethanol at closed container for 3x24 hours, and stirred occasionally. Solvent was changed daily. The solution was allowed to stand for 4 days until the solute settles. The extraction process was continued using a water bath until a thick extract was obtained.

Basil Leaves Extract

2 kg of basil leaves were taken and cleaned, then dried using an oven at a temperature of 40-50℃ for 2x24 hours. The dried basil leaves were then mashed using a blender and soaked with 70% ethanol in a closed container for 3x24 hours, stirred occasionally. Solvent was changed daily. The solution was allowed to stand for 3 days until the solute settles. The extraction process was continued using a water bath until a thick extract was obtained.

Quantitative Phytochemical Test

The test for tannin levels was carried out by weighing 0.5 grams of the sample and diluted with aqua bidestilata. After dissolving, the sample was transferred to 1 ml of a 10 ml container which already contained 7.5 ml of aqua bidestilata. 0.5 ml of Folin denis reagent was added to the solution, stand 3 minutes, then added saturated $Na_2\ CO_3$ solution. After that, it was incubated for 15 minutes, then the absorption was read by UV-Vis spectrophotometry at a wavelength of 740 nm.

Testing the levels of alkaloid compounds using a sample of 10 grams was put in a 250 ml beaker glass, then 200 ml of 10% acetic acid was added. The beaker glass was closed and allowed to stand for 4 hours, then filtered. A quarter of the extract was evaporated with a water bath and added ammonium hydroxide and then precipitated. The precipitate was washed with dilute ammonium hydroxide and filtered. The residue was evaporated until a constant weight was obtained.

Testing the levels of flavonoid compounds was carried out by pouring 500 L of sample using a micropipette poured into a test tube and adding 2 ml of distilled water. 150 L of 5% NaNO2 was added and allowed to stand for 6 minutes, then 150 L of 10% AlCl3 was added and allowed to stand for 6 minutes. 2 ml of 4% NaOH was added, then diluted with distilled water until the volume of the tube reached 5 ml and allowed

to stand for 15 minutes. The absorbance was measured at a wavelength of 520 nm.

The test of phenolic compounds used a sample of 0.5 ml, added Folin Ciocelteau reagent (1:10) and 4 ml of 1 M sodium carbonate was left for 15 minutes. Measurements were carried out using spectrophotometry at a wavelength of 750 nm. Measurements were carried out three times. Total phenol was calculated using the linear regression equation of the gallic acid calibration curve.

A sample of 40 ml was poured into a separating funnel, and a layer of water was removed to test the presence of alkaloid compounds. The sample was mixed with 60 ml of n-butanol, then washed with 10 mL 5% NaCl, which was then evaporated using a rotary evaporator. The sample was dried in an oven, until a constant weight was obtained which was determined as the weight of the saponin.

Testing of steroid levels was carried out using spectrophotometry in the wave range of 200-800 nm, so that the maximum spectrum and wavelength would be obtained.

Triterpenoid levels were tested by mixing 1 ml of sample with 1.5 ml of chloroform and allowed to stand for 3 minutes. The solution was added 100 m concentrated H2SO4. The solution was then incubated for 1.5-2 hours in the dark until a brown precipitate was formed. The solution was added 1.5 ml of 95% methanol. The absorbance was measured at a wavelength of 538 nm using UV-Vis.

RESULTS

Based on the research conducted, the quantitative phytochemical test results obtained from a mixture of Mauli banana stem and basil leaves extract are listed in Table 1.

Table 1. Average Content of Total Triterpenoids, Saponin, Alkaloid, Flavonoid, Phenolics, Steroids, and Tannin of Mauli banana stem and basil leaves mixture extract.

No.	Bioactive Compound	Quantitative	
		(%)	(mg/g)
1.	Triterpenoid	30,00	304,46 ± 0,57
2.	Saponin	24,75	247,53 ± 0,57
3.	Alkaloid	22,00	219,87 ± 0,98
4.	Flavonoid	15,00	152,83 ± 0,57
5.	Phenolic	8,00	80,68 ± 0,70
6.	Steroid	4,62	46,28 ± 0,14
7.	Tannin	0,03	0,34 ± 0,08

Based on table 1, the results of quantitative phytochemical tests carried out on a mixture of 60% concentration of Mauli banana stem extract and 100% concentration of basil leaves extract showed the total levels of bioactive compounds contained 30% triterpenoids, 24.75% saponin, 22% alkaloid, flavonoid 15%, fenolik 8%, steroid 4,62%, tannin 0,03%. The table shows that the highest total levels of bioactive compounds in the mauli banana stem and basil leaves mixture extract were triterpenoid with 30%, and the lowest is tannin with 0,03%.

DISCUSSION

The results of quantitative phytochemical tests carried out on a mixture of 60% concentration of mauli banana stem extract and 100% concentration of basil leaves extract showed levels of bioactive compound, namely triterpenoids (30%), saponin (24.75%), alkaloid (22%), flavonoid. (15%), phenolics (8%), steroids (4.62%) and tannin (0.03%). In previous studies, mauli banana stem have been investigated for their secondary metabolite content with two different solvents and extraction methods. The methanol extract of the mauli banana stem with the maceration extraction method contained tannin (67.59%), saponin (14.49%), alkaloid (3.44%), and flavonoid (0.25%). The water extract of the mauli banana stem with the digestion extraction method contains alkaloid (4.15%), hydrolyzed tannin (1.055%), condensed tannin (0.42%), and flavonoid (0.31%).[5,6]

In the methanol extract of basil leaves using the soxhletation extraction method, it contains saponin (2.23%), phenolic (1.27%), steroids (1.18%), alkaloid (1.4%), triterpenoids (2%), and tannin (1.07%).[7] This indicates that different solvents and extraction methods can produce variations in the total levels of bioactive compound. The level of dissolved bioactive compound at the time of extraction is influenced by the degree of polarity of the solvent. The solvent polarity index for ethanol is (5.2), metabolized (5.6), water (9.0), and 70% ethanol (7.3).[11]

The difference in the total levels of saponin, alkaloid, flavonoid, and tannin in the two extracts showed that saponin, alkaloid, and flavonoid were more soluble in 70% ethanol extract than methanol extract because 70% ethanol solvent was more polar than methanol. Tannin showed higher levels in the methanol extract of mauli banana stem because their polarity was closer to the polarity of methanol than that of 70% ethanol. This was also caused by differences in the mauli banana stem extract concentration in the two extracts. In this study, the mauli banana stem extract had a concentration of 60%, and the study of the mauli banana stem methanol extract alone had a 100% concentration so that the tannin content was

reduced. Palekahelu research (2018) supports this result, which compares the total tannin content of the kapehu leaves methanol extract of 36.8% and the ethanol extract of kapehu leaves of 6.6%. Kusumo et al., (2017) research also showed that the total content of tannin compounds in the methanol extract of kemuning leaves was 31.5%, so it was more than the ethanol solvent which had a tannin content of 2.52%. [11,12,]

Other factors that affect the levels of bioactive compound are compound interactions and extraction methods. Bhargavi and Shankar (2020) stated that mixing two plant extracts in inappropriate ratios can lead to the formation of hydrogen bonds in the hydroxy groups, thereby reducing the levels and biological effects of the compounds.[13]

The water extract of the Mauli banana stem used the digestion method and the methanol extract of the basil leaves used the soxhletation method. In the digestion and soxhletation methods, there is a heating process to reduce the total levels of secondary metabolite compounds. This is because most bioactive compound are thermolabile such as flavonoid, alkaloid, tannin, phenolics and have limited solubility in extraction solvents at room temperature of 27-30°C. The maceration extraction method used in the mixed extract of the Mauli banana stem with a concentration of 60% and a concentration of 100% basil leaves is not heated so that the natural ingredients do not decompose and allow a lot of secondary metabolite compounds to be extracted. [14.15]

Most of the secondary metabolite compounds in the mixed extract of Mauli banana stem and basil leaves were greater than the single extract. This proves that a mixture of mauli banana stem and basil leaves can work synergistically.

Triterpenoids as bioactive compound with the highest levels in a mixture of mauli banana stem and basil leaves extract can potentially be anti-inflammatory, wound healing, and antibacterial. Plants with high triterpenoid content are dicotyledonous plants.[16] This indicates that the triterpenoid content in the extract mixture comes from basil. Another factor that affects the high levels of triterpenoids in a mixture of extracts of Mauli banana stem and basil leaves is the extraction method used.

Triterpenoids are compounds that are not heat resistant. Maceration method is a cold extraction method so that triterpenoid compounds can be extracted maximally. In the extraction process, the solvent is replaced every day. This affects the secondary metabolite compounds that will be extracted completely. There is an exchange of fluid in the cell during immersion with a solvent having a lower concentration. This process continues until an equilibrium is reached between the concentration inside and outside the cell.

The second highest content in the mixture of Mauli banana stem and basil leaves extract is saponin compounds. Saponin function as antioxidants, antibacterials, antibiotics, accelerate the growth of new cells, antifungal, and stimulate the formation of fibroblasts. Saponin have an effect as a secondary antioxidant by increasing the formation of catalase and superoxide dismutase, and can form hydroperoxides, so that lipid peroxidation is inhibited. [17]

Alkaloid as the third-highest secondary metabolite compound with 22% have the potential for wound healing. Alkaloid have been known to have good wound healing activity. In an in vivo study, topical application of an alkaloid enriched ointment showed high skin healing activity in rat wounds. In in vitro studies, alkaloid have the ability to accelerate the initial phase of wound healing by stimulating chemotaxis for fibroblasts. Alkaloid have been observed to significantly increase wound healing activity ($P < 0.05$), as evidenced by an increase in the rate of wound contraction and an accelerated epithelialization phase. Alkaloid also have antibacterial and antioxidant activity

The fourth highest content is flavonoid. Flavonoid have the potential as antioxidants by capturing reactive oxygen species (ROS) directly, preventing ROS regeneration, and indirectly increasing the antioxidant activity of cellular antioxidant enzymes. [17]

Phenolic compounds as the fifth-highest content have higher antioxidant activity than other group compounds. The role as an antioxidant is related to the number of OH functional groups and the conjugate bonds in the benzene aromatic ring. The hydroxy group in phenolic compounds has a lower bond dissociation energy than other bonds, so that phenolic compounds easily release and donate hydrogen. [18]

The sixth highest content is steroids. Steroids have antibacterial potential, help maintain blood pressure, regulate water and salt balance, and have anti-inflammatory properties. The mechanism of steroids as antibacterial causes leakage of liposomes because they interact with cell membrane phospholipids which causes decreased membrane integrity and changes in cell membrane morphology, causing cell fragility and lysis. [19]

The content with the lowest total content in the mixture of mauli banana stem and basil leaves extract was tannin. Low levels of tannin can be influenced by the length of the extraction time. Based on research conducted by Naima et al (2015), it was stated that the extraction of tannin

with the highest levels was obtained in 2 hours of extraction. Extraction that is too long can damage the tannin. In measuring the total tannin content, folin denis reagent was added. Formation of color based on oxidation-reduction reaction, tannin as reducing agents and folin denis as oxidizing agents.[10,14,20]

The principle of the folin denis method is the formation of a complex blue compound whose absorption can be measured in the visible light region.[20] Tannin have antibacterial and antifungal properties. Apart from being antibacterial and antifungal, tannin also have the potential as an anti-inflammatory. Tannin can increase the activity and number of macrophages. Macrophages will induce growth factor production.[11,16]

It can be concluded that the content of bioactive compounds of mauli banana stem and basil leaves extract mixture increased in most of the bioactive compounds compared to single extracts and had potential as herbal medicinal ingredients. The highest bioactive compounds were triterpenoids (30%), while the lowest bioactive compounds were tannin (0.03%).

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