Indonesian Journal of Cosmetic
E-ISSN: 3026-3352

THE EFFECT OF DIFFERENCES IN CHARACTERISTICS OF BIDARA LEAF EXTRACT (ZIZIPHUS MAURITIANA L.) OBTAINED FROM MACERATION, ULTRASONIC, AND SOXHLET METHODS

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ABSTRACT

Bidara leaves are known to contain alkaloids, flavonoids, steroids, tannins, and saponins. This study aims to analyze how the three extraction methods used can affect the characteristics of bidara leaf extract. The extraction methods used in this study were the maceration method, the ultrasonic method, and the soxhlet method. The calculation of the yield value was carried out and the difference was seen with the largest number in the ultrasonic method 10.37% w/w, the soxhlet method 3.49% w/w, and the maceration method 3.68% w/w, then the stock solution calculation was carried out and the results were obtained ultrasonic method 0.1%, soxhlet method 0.0044%, and maceration method 0.012%. The evaluation used was phytochemical screening. The results of phytochemical screening showed that the leaf extract contained secondary metabolites, namely alkaloids, flavonoids, steroids, tannins, and saponins. It can be concluded that the three extraction methods in bidara leaves did not show differences in the presence of secondary metabolites, but affected their yield values.

Key words: Extraction, Bidara Leaves, Maceration, Soxhletation, Ultrasonic, Phytochemical Test

INTRODUCTION

Plants as traditional medicinal ingredients have been widely used for health maintenance, treatment, and beauty (Sakka & Muin, 2023). One of the plants that can be used as an active ingredient in medicines and cosmetics is bidara leaves (Zizhipus mauritiana L) (Masliyah et al., 2021). Bidara leaves (Zizhipus mauritiana L) are a type of tree that grows with green leaves, has fruit and is widely found in the Sumbawa area (Hermawati et al., 2022). Bidara leaf research has become interesting in recent years due to its bioactive

compound content which has the potential to be used as an antioxidant, antifungal, and antibacterial (Maiza et al., 2022). This is because bidara leaves have beneficial content including protein, calcium, iron, magnesium, vitamins, active compounds such as phenols, flavonoids, carotenoids, alkaloids, quercetin, methyl esters, terpenoids, saponins, and so on which are rich in benefits (Chairunnisa et al., 2019b). Secondary metabolite compounds of bidara leaves can be obtained through various extraction methods, the types of extraction methods that are often used include the maceration method, the soxhletation method, and the ultrasonic method (Fauziyah et al., 2022). Extraction is a technique for separating compounds based on differences in their solubility (Angriani, 2019). There are several types of extraction methods that do not utilize temperature, one of which is the maceration extraction method. Maceration is an easy and simple extraction process, which is carried out by soaking the simplicia material in a solvent at cold temperatures discontinuously to extract the desired compounds (Usman et al., 2021). However, the disadvantages of the maceration method are the long extraction time and the use of quite a lot of solvent in the process. During the soaking process, the cell walls and cell membranes will break down due to the difference in pressure between the outside of the cell and the inside of the cell so that the secondary metabolites in the cytoplasm will break down and dissolve in the organic solvent used (Badaring et al., 2020). Generally, the maceration method extraction uses room temperature in the process (Ambaro et al., 2021). In a study conducted by Sarah Chairunnisa et al. from the extraction of bidara leaf simplicia, a yield of 28.92% was obtained (Chairunnisa et al., 2019a). Maceration is divided into several variations of methods such as digestion (low temperature heating), maceration through continuous stirring, remaceration, circular maceration, and multilevel circular maceration, while reflux has only one variation, namely using a Soxhlet apparatus called the soxhlet method (Yuwana & Leseni, 2022). The soxhlet extraction method is a method of separating substances from their mixtures by heating, the solvent used will circulate, compared to the maceration method, soxhlet extraction provides higher extract yields because all the desired components in the sample are perfectly isolated (Wijaya et al., 2019). The advantages of this method include using less solvent because the solvent will be used to repeat the extraction and the hot steam does not go through the powdered simplicia, but through the side pipe (Candra et al., 2021). However, the soxhlet extraction method has several disadvantages, including not being able to be used on materials that have a hard texture, besides the process is complicated and takes a long time, because it must be evaporated in a rotary evaporator to obtain a thick extract (Triesty & Mahfud, 2017). In

a study conducted by Anike et al. who used the soxhlet extraction method of bidara leaves, the yield value was 11.19% (Putri et al., 2022). In general, extraction will be better if the surface of the simplicia powder in contact with the solvent is wider. Thus, the finer the powder of the herbal medicine, the better the extraction. Ultrasonic extraction is a modification of the maceration method. The extract is processed using ultrasound (ultrasonic waves) with a high frequency, with high vibrations, namely 20kHz. The working principle is by observing the acoustic properties of ultrasonic waves propagated through the medium passed through. When the wave propagates, the medium passed through will experience vibrations. The propagation medium with liquid is known as ultrasonic bath extraction (Rizki et al., 2024). Vibration will provide intensive stirring to the extraction process. Stirring will increase osmosis between the material and the solvent so that it will increase the extraction process. The ultrasonic extraction method is also known as sonochemistry, which is the use of the effects of ultrasonic waves to influence changes that occur in chemical processes (Susiloningrum et al., 2023). The main advantages of ultrasonic wave extraction include greater efficiency, shorter operating time and usually faster mass transfer rates when compared to extraction (Edi Setyantoro & Budi Wahjuningsih, 2019). The advantages of extraction using Ultrasound technology are minimal use of extraction time, energy and solvent use. Extraction with Ultrasound technology also facilitates more effective mixing, faster energy transfer, reduced thermal gradients and extraction temperatures, selective extraction, faster response, and greater yields (Badai et al., 2022). This study focused on testing the ultrasonic extraction method because in previous studies this method produced a higher yield and had a higher stock solution concentration than the maceration and soxhlet methods.

MATERIAL AND METHODS

Material

Ziziphus mauritiana L leaves were obtained from the residents' plantation, Sumber Fajar Village, Seputih Banyak, Central Lampung. 96% methanol as an extraction solvent, ice cubes as a cooling medium in soxhletation, and Whatman No. 42 filter paper as a filter. The important tools used are: a set of maceration tools, a set of soxhletation tools (Biobase Soxhlet Extractor Fat), a set of ultrasonic tools (Ovan-Ultrasonik Batch), a vacuum Rotary Evaporator (Ika Rotary Evapolator Rv 10 Digital), and a blender.

Methods

a. Method of Making Simplisia ziziphus mauritiana L

Fresh ziziphus mauritiana L. leaves are sorted wet and then washed until clean. The drained leaves are then dried by airing them protected from direct sunlight at room temperature for one week. The dried leaves are then mashed using a blender. The simplisia is then stored in a closed dry container.

b. Extraction by Maceration Method

Extraction of dry ziziphus mauritiana L leaves (5 grams) was carried out by the maceration method at room temperature using 96% methanol solvent (1:5 w/v). The process was carried out for 30 minutes. The filtrate was separated from the residue using Whatman filter paper. The filtrate was collected and evaporated using a vacuum Rotary Evaporator with a temperature of 60℃ and a speed of 120 rpm until a thick extract was obtained.

c. Extraction by Soxhletation Method

Ziziphus mauritiana L leaves (5 grams) were extracted using a set of Soxhletation apparatus that had been assembled previously. The leaf powder was wrapped in filter paper and placed in a Thimble. 96% methanol solvent (200 ml) was put into a round bottom flask which had been filled with boiling stones. The methanol extract obtained was evaporated using a vacuum rotary evaporator at 60 ℃ until a thick extract was obtained.

d. Extraction by Ultrasonic Method

Ziziphus mauritiana L. leaf simplicia (5 grams) was extracted with 96% methanol solvent (1:10 w/v). The mixture was put into an Erlenmeyer flask (100 mL) and sonicated for 60 minutes at an ultrasonic frequency of 60 kHz. The filtrate was filtered against solid particles with Whatman No. 42 filter paper. Then, it was evaporated using a vacuum rotary evaporator at 60 ℃ until a thick extract was obtained.

e. Phytochemical Test

Phytochemical tests include flavonoid tests, alkaloid tests, terpenoid tests and tannin tests as follows:

Alkaloid-Dragendorff Test

The Alkaloid test was carried out by adding 0.5 mL of the extract sample to 5 mL of aquadest. After that, 5 drops of dragendorff reagent were added to the mixture and the sediment was observed before and after the reaction (Tandi et al., 2020).

Flavonoid Test

The Flavonoid test was carried out by adding 1 mL of the extract sample to 9 mL of methanol solvent, after which 3 drops of NaOH (50%) were added and the color changes that occurred were observed (Susiloningrum & Mugita Sari, 2021).

Steroid/Triterpenoid Test

The Steroid/Triterpenoid test is carried out by adding 5 drops of Libermann-Burchard (LB) reagent to 1 mL of the extract sample, the blue color formed indicates (+) steroids and the red color formed indicates (+) triterpenoids (Kirana Jati et al., 2019).

Tannin Test

The tannin test is carried out by heating 2 mL of the sample in a water bath, then adding 3 drops of FeCl3 (10%). Observe the color change before and after the reaction (Muhammad Nur Fauzi et al., 2021).

Saponin Test

The saponin test is carried out by adding 5 mL of aquadest to 0.5 mL of the sample, shaking vigorously, then adding 2 drops of HCL (2N), then observing the foam for 30 seconds (Kumalasari & Andiarna, 2020).

RESULT AND DISCUSSION

Samples of bidara leaf plants were obtained from the plantations of residents of South Lampung, Lampung, Indonesia. Plant termination showed that the leaves used in this study came from the bidara plant Ziziphus mauritiana L which is a member of the Rhamnaceae family. Ziziphus mauritiana L. is a plant that can grow to a height of about 2 meters and with a stem diameter of approximately 40 cm (Noviyanti et al., 2019). It has round-shaped fruit with a green color, there are seeds and leaves that are green on the front and gray on the back of the leaf, with a single leaf type, and are located alternately on the stalk segments, and the stems and stalks are covered with fine thorns (Maria Ulfa & Junaida, 2023). For the morphology of the ziziphus mauritiana L plant used for sampling, it can be seen in Figure 1. The bidara leaves taken are dark green leaves, this indicates that the bidara leaves are old so that it is expected that there is an optimal chemical content or secondary metabolite compounds in the simplicia (Desni Yetti et al., 2023). Then wet sorting is carried out which aims to ensure that the bidara leaves are not perforated or damaged, and there are no pests on the leaves and the sorting of unnecessary plant parts. Then the washing process is carried out with the aim of removing unwanted foreign substances attached to the leaves, then the bidara leaves are dried using room temperature and indirect exposure to sunlight so that the active substances or secondary metabolite compounds contained in them are not damaged and prevent chemical

changes. A special room used for drying is carried out so that the bidara leaves to be dried are hygienic and free from contamination by foreign substances. Bidara leaves that are in the drying process must be routinely sprayed with 96% alcohol evenly every 2 days. This aims to inhibit the growth of destructive microorganisms that grow on the simplicia sample, so that they can be stored for a relatively long time. Drying at room temperature takes one week to dry completely.

Figure 1. Morphology of the bidara plant (ziziphus mauritiana L)

The final step of the preparation of the next simplicia is dry sorting, this is done to separate dirt and foreign objects such as unnecessary plant parts that are still left in the dry leaf sample that has been dried. The bidara leaf sample that has gone through dry sorting is smoothed using copper which aims to enlarge the surface area of the simplicia so that the extraction process is more optimal.

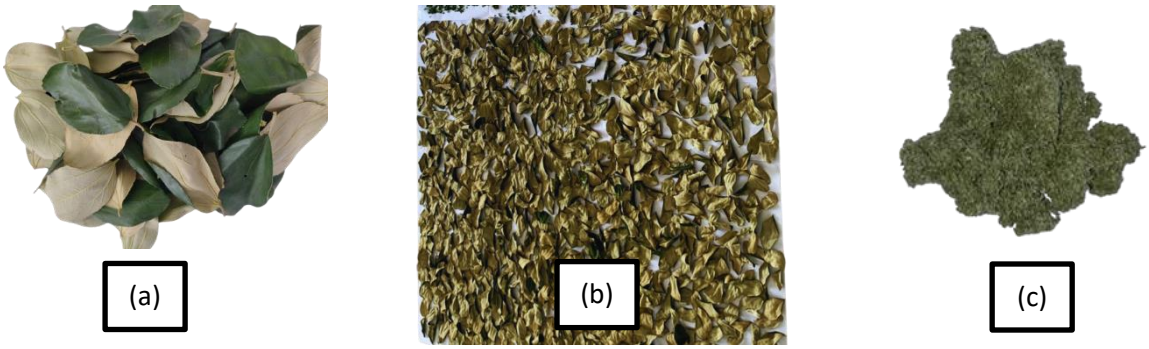
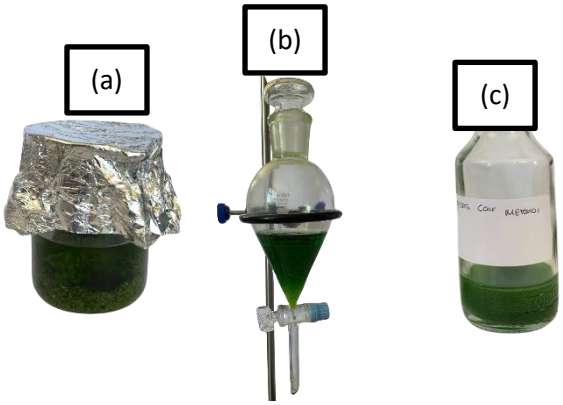


Figure 2. (a) Bidara leaves after wet sorting, (b) Drying process of bidara leaf simples (c) Bidara leaf simples powder

Based on the results of wet and dry sorting until the refinement of the bidara leaf simplicia, differences in characteristics were obtained in the samples. The characteristics of the dried bidara leaves were seen and showed that the results of the dried bidara leaves had a light green color and experienced physical changes in the form of wrinkling, and then the yield value was calculated using the formula and the yield value was obtained as much as 50.05%. % Yield = (Final Weight) / (Initial Weight) × 100%.
The sorted bidara leaf simplicia ziziphus mauritiana L was taken weighing 5 g each which would be used in the extraction process using three different methods: maceration, soxhletation, and ultrasonic. 96% Methanol solvent was used as the solvent medium because it has the ability to dissolve various types of substances, both polar and non-polar.
In the maceration method, Methanol is dissolved in n-hexane so that two phases are formed, namely the methanol phase which is below and the n-hexane phase which is above, this is due to the difference in density of the two solvents. Methanol has a higher density (about 0.79 g/mL) compared to n-hexane (about 0.66 g/mL). This causes methanol to fall to the bottom and n-hexane to be on top. This separation process can be seen in Figure 3.



(Figure 3. (a) Maceration process of bidara leaves, (b) partition of n-hexane extract solution with methanol extract, (c) Maceration results after separation.

The next extraction method is extraction using the soxhlet method. The soxhlet method is carried out for 2 hours and the circulation is calculated as many as 2 times. This circulation process allows the solvent to continue to flow through the sample repeatedly. In the soxhlet method, the extraction process takes quite a long time, which is around 1 hour for 1 cycle. Energy and time play key roles in the soxhlet extraction of bidara leaf simplicia. Energy is needed to heat the solvent used in the extraction, such as water or methanol. Time is also important because the extraction process requires

sufficient duration to allow the active substances to completely dissolve into the solvent in each cycle.

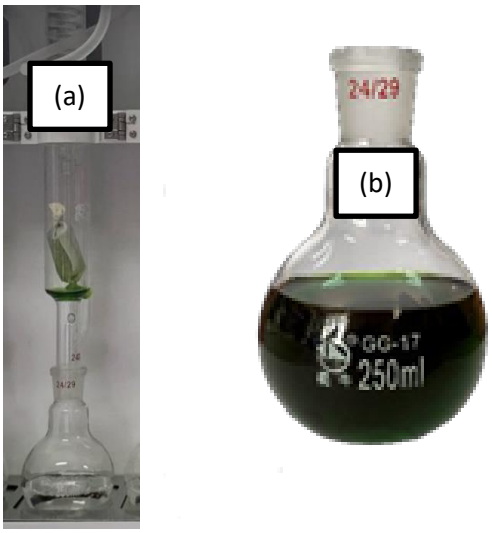


Figure 4. (a) Soxhletation process of bidara leaves, (b) Soxhletation extraction results.

Next, the ultrasonic extraction method was carried out using a sonicator for 1 hour. This process creates conditions that allow the solvent to penetrate the cell tissue and extract the desired compounds more quickly and effectively. After sonication, the mixture turned dark green. This indicates that the active compounds have been extracted into the methanol solvent used. Ultrasonic extraction and extraction results can be seen in Figure 5.

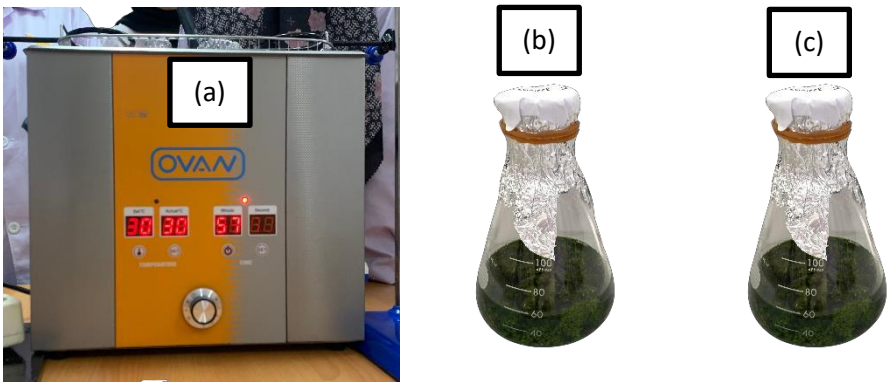


Figure 5. (a) Ultrasonic extraction process, (b) Ultrasonic extraction results.

Vacuum evaporation was carried out to obtain thick bidara leaf extract by evaporating the methanol solvent in it using a temperature of 65oC so that

the secondary metabolite compounds of the plant were not damaged or degraded (Desni Yetti et al., 2023). The sample using the soxhlet maceration and ultrasonic techniques had a dark green color, a leafy aroma and a slightly thick liquid. Based on the extraction method that had been carried out, the yield value was obtained. Determination of the yield results from the maceration, ultrasonic, and soxhlet extraction methods was carried out by comparing the mass of the dry extract (g) with the initial mass of the material before the extraction process (g) through the following calculation: % Yield = (Total final mass of the extract (g))/(mass of the initial simplicia (g))×100% From the calculation above, the yield value for the maceration method was 3.68%, the soxhlet method 3.49%, and the ultrasonic method 10.37%. This calculation was carried out to determine the percentage of the amount of material remaining from the extraction process and to determine the level of effectiveness of the resulting process. After that, it was continued with the calculation of the stock solution which aims to determine the initial concentration of a solution and to avoid repeated weighing using the formula: % stock solution = (Final Mass) / 1 × 100%

The results of the calculation above produce the amount of stock solution with the maceration method of 0.012%, the soxhletation method of 0.044% and the ultrasonic method of 0.1%, seen from the results of the stock solution calculation, it was found that the ultrasonic method had the highest stock solution value among the three, because the extract obtained was more concentrated than other extraction methods.

Phytochemical tests were carried out using bidara leaf extract from ultrasonic extraction because this method has the highest yield value than other extraction methods. Phytochemical screening tests carried out included alkaloid, flavonoid, steroid / triterpenoid, tannin, and saponin tests, the results of which are shown in table 1.

Table 1. Results of Phytochemical Screening of Simple Drugs from Bidara Leaf Extract (ziziphus mauritiana L.)

Nama Senyawa	Ekstrak sebelum ditambahkan reagen	Ekstrak setelah ditambahkan reagen	Keterangan (+)/(-)

Alkaloid			(+)
Flavonoid			(+)
Steroid dan Triterpenoid			(-)
Tanin			(-)

Saponin			(+)

Phytochemical screening is a method used to identify the chemical compounds contained in the extract of bidara leaf simplicia (Ziziphus mauritiana L.) (Mauludiyah et al., 2020). Phytochemical screening is carried out using reagents to detect compounds including alkaloids, flavonoids, steroids/triterpenoids, tannins, and saponins (Alydrus et al., 2023). The bidara leaf simplicia extract is placed in a test tube and then added with a detection reagent. The changes that occur after the addition of the reagent determine the presence or absence of compounds contained in the simplicia extract. Based on table 1 above, it shows that the bidara leaf extract that went through phytochemical screening by observing the color changes and precipitation that occurred, contained secondary metabolites of alkaloids, flavonoids and saponins. In the alkaloid test, sediment was found after the reagent was given, this indicates a positive result. In addition, in the flavonoid test there was a color change from green to yellow after the reagent was given, this indicates a positive result. Furthermore, in the steroid or triterpenoid test, there was no color change after being given the LB reagent, indicating a negative result. Likewise, in the tannin test, there was no color change after being given the reagent, indicating a negative result. Meanwhile, the saponin test showed foam for > 30 seconds after being dripped with HCL, indicating a positive result. The results of the phytochemical screening determined that the extract of bidara leaf simplicia (Ziziphus mauritiana L.) obtained from the ultrasonic extraction method was proven to contain secondary metabolite compounds, namely alkaloids, flavonoids, and saponins. However, for the secondary metabolite compounds tannin and steroids or triterpenoids showed negative results, proving that there were no secondary metabolite compounds in the bidara leaf simplicia extract.

CONCLUSION

Based on the results of research on bidara leaf samples with various methods, the highest yield was obtained in the ultrasonic method with a stock solution concentration of 0.1% and a total yield of 10.37% w/w. Secondary metabolites contained in bidara leaves using phytochemical screening are alkaloids, flavonoids, and saponins. Therefore, bidara leaves can potentially be antioxidant, anti-fungal, and anti-bacterial compounds, in addition, bidara leaf compounds can be useful for active ingredients in cosmetics and medicines.

ACKNOWLEDGEMENT

Thanks for Cosmetic Technology Laboratory, Department of Cosmetic Engineering

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