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Identification of Secondary Metabolite Compounds from *Melandean* (*Bridelian micrantha*) Leaf Extract

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Abstract

The *Melandean* plant or *Bridelia micrantha* (Hochst.) Baill is traditionally used by the community to treat various human diseases, such as poisoning, paralysis, digestive diseases, diabetes, syphilis, and other diseases. *Bridelia micrantha* is believed to have active chemical compounds in the form of secondary metabolites which are useful for treatment. This active chemical compound is contained in the roots, leaves, seeds, skin, stems, and fruit. *Bridelia micrantha* leaves were used as samples in this study to analyze the secondary metabolite compounds contained therein. Leaf extraction was carried out using 80% methanol solvent through a maceration process. Analysis was carried out qualitatively using the phytochemical screening test method and quantitatively using the thin layer chromatography test method. Based on the results of the phytochemical screening test carried out, it is known that *Bridelia micrantha* is positive for containing active compounds in the form of flavonoids, tannins, saponins, and alkaloids. The results of the thin layer chromatography (TLC) test showed that flavonoid compounds were found with an Rf value of 0.437, tannin compounds with an Rf value of 0.312, saponin compounds with an Rf value of 0.625, and alkaloid compounds with Rf values of 0.125, 0.187, 0.312 and 0.5.

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INTRODUCTION

The use of plants or vegetation as medicinal resources for various ailments has been long-standing. Plants are considered a crucial natural source for treating both humans and animals (Petrovska, 2012). In Indonesia, the utilization of plants as medicine is prevalent in both traditional and modern forms of processing (Cahyaningsih et al., 2021; Yanuar et al., 2011). Approximately 80% of medicinal plants in Southeast Asia can be found in Indonesia. Given Indonesia's status as the third-largest tropical forest nation globally after Brazil and Zaire, it serves as a foundation for various medical treatments and potential pharmaceutical discoveries in the future. According to WHO, about 21,000 plant species have the potential for medicinal use, with over 30% of all plant species already employed for medicinal purposes. The estimated number of effective medicinal plants in Indonesia is around 1,260 species (Khan, 2016).

Active chemical compounds used for medicinal purposes are found in various parts of plants such as roots, leaves, seeds, bark, stems, and fruits. The active compound content within plants differs across different tissues (Dias et al., 2016; Espinosa-Leal et al., 2018).

Generally, different parts of plants possess distinct active compound contents, leading to varied effects in combating diseases or neutralizing free radicals entering the body (Balasaheb Nimse & Pal, 2015; Sorokina & Steinbeck, 2020). Plant cells in these different parts contain active chemical compounds like alkaloids, steroids, terpenoids, saponins, and flavonoids (Mir et al., 2016; Nurhasnawati et al., 2019). These plant parts rich in phytocompounds with therapeutic benefits can be developed into potent antimicrobial agents (Atanasov et al., 2020).

Natural or bioactive chemical compounds responsible for the therapeutic properties of plants are known as phytochemicals. Phytochemicals are broadly classified into two categories: primary and secondary metabolites (Asha et al., 2015). Primary metabolites are necessary for the growth and maintenance of plant cells, such as vitamins, amino acids, lipids, carbohydrates, proteins, and nucleotides (Zimmermann et al., 2019). Secondary metabolites, on the other hand, are the end products of primary metabolites and possess several valuable biological properties such as antioxidant, antimicrobial, antibacterial, antifungal activities (Karadağ et al., 2019; Prasathkumar et al., 2021; Ralte et al., 2022; Toiu et al., 2019). Secondary metabolites encompass alkaloids, steroids, flavonoids, tannins, terpenoids, saponins, etc. (Rachana, 2017; Asha et al., 2015). These secondary metabolic products are employed as the plant's defense against attacks from viruses, bacteria, and free radicals that can cause damage or death to the plant.

Secondary metabolites are naturally formed as part of the plant's defense mechanism against various environmental threats. Phenolic compounds like flavonoids, tannins, and lignin play crucial roles in defending against UV radiation, oxidative stress, and pathogen attacks. Flavonoids act as antioxidants, reducing cell damage caused by free radicals (Agati et al., 2020; Terao, 2009). Flavonoids and tannins are two classes of phytochemical compounds commonly found in various plant types. Flavonoids, such as quercetin, kaempferol, and catechin, are present in various foods like fruits, vegetables, tea, and grapes. They offer benefits like protecting cells from oxidative damage, supporting the immune system, and having anti-inflammatory potential (Al-Khayri et al., 2022). Oxidative damage occurs when there's an imbalance between free radical production that harms cells and the body's ability to neutralize them using antioxidants. Free radicals are molecules with unpaired electrons, prone to reacting with other molecules in the body, causing damage to DNA, proteins, and fats within cells.

Tannins are polyphenolic compounds found in various plants like tea, grapes, and some types of fruits. Tannins have a bitter taste and can influence the taste and color of beverages like tea and wine. They also possess astringent properties that can create a dry or puckering sensation in the mouth after consumption. Tannins also have antioxidant properties and are believed to have several health benefits, including potential anti-inflammatory effects and possible protection against heart disease (Zahouani et al., 2021). Both groups of secondary metabolites are extensively used in herbal medicine. Their ability to protect cells from oxidative damage leads to their application as anti-cancer agents. Oxidative damage that can lead to DNA damage causes genetic mutations, potentially leading to the development of cancerous cells due to the loss or weakened control over cell growth (Caliri et al., 2021; Preci et al., 2021; Vodicka et al., 2020). Secondary metabolite groups found in plants also include terpenoids and alkaloids.

Terpenoids comprise compounds such as essential oils and some pigments like carotenoids. They can act as repellents against insects or pests and have antimicrobial effects to protect plants from infections (Abdulaziz et al., 2019; Islam et al., 2023; Sharaf et al., 2022). Alkaloids are compounds commonly exhibiting pharmacological activity in plants. Some alkaloids act as defense mechanisms against herbivores due to their toxic nature, deterring

feeding from pests or herbivorous animals. These compounds have toxic potential and can produce cyanide when hydrolyzed. Despite their danger to consumers, for plants, cyanogenic glycosides can act as defenses against herbivore attacks (Naz et al., 2020). Secondary metabolites involved in environmental defense in plants can be produced in response to specific environmental pressures such as excessive light, water scarcity, or pathogen attacks. These secondary metabolite compounds assist plants in surviving and adapting to changing environmental conditions (Akhi et al., 2021; Isah, 2019; Zaynab et al., 2019). The importance of secondary metabolite compounds in the field of medicine, amidst the emergence of various global health issues, underscores the significance of exploring medicinal plants.

One such plant producing secondary metabolite compounds is the *Melandean* plant or Bridelia micrantha (Hochst.) Baill, which belongs to the Phyllanthaceae family (Asumang et al., 2021; Ce et al., 2020; Maroyi, 2017; Okeleye et al., 2011). The B. micrantha tree ranges in size from small to medium and is considered semi-deciduous. Commonly known as Mitzeerie or coastal golden leaf, this plant can grow up to 20 meters in height with a thick, round trunk and crown. The Bridelia genus includes around 60-70 species found in tropical and subtropical regions worldwide, particularly in Africa and Asia. Some Bridelia species are utilized in traditional medicine worldwide for their anthelmintic, antiamebic, antibacterial, anticonvulsant, antidiabetic, antidiarrheal, anti-inflammatory, antimalarial, antinociceptive, antiviral, hypoglycemic properties, and for ailments related to the stomach, cardiovascular system, gynecology, and sexual diseases (Mahomoodally et al., 2020; Oluwagbamila et al., 2023; Sahithya & Krishnaveni, 2022).

(Ngueyem et al., 2009; Yeboah et al., 2022) reported around 60 Bridelia species (Phyllanthaceae) found in tropical and subtropical regions worldwide, especially in Africa and Asia. Some Bridelia species are employed in popular medications as antiamebic, anti-anemic, antibacterial, anticonvulsant, antidiabetic, antidiarrheal, anthelmintic, anti-inflammatory, antimalarial, antinociceptive, antiviral, hypoglycemic agents, and for conditions related to the stomach, cardiovascular system, gynecology, and sexual diseases. The Bridelia genus, rich in flavonoid and tannin phytochemical groups, serves as a basis for this plant's ability to protect cells from oxidative damage (Mahomoodally et al., 2020; Mondal, Hossen, et al., 2021; Mondal, Kundu, et al., 2021).

Several parts of the Bridelia micrantha plant are traditionally used by African and Asian communities (Maroyi, 2017; Yeboah et al., 2022). In Ivory Coast, this plant is used as a potent laxative in cases of severe constipation and stubborn poisoning. Similarly, in South Africa, the bark of B. micrantha is used in folk medicine to treat paralysis, digestive ailments, and joint pain, while in Nigeria, the bark's utilization is for managing diabetes. In Central Uganda, the plant's leaves and bark are used by boiling to treat syphilis, and the wood is used for early jaundice (Ngueyem et al., 2009 Maroyi, 2017). However, the use of this plant as traditional medicine is not widely practiced in Indonesia. The lack of familiarity among the population with this plant is one reason why it hasn't been extensively used as traditional medicine.

In Indonesia, particularly in West Nusa Tenggara, this plant is only found in East Lombok. The people in East Lombok also use the *Melandean* plant (Bridelia micrantha) as a traditional medicine ingredient for various diseases. The commonly utilized parts of the *Melandean* plant by the locals are its leaves and bark. The leaves are boiled and consumed to treat diarrhea. The bark is dried and used as a therapeutic drink for various illnesses. Previous interviews were conducted with a person suffering from lymph node disease. Based on the interview results, after undergoing therapy by consuming a brew made from the *Melandean* plant's bark, the lymph nodes, initially measuring 0.8 cm x 2 cm, disappeared and there were no further symptoms detected (Nustariza, 2023).

The results of the interviews with individuals who have used Bridelia micrantha as an anti-inflammatory agent and for lymph node symptoms have shown highly positive effects in curing their illnesses. Exploration of this plant needs continuous development because existing literature studies have not yet determined whether this plant possesses the ability to prevent or treat lymph node cancer symptoms. Exploring this plant is crucial to provide assurance to the public if it's scientifically proven safe for consumption as traditional medicine. This native African plant would likely contain different secondary metabolite compounds if grown on another continent or in different environmental conditions. Secondary metabolite compounds are produced as one way for plants to adapt or protect themselves from the influence of their surrounding environment.

METHOD

This research follows the principles of laboratory experimental research with a full replication design. Each treatment in this study will be completely replicated three times. This is done to reduce random errors or the influence of random variability and enhance the validity of the research results. The sample used in this study consists of leaves from the *Melandean* plant (Bridelia micrantha) obtained from Rakam Village, Selong Sub-district, East Lombok Regency. Leaf samples were collected directly from the *Melandean* trees in April 2023 in Selong, East Lombok. The research instruments include tools and materials. The tools used in this research include containers, a blender, analytical scales, black cloth, round-bottom flasks, measuring cups, jars, cheesecloth, aluminum foil, droppers, test tubes, a 50mm mesh, a stirring rod, a hotplate, a stirring rod, and a rotary evaporator. The materials used in this research include *Melandean* leaf samples, 80% methanol, Mg powder, HCl, FeCl3, distilled water, and Mayer's reagent.

Plant Determination

The determination of *Melandean* (Bridelia Micrantha) bark was conducted by bringing fresh or undried parts of the *Melandean* bark to identify the species and ensure the accuracy of the raw material. Determination was carried out at the Advanced Biology Laboratory of Mataram University.

Preparation of Raw Materials

Melandean leaves were sorted when wet to separate foreign materials such as dirt, damaged leaves, or accidentally included other plants. After wet sorting, they were washed to remove adhering dirt using running water. Subsequently, the rinsing process was conducted in perforated containers to facilitate drainage. The rinsing process continued until the residual washing water dried up. Drying was performed by sun-drying covered with black cloth for three days or until completely dry. The dried leaves were then sorted when dry to eliminate impurities like hairs and others that might be present due to physical contact during the drying process. When ready for use, the *Melandean* leaves were pulverized using a blender and sieved using a 40-60 mesh sieve to obtain fine powder for a cleaner extract (Uddin & Alam, 2019).

Methanol Extraction of *Melandean* Leaves

The *Melandean* leaf powder was extracted using the maceration method. Firstly, 400 grams of raw material were weighed and placed in a maceration vessel. Then, 2500 ml of methanol solvent was added. This soaking process was carried out for 3 x 24 hours (72 hours) with stirring once daily. After the first maceration process, the obtained macerate was filtered and subjected to two additional macerations with the same solvent. The resulting macerate was

then evaporated using a rotary evaporator at 50°C until the solvent disappeared (Ulandari & Sani, 2023).

Phytochemical Screening

Qualitative analysis of secondary metabolites in the raw material was conducted using phytochemical screening. Phytochemical screening of the *Melandean* leaf extract (Bridelia micrantha) was performed using a color reaction test method. This test employed specific chemical reagents to produce color changes indicating the presence of particular compounds. The results of each test were recorded in Table 1 (Appendix 1). The groups of compounds tested included flavonoids, saponins, tannins, and alkaloids, conducted as follows (Novilda et al., 2022):

Flavonoid Test. 1 mL of the methanol extract of *Melandean* leaves was added to a test tube, followed by the addition of Mg powder and 1 mL of concentrated HCl. A change in color to reddish-yellow or orange indicates the presence of flavonoids in the extract. **Tannin Test.** 1 mL of the methanol extract of *Melandean* leaves was added to a test tube and mixed with 1 mL of 10% FeCl3. The formation of dark blue or dark greenish color indicates the presence of tannin compounds in the extract**.. Saponin Test.** 1 mL of the methanol extract of *Melandean* leaves was added to a test tube and mixed with 1 mL of concentrated HCl. The mixture was vigorously shaken to produce foam. If the foam remains stable or does not disappear for 10 minutes, it indicates the presence of saponin compounds in the extract. **Alkaloid Test.** 1 mL of the methanol extract of *Melandean* leaves was added to a test tube with 1 mL of 1% HCl and 1 mL of Mayer's reagent. The compound mixture was heated in a water bath for 1 minute. The formation of white precipitate indicates the presence of alkaloid compounds.

Thin Layer chromatography

Phytochemical testing using TLC (Thin Layer Chromatography) was performed on the compound groups that tested positive in the phytochemical screening using specific reagents. Identification with TLC utilized silica gel GF254 plates. Each plate measuring 1x10 cm2 was initially marked with elution boundary lines ± 1 cm from the bottom and top edges. The methanol extract of *Melandean* leaves (Bridelia micrantha) was spotted approximately ± 1 cm from the bottom edge of the plate using a capillary tube, dried, and then eluted with the respective mobile phases of its compound groups. The resulting spots on the TLC plate were measured using UV light at 366 nm. Subsequently, the Rf (Retardation Factor) was calculated for each sample. The calculated Rf values were recorded in Table 2 (Appendix 1). The formula for calculating the Rf is as follows (Fajriaty, et al., 2018).

$$\boldsymbol{Rf = \frac{Spots\ Milliage}{Solvent\ Milliage}}$$

Here are the compound mixtures in the mobile phase used for TLC testing for each compound. **Flavonoid compound**, a mobile phase was prepared consisting of n-hexane:ethyl acetate (3:7), which was then introduced into the chamber and left until saturated. The flavonoid extract was spotted on the TLC plate and placed inside the chamber. **Tannin compound**, a mobile phase was prepared consisting of n-hexane:ethyl acetate (3:7), introduced into the chamber, and left until saturated. The tannin extract was spotted on the TLC plate and placed inside the chamber. **Saponin compound**, a mobile phase was prepared consisting of chloroform-methanol-water (8:2:1), introduced into the chamber, and left until saturated. The saponin extract was spotted on the TLC plate and placed inside the chamber**.. Alkaloid compound**, a mobile phase was prepared consisting of n-hexane:ethyl acetate (7:3), introduced into the chamber, and left until saturated. The alkaloid extract was spotted on the TLC plate and placed inside the chamber.

Data Analysis

The data obtained from the phytochemical screening and thin layer chromatography (TLC) were analyzed descriptively. The results of the phytochemical screening were interpreted to determine the types of compounds present in the sample and their relative content. This data can be used as a basis to assess the phytochemical potential of the sample in terms of biological activity or other potential applications. The Rf values calculated from the TLC test were used for further descriptive analysis to illustrate how the compounds in the sample move on the chromatographic media.

RESULTS AND DISCUSSION

The *Melandean* leaves used to produce the extract in this research amounted to 1 kg. After undergoing wet sorting, drying, dry sorting, grinding, and sieving processes, the result obtained was in the form of powdered raw material. Following these processes, the amount of powdered raw material obtained was 400 grams (figure 1.a).

(a) (b)

Figure 1. (a) Simplicia Powder; (b) Simplicia Exstract

A total of 400 grams of raw material was then subjected to extraction using the maceration method. The maceration process involved the use of methanol as a solvent. The maceration process was repeated twice, and the resulting macerate was concentrated using a vacuum rotary evaporator. Based on this process, the final outcome obtained was a concentrated extract totaling 9.894 grams (figure 1.b). The yield percentage derived from these procedures was calculated to be 2.47%.

The extraction process of *Melandean* leaf powder in this study utilized the maceration method, known for its relatively simple procedures and cost-effectiveness. Additionally, this extraction method does not involve heating, preventing sample degradation and allowing for a higher extraction yield of compounds (Nurhasnawati, et al., 2017).

The initial step in this extraction involved soaking 400 grams of powdered raw material in 2500 mL of 80% methanol solvent for three rounds of 24-hour periods with occasional stirring each day. The utilization of 80% methanol as the extraction solvent in this research was due to its efficiency in extracting bioactive compounds from plant tissues, facilitating further exploration of its potential biological activity (Ferreira & Souto, 2019). After the first soaking process, filtration was conducted to separate the filtrate from the residue. The obtained residue was then subjected to two additional maceration cycles with fresh solvent to maximize compound extraction. The combined filtrates from these two macerations were collected.

The total filtrate obtained from the maceration using 80% methanol amounted to 5 liters. Subsequently, the filtrate was evaporated using a rotary evaporator at a relatively constant temperature of 50°C, resulting in 9.894 grams of concentrated *Melandean* leaf extract. Evaporation aimed to remove the solvent and concentrate the secondary metabolite compounds within the extract. The obtained concentrated *Melandean* leaf extract displayed a dense, dark greenish color, possessed the characteristic aroma of *Melandean* leaves, and exhibited a thick consistency. The extraction yield obtained was 2.47%. According to Hasnaeni, et al. (2019), the extraction yield data is correlated with the active compounds within a sample—the higher the yield, the assumption is that there's a higher amount of active compounds present in the sample.

The Phytochemical Screening Results

The phytochemical screening results indicated that the methanol extract of *Melandean* leaves contained secondary metabolite compounds, specifically showing positive presence of flavonoids, tannins, saponins, and alkaloids. Table 1 illustrates the phytochemical screening results for the methanol extract of *Melandean* leaves.

Table 1. Results of phytochemical screening tests on *Melandean* leaf samples

No.	Tested Compound	Reactant	Figure	Observation Result	Conclusion
1.	Flavonoid	Mg + HCl pekat		Discoloration to red	Positive
2.	Tannin	$FeCl_3$ 10%		Discoloration to blackish-green	Positive
3.	Saponin	HCl pekat		Formed stable foam	Positive
4.	Alkaloid	HCl 1% + Pereaksi Mayer		There is a white precipitate	Positive

The flavonoid test showed a color change to red upon the addition of Mg + concentrated HCl, indicating that the methanol extract of *Melandean* leaves contains flavonoids. In the tannin test, a color change to dark green occurred after adding 10% FeCl3, signifying the presence of tannins in the methanol extract. The saponin test resulted in stable foam formation for approximately 10 minutes, indicating the presence of saponins. Additionally, the alkaloid test

revealed the formation of a white precipitate, indicating the presence of alkaloids in the compound.

The flavonoid test conducted on *Melandean* leaf extract showed positive results for flavonoids. This conclusion was drawn from the intense red color change observed in the methanol extract after treatment with magnesium and concentrated hydrochloric acid. Flavonoids come in various forms, either free as aglycones or bound as glycosides. When flavonoids react with Mg and concentrated HCl, a complex forms between flavonoid ions and magnesium ions, causing the color change. This reaction is characteristic in the phytochemical screening of flavonoids (Kurniati, 2020).

The saponin test conducted in this study showed positive results, indicated by the presence of foam that remained stable for about 10 minutes. Saponins, which are glycosides, can yield sugars and sapogenins upon hydrolysis using water or acidic solutions. Saponins are surface-active compounds capable of forming colloidal solutions. When agitated, they produce foam and bubbles, thus the presence of foam and bubbles indicates the presence of glycosides, signifying the presence of saponin in the sample (Kurniati, 2020).

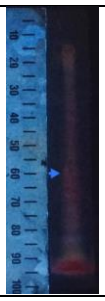
The tannin test conducted through phytochemical screening in this study yielded positive results, evidenced by the color change to dark green. This change occurs due to the reaction between tannin and iron ions (Fe3+). During this reaction, Fe3+ ions bond with phenolic groups in the tannin structure, forming a colored complex. The color change typically ranges from blue to dark green, depending on the type of tannin present in the sample (Newman & Cragg, 2016).

The alkaloid test conducted in this study showed positive results, indicated by the formation of a precipitate. The formation of a brown-colored precipitate serves as an indicator of the reaction between hydrochloric acid (HCl) and alkaloid compounds present in the sample. Additionally, the addition of Mayer's reagent leads to the formation of a white or yellow precipitate. This occurs due to the reaction between alkaloids, potassium iodide (KI), and mercuric chloride (HgCl2), resulting in the formation of a potassium-alkaloid complex that precipitates (Putri & Lubis, 2020).

Based on the Thin Layer Chromatography (TLC)

After the secondary metabolite extraction from Mlandeyan was performed, a subsequent thin-layer chromatography (TLC) test was conducted. This test involved comparing the retention time with the established standards. The results of the TLC test can be seen in the table 2 below.

Table 2. Table of Rf calculation results in thin layer chromatography

No.	Tested Compound	Figure	Stain spacing	Solvent distance	Rf	Stain color
1.	Flavonoid		35	80	0,437	Yellowish orange

2.	Tannin		25	80	0,312	Crimson
3.	Saponin		50	80	0,625	Yellowish orange
4.	Alkaloid		10 15 25 40	80	0,125 0,187 0,312 0,5	Orange

Results in Table 2, it's evident that the flavonoid compound has an Rf value of 0.437, indicating a relatively high affinity towards the stationary phase in the chromatographic media. The tannin compound has an Rf value of 0.317, suggesting similar affinity to sample A1. Meanwhile, saponins exhibited the highest Rf value of 0.625, signifying a high affinity for the stationary phase and a tendency to move further with the solvent. In the case of alkaloids, four spots were identified with Rf values of 0.125, 0.187, 0.312, and 0.5, indicating different affinities among the alkaloid compounds present in the methanol extract of *Melandean* leaves.

The results of the Thin Layer Chromatography conducted on the methanol extract of *Melandean* leaves indicated the presence of flavonoids, tannins, saponins, and alkaloids. This was demonstrated in Table 2, where spots formed, indicating the presence of these compounds within the plate under illumination with a UV lamp at 366 nm. The colors of the spots for samples A1, A2, B, and C were orange-yellow, purple, orange-yellow, and orange, respectively (Table 2). Table 2 presents the calculated Rf values and the colors of the spots formed by compounds contained in the methanol extract of *Melandean* leaves. The spot distance refers to the horizontal distance from the sample's starting point to the middle of the spot formed on the chromatogram. Rf is the value calculated by dividing the distance traveled by the spot with the distance traveled by the solvent on the chromatographic media.

The identification process using TLC aimed to observe the separation of samples based on differences in polarity between the sample and the solvent (eluent). It provided an initial representation of the chemical composition based on the chromatogram pattern (Fajriaty, et al., 2018). TLC was conducted to confirm the positive results obtained from phytochemical screening showing the presence of compound groups. Determination of the best eluent was performed using analytical TLC with various eluent variations in all phytochemical test results.

The extract was applied to the TLC plate, then placed in a chamber containing a saturated solvent combination. Thin Layer Chromatography separation employed a spraying reagent or

a fluorescence indicator to aid in visualizing fluorescent spots on the eluted layer. Fluorescence indicators are compounds emitting visible light when exposed to a specific wavelength like UV light. The color appearance at that wavelength is caused by interactions between UV light and chromophore groups attached by auxochrome in the spots. The visible light fluorescence is the emission of light when the electrons transition from their ground state to higher energy levels and subsequently return while releasing energy (Maulana, 2018).

The optimal separation results are determined by the number of distinct spots, round spot shapes, clear spot separation, and indicative colors of positive compounds. This aligns with literature suggesting that effective separation results in the generation of numerous compounds, absence of tailing in spots, and clear separation. Compounds with low Rf values are more polar compared to those with high Rf values; hence, compounds with low Rf values have a higher distribution coefficient due to their strong retention on the stationary phase (polar) compared to the mobile phase (non-polar) (Maulana, 2018).

Flavonoid Compound

Separation of flavonoid compounds in the methanol extract of *Melandean* leaves was conducted using an eluent composed of n-hexane:ethyl acetate (3:7). After introducing the eluent into the chamber and allowing it to saturate, the flavonoid extract was applied to the TLC plate and inserted into the chamber. After the mobile phase completed, the spots generated were detected by observing under a UV lamp at 366 nm. As per the research results presented in Table 2, the resulting spot color detected under the UV lamp at 366 nm was orange-yellow, indicating a positive presence of flavonoid compounds. Maulana (2018) stated that flavonoids fluoresce and exhibit colors such as yellow, green, or blue. The obtained Rf value was 0.437 (Table 2). According to Rahayu et al. (2015), an Rf value between 0.2 – 0.75 indicates the presence of flavonoids. The relatively low Rf value suggests that the compound is more distributed in its stationary phase, indicating a tendency towards polarity (Maulana, 2018).

Tannin Compound

Separation of tannin compounds in the methanol extract of *Melandean* leaves utilized an eluent of n-hexane:ethyl acetate (3:7). After introducing the eluent into the chamber and allowing saturation, the tannin extract was applied to the TLC plate and inserted into the chamber. Upon completion of the mobile phase, the resulting spots were detected by observing under a UV lamp at 366 nm. The presented results in Table 2 indicate purple-colored spots upon detection under the UV lamp at 366 nm, signifying the positive presence of tannin compounds according to Maulana (2018), which fluoresce purple under UV 366 nm. The Rf value obtained from this tannin test was 0.312 (Table 2). In the study by Ferdinan, et al. (2022), it's mentioned that the Rf value for tannin compounds ranges from 0.07 to 0.77. Based on the Rf value, it can be inferred that the compound positively contains tannins.

Saponin Compound

Separation of saponin compounds in the methanol extract of *Melandean* leaves was achieved using an eluent of chloroform-methanol-water (8:2:1). After introducing the eluent into the chamber and allowing saturation, the saponin extract was applied to the TLC plate and inserted into the chamber. Once the mobile phase concluded, the resulting spots were detected by observing under a UV lamp at 366 nm. The research results in Table 2 showed orange-yellow-colored spots. The positive appearance (saponin) after detection under a UV lamp at 366 nm is characterized by a yellow color (Maulana, 2018), indicating the presence of saponin compounds. The Rf value obtained was 0.625 (Table 2), aligning with the

standard saponin Rf value of 0.62 (Wijaya, et al., 2020), affirming the positive presence of saponins in the sample extract.

Alkaloid Compound

Separation of alkaloid compounds in the methanol extract of *Melandean* leaves was carried out using an eluent composed of n-hexane:ethyl acetate (7:3). After introducing the eluent into the chamber and allowing saturation, the alkaloid extract was applied to the TLC plate and inserted into the chamber. Upon completion of the mobile phase, the resulting spots were detected by observing under a UV lamp at 366 nm. The research presented in Table 2 revealed Rf values of 0.125, 0.187, 0.312, and 0.5 (Table 2). In the study by Ferdinan et al. (2021), the Rf values for the most common 12 alkaloids range from 0.07 to 0.62. These Rf values indicate the presence of alkaloids in the sample extract, ranging from low to high, suggesting the compounds possess varying polarities. The spots formed were round, had clear separation, and exhibited a positive appearance (alkaloid) when detected under a UV lamp at 366 nm, appearing orange. According to Maulana (2018), the colors that emerge under UV observation at a wavelength of 366 nm include orange, bluish-purple, and brown.

CONCLUSION

Based on the research conducted on the methanol extract of *Melandean* leaves (Bridelia micrantha) through phytochemical screening, it was found that there are secondary metabolite compounds present in the methanol extract of *Melandean* leaves, namely flavonoids, tannins, saponins, and alkaloids. The results of thin layer chromatography (TLC) indicate the presence of flavonoid compounds with an Rf value of 0.437, tannin compounds with an Rf value of 0.312, saponin compounds with an Rf value of 0.625, and alkaloid compounds with Rf values of 0.125, 0.187, 0.312, and 0.5.

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