EXPLORING THE THERAPEUTIC POTENTIAL OF METHANOL LEAF EXTRACT FROM *Ziziphus mauritiana* L. : A CANDIDATE FOR TRADITIONAL MEDICINE

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ABSTRACT

The tropical herbaceous plant known as bidara (*Ziziphus mauritiana* L.) offers a number of health advantages. Bidara leaves are traditionally used in Indonesia, particularly in Aceh, to treat a variety of illnesses. Considering its potential health benefits, this research aims to examine the potential of methanol extract of bidara leaves as a candidate for traditional medicine. This research focuses on the results of screening for phytochemical activity in the methanol extract of bidara leaves and the results of GC-MS analysis of secondary metabolites in the methanol extract of bidara leaves as potential candidates for traditional medicine. This research is expected to be able to identify the compounds contained in bidara leaves as a whole and find out the results of identifying secondary metabolites that have the potential to be candidates for traditional medicine. This study used qualitative descriptive methods for phytochemical screening and GC-MS analysis. Bidara leaf extract was obtained by methanol extraction, and its concentration of secondary metabolites, including alkaloids, flavonoids, saponins, tannins, and terpenoids, was then determined by testing the extract with a number of reagents. To determine the chemical structures of the extract's constituents, GC-MS analysis was performed. The findings demonstrated the presence of several secondary metabolites, including alkaloids, steroids, saponins, flavonoids, and phenolics, in the methanol extract of bidara leaves. Several active substances with potential applications in traditional medicine were found through GC-MS analysis. These compounds have various health benefits including anti-inflammatory, antioxidant, and antimicrobial. This research succeeded in identifying active compounds in the methanol extract of bidara leaves which have potential as candidates for traditional medicine. The results of phytochemical screening and GC-MS analysis show that bidara leaves can be further developed as an effective and safe raw material for traditional medicine.

Keywords: *Ziziphus mauritiana* L., Methanol Extract, Traditional Medicine Candidate, Phytochemical Screening, GC-MS Analysis.

A. INTRODUCTION

The bidara plant, *Zhizipus mauritania* L., is a kind of shrub or small tree that grows in North and tropical Africa, West Asia, and Israel. It produces fruit and is usually green, except in the spring when it sheds its leaves. It thrives in valleys that are up to 500 meters above sea level. Phenolics and flavonoids found in the

bidara plant have anti-inflammatory, antioxidant, antibacterial, and tumor-prevention properties (Lestari *et al*., 2020).

The Bidara plant, also called Widara in Javanese (*Zhizipus mauritania* L.), is a tropical herbaceous plant that, when pressed, generates foam and an extremely fragrant perfume similar to soap, which is used to bathe feverish patients. In Islamic law, the leaves of the bidara leaf plant are sunnah to be used to bathe corpses in order to remove impurity from the corpse's body.used when bathing is mandatory for women who are newly pure after menstruating, then used in the ruqyah process to treat people who are possessed, and as an antidote to jinn such as bay or black magic when planting trees in front of the house(Hadizadeh *et al.,* 2009; Hussen *et al.,* 2010; Michel *et al.,* 2011).

Thousands of different plant species can be found throughout Indonesia. Both conventional and modern medications can be made from the biodiversity that now exists. Traditional medicine has long been known to and utilized by Indonesians to treat a wide range of illnesses. Plants as traditional medicine ingredients have been widely used for health maintenance, treatment and beauty. The medical world has also studied traditional medicine a lot and the results support that medicinal plants contain substances that are clinically beneficial for health. Indonesia has a wide variety of medicinal plants that are utilized as raw materials for pharmaceuticals. Some of these plant species have even undergone clinical testing to determine their phytochemical composition, effectiveness, and safety. (Akhyar, 2010).

Aceh is one of the provinces where people still predominantly use traditional medicine from plants. One of them is that the people of Teu Dayah Village, Kuta Malaka District, Aceh Besar Regency, Aceh often use bidara leaves (*Zhizipus mauritania* L.) for treating boils, cuts, fever, skin diseases, eliminating jaundice, smoothing the skin, and many others. . Based on previous references, bidara leaves (*Zhizipud mauritania* L.) have been proven to be able to eradicate bacteria, fungi and other pathogens.

People also frequently utilize bidara leaves (*Zhizipus mauritania* L.) to cure a variety of internal and external medical conditions. Additionally, bidara leaf powder works well as a face mask. Because its leaves contain saponins, bidara is also very beneficial. Plant-derived saponin chemicals are useful as foaming agents for soap (Lumbanraja, I., Wartini, N., & Suhendra, L., 2019). In other research, it was stated that *Zhizipus mauritania* L. can be an excellent source of carbohydrates, protein and fiber. *Zhizipus mauritania* L.'s leaves, fruit, and seeds have the potential to be used as nutraceutical ingredients in food and pharmaceutical applications. (Jailani, F. et al., 2019). The author is interested in researching the topic "Potential of Methanol Extract of Bidara Leaves (*Ziziphus mauritiana* L.) Aceh as a Candidate for Traditional Medicine" in light of the background information mentioned above."

B. RESEARCH METHOD

1. Research Design

The results of phytochemical screening and identification of GC-MS analysis of secondary metabolites from bidara leaves (*Ziziphus mauritiana* L.) were determined in this work using a qualitative descriptive research approach. Several reagents are used in phytochemical screening, with the types and concentrations varied according to the type of phytochemical test. The following phytochemical

tests are performed: terpenoid, alkaloid, polyphenol, flavonoid, saponin, and tannin assays. Meanwhile, identification of the overall compound uses GC-MS/MS (Gas Chromatograph-Tanden Mass Spectrometry) analysis.

2. Time and Place of Research

The study was carried out between November 2023 and April 2024. *Ziziphus mauritiana* L., often known as bidara leaves, were procured from Teu Dayah Village in Kuta Malaka District, Aceh Besar Regency, Aceh. At Syiah Kuala University's Research Laboratory of the Chemistry Study Program, Faculty of Mathematics and Natural Sciences, phytochemical analysis tasks and secondary metabolite LC-MS analysis tests were conducted.

3. Research Procedures

3.1 Sample Preparation

Bidara leaves that have been washed and cleaned using running water to remove sticky dirt on the leaves are then dried using an oven at a temperature of 50 ± 2 oC. The bidara leaves that have been aired are then arranged on a baking sheet with the same thickness so that the leaves dry evenly. This drying process is carried out until the bidara leaves are easily crushed (moisture content ± 7.56 percent). The resulting dry leaves are then crushed using a blender and sifted using a 60 mesh sieve to produce bidara leaf powder (Bintoro et al., 2017).

3.2 Extraction Process

Bidara leaf simplicia powder (*Zhizipus mauritania* L.) weighing 150 grams was macerated for a day at room temperature with 450 milliliters of methanol before being filtered. After a day of remaceration at room temperature with 450 milliliters of methanol, the residue is filtered. After that, the filtrate was further evaporated in a vacuum rotary evaporator to produce a thick extract. The extract was then concentrated by heating it to 300 degrees Celsius and letting the solvent evaporate. The resulting extract is kept in an extract glass and is used as stock extract. After that, use an analytical balance to weigh the outcomes.

3.3 Phytochemical Screening

1) Alkaloid Test

Bidara leaf simplicia (*Zhizipus mauritania* L.) weighing 150 grams was macerated for a day at room temperature with 450 milliliters of methanol before being filtered. Following a day of remaceration at room temperature with 450 ml of ethanol, the residue is filtered. After that, the filtrate was further evaporated in a vacuum rotary evaporator to produce a thick extract. The extract was then concentrated by allowing it to sit at 30 0C for the solvent to evaporate. The resulting extract is kept in an extract glass and is used as stock extract. Once the thick extract has been produced after 8 days, weigh the data using an abalistic scale.

2) Flavonoid Test

After dissolving 0.1 g of thick ethanol extract in 10 ml of ethanol, the mixture was split among four test tubes. Concentrated H2SO4, concentrated Mg-HCl powder, and NaOH were progressively introduced to the first, second, third, and fourth tubes, which served as the control tube. Each tube's color is compared to

the control tube; if there is a difference in color, the tube contains flavonoids. (Harbone, 2008 in Taher, 2011).

3) Saponin Test

In a test tube, 10 ml of thick extract was agitated vertically for 10 seconds and then left. A foam that is 1–10 cm high and stays steady for at least 10 minutes is a sign of saponin. One drop of 2N HCl is added, but the foam does not go away (Ministry of Health, 1995).

4) Triterpenoid Test

Steroid and triterpenoid examination was carried out using the Liebermann-Burchard reaction. 2 ml of thick extract was evaporated in an evaporator cup. The residue was dissolved in 0.5 ml of chloroform, then 0.5 ml of anhydrous acetic acid was added. Next, 2 ml of concentrated sulfuric acid was added through the tube wall. The formation of a brownish or violet ring at the border of the solution indicates the presence of sterols (Ciulei, 1984).

5) Polyphenol and Tannin Test

1 ml of thick extract is reacted with 10% iron (III) chloride solution, if it changes color to dark blue or greenish black, it indicates the presence of tannin (Robinson, 1991).

3.5 GC-MS analysis

For the GC-MS analysis, 3 grams of extract were dissolved in 1 milliliter of NaOH and heated for two hours at 800 degrees Celsius. The solution was mixed with 9 HCl until the pH was between 7 and 8. Add acetonitrile next, centrifuge for 10 minutes at 4500 rpm, and filter the supernatant using a 0.2 µm filter. After it is ready, the filtrate is placed in the autosampler to be analyzed. A linear gradient solvent system comprising solvent A (water with 0.1% formic acid) and solvent B (acetonitrile with 0.1% formic acid) was used to perform gradient elution. With an injection volume of 2 µm and a temperature maintained at 400C, Hypersil Gold was the column utilized. Samples underwent MS/MS analysis in.

3.6 Data Analysis

Tables and graphs are created using the data gathered from the screening results, and the outcomes are then explained. In the meantime, data is provided in the form of a chromatogram specified in TIC (Total ion chromatogram) and XIC (Extraid ion chromatogram) for GC-MS analysis identification of secondary metabolites.

C. RESULTS AND DISCUSSION

1. Research result

Table 1. Phytochemical screening test results of bidara leaf extract (*Ziziphus mauritania* L.)

Metabolite Compounds	Reagent	Test results	Observation result

Alkaloids	Mayer	+	A white precipitate is formed
	Wagner	+	A brown precipitate forms
	Dragendorff	+	A red precipitate is formed
Steroids	Liebermann-Burchard test	+	A green color is formed
Terpenoids	Liebermann-Burcahrd test	−	No red color is formed
Saponin	Shuffling	+	Foamy
Flavonoids	HCl and Mg Metal	+	A red color forms
Phenolic	FeCl3	+	A green color is formed
Tannin	Gelatin + H2SO4	−	No white precipitate is formed

Information:
+ :Positive for containing metabolite compounds
_ :Negative does not contain metabolite compounds

Table 2. GCMS test results of methanol extract of bidara leaves (*Ziziphus maurtiania* L.)

No.	Retention Time (minutes)	Compound name	Area (%)	Height (%)
1.	5,394	Ethanol, 2-butoxy-	85.97	77.33
2.	6,447	1-[(Trimethylsilly) oxy] propan-2-ol	1.82	2.22
3.	7,487	I-Limonere	3.50	6.32
4.	11,945	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	1.89	2.83
5.	13,241	Trans-Caryophyllene	0.41	1.11
6.	14,278	Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-din	0.69	1.79
7.	15,455	Heneicosane	0.90	2.04

8.	18,807	Hexadecanoic acid, mthyl ester (CAS) Me	4.82	6.36

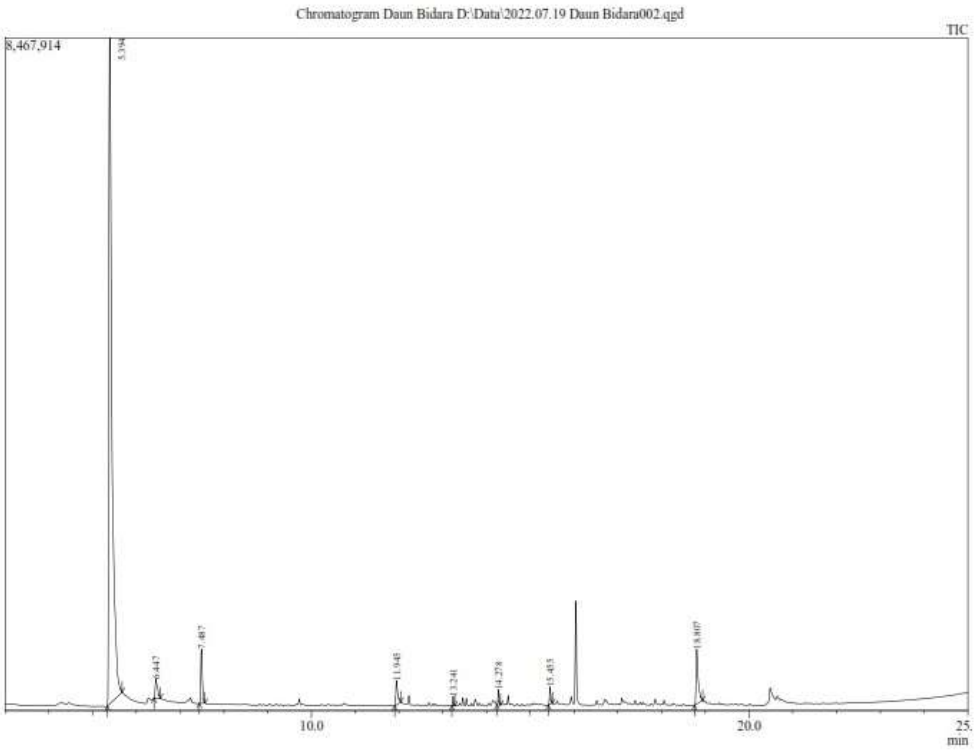

Figure 1. Chomatogrammethanol extract of bidara leaves (Ziziphus mauritania L.)

Figure 2. Bidara leaves(*Zhizipus mauritania* L.)
Source: Personal Documents

2. Discussion of Research Results

Based on the results of the phytochemical screening test, there is secondary metabolite content in the methanol extract of bidara leaves (*Ziziphus mauritania* L.) in the form of alkaloids, steroids, saonins, flavonoids and phenolics.

a. Alkaloids

From the analysis results it is known that the methanol extract of *Zizhipus mauritania* L leaves contains alkaloids. The alkaloid test was carried out using Mayer, Wagner and Dragendorff reagents. Positive test results with Mayer's reagent formed a white precipitate, Wagner's reagent formed a brown precipitate while Dragendroff's reagent formed a red precipitate, HCl was added to the sample because alkaloids are basic so they need to be extracted using an acidic solvent, almost all alkaloid compounds come from plants and are widely distributed in various types of plants. All alkaloids contain nitrogen which is often found in the etherocyclic ring, but some are found in aliphatic structures, which are basic (Lenny.2008).

b. Flavonoids

From the results of the analysis it was found that the methanol extract of Zizhipus Maurtania L. leaves contained positive flavonoids. The flavonoid test was carried out using HCl reagent and Mg metal, the test results were positive and a red color was formed. Plants act as body regulators, regulators of the photosynthesis process, as microbial, antiviral and anti-insecticide substances. Some flavonoids are deliberately produced by plant tissue in response to infection or injury, which then functions to inhibit the fungi that attack them. The reagent commonly used for flavonoids is concentrated HCl which will change the color of the sample to red or orange if the sample contains flavonoids. (Kristanti, et al., 2008).

c. Tannins and Polyphenols

From the analysis results it is known that the methanol extract of *Zizhipus mauritania* L. leaves is negative for tannins and positive for phenolics. The tannin and polyphenol test was carried out using the FeCl3 reagent, the positive test results formed a green color and while the Gelatin + H2SO4 test results were negative, no white precipitate was formed. Tannin is an active plant compound which belongs to the flavonoid group, has an astringent taste and has the ability to tan the skin. When tannin is reacted with 1% FeCl3, the color changes to bluish green (Harbone, 2008).

d. Saponin

From the results of the analysis it is known that the methanol extract of *Zizhipus mauritania* L. leaves is positive for containing saponin. The saponin test was carried out using shaking reagent, the test results were positive for foam. Saponin in a very dilute solution can be a fish poison, apart from that, saponin also has the potential to be an antimicrobial, and can be used as a raw material for the synthesis of steroid hormones. Samples containing saponin will produce foam that lasts for 10 minutes when reacted with 1 M HCl (Hendayana, 2006).

e. Triterpenoids

From the results of the analysis it was found that the methanol extract of *Zizhipus mauritania* L. leaves contained negative triterpenoids. The triterpenoid test was carried out using the Liebermann-Burchard reagent. The negative test results did not produce a red color. Triterpenoids consist of a framework with 3 cyclic 6s combined with cyclic 5 or in the form of 4 cyclic 6s which have a group on a particular cyclic. The test that is widely used is the Lieberman-Burchard reaction (concentrated acetic anhydride-H2SO4) which with most triterpenes and sterols gives a green color. -blue (Abraham, et al., 2006).

f. Steroids

From the results of the analysis it is known that the methanol extract of *Zizhipus mauritania* L. leaves is positive for containing steroids. The steroid test was carried out using the Liebermann-Burchard reagent. The positive test results produced a green color. A group of lipids derived from a saturated compound called cyclopentano perhydro phenanthrene, which has a core with 3 integrated cyclohexane rings and 1 cyclopentane ring joined at the end of the cyclohexane ring. Some important steroid derivatives are alcohol steroids or strols (Effendi, 2014).

The working principle of GC-MS is a partition between the stationary phase and the mobile phase (gas), a liquid sample is injected into the injector and then evaporated. The GC-MS instrument consists of a carrier gas, pressure flow regulator, injection site, column and detector. The carrier gas functions as the mobile phase, the flow and pressure regulator functions to channel the sample vapor into the column. The column is the heart of chromotography. The detector functions as a separate sample detector (Sastrohamidjojo, 2005).

Identification of active compounds using Gas Chromotography Mass Spectrometry (GC-MS) was carried out to determine the active compounds contained in the bidara plant extract. The crushed bidara leaves are extracted in the form of a paste, using ethanol solvent and soaking (maceration) technique. A sample of 47.8 grams of daum bidara and 1.25 ml of methanol solvent, the resulting extract was then continued with GC-MS analysis, the GC-MS data showed that more than 21 bioactive compounds were obtained with RT and Area values which were divided into 8 groups. from 4.82 to 85.97.

Based on the data in the picture. 1 and table. 2 it can be seen that the methanol extract of bidara leaves contains very many bioactive compounds. The high content of bioactive compounds shows the great potential of bidara leaves to be used as an alternative source of antioxidants. In table. 2 it can be seen that the bioactive compound content contained in bidara leaves with the lowest area percent is Trans-Caryophyllene with a molecular weight of 0.41 g/mol.

In order to utilize this potential, further research needs to be carried out to analyze one by one or in groups the bioactive compounds that can be utilized and their activity known both in vitro and in vivo as well as which bioactive compounds act as antioxidants in traditional community medicines.

In vitro activity can be carried out by testing in cell cultures. Meanwhile in vivo. From the database produced in this research, we can also hope that this research has provided an important picture of the content of bioactive compounds

contained in bidara leaves and in the future it is also necessary to carry out research on other plant parts besides the bidara plant.

D. CONCLUSION

The results of phytochemical screening on the methanol extract of bidara leaves (*Zizhipus mauritania* L.) contained secondary metabolite compounds in the form of alkaloids, steroids, saponins, flavonoids and phenolics. Based on the GCMS test results, 21 chemical compounds were produced which act as candidates for traditional Acehnese medicine.

It is intended that more research may be done to examine individual or group bioactive compounds that are useful and whose in vitro and in vivo activities are well-understood, as well as which bioactive compounds function as antioxidants in conventional community medications. Testing in cell cultures is one method of doing in vitro activity. In the interim, in vivo. From the database produced in this research, we can also hope that this research has provided an important picture of the content of bioactive compounds contained in bidara leaves and in the future it is also necessary to carry out research on other plant parts besides the bidara plant.

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