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Botany, Pharmacognosy, and Phytochemical Study of *Gymnanthemum amygdalina* (Delile) Sch.Bip. Leaves from Bandung, West Java, Indonesia

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ABSTRACT

Gymnanthemum amygdalina (Delile) Sch.Bip. possesses antioxidant, antibacterial, anticholesterol, anticancer, and antidiabetic properties. This study aimed to determine the morphology of leaves, stems, flowers, and roots, as well as the microscopic powder characteristics, phytochemical profile, and Thin Layer Chromatography (TLC) pattern of *G. amygdalina* leaves. The samples were collected from Bandung City, West Java, Indonesia. Methods included morphological examination, microscopic powder analysis, phytochemical screening, and TLC. The morphological characterization of *G. amygdalina* showed that the leaves measured 10–22 cm in length and 2–8 cm in width, with an oblong shape, acuminate tips, acute bases, pinnate venation, serrated margins, rough surfaces, woody stems, and a taproot system. Microscopic examination revealed fragments of trichomes. Phytochemical screening indicated the presence of alkaloids, flavonoids, saponins, tannins, and terpenoids/steroids. TLC analysis showed five red spots with an average Rf value ranging from 0.06 to 0.58. The novelty of this study lies in the comprehensive pharmacognostic characterization of *G. amygdalina* collected from Indonesia, particularly West Java, which has never been previously reported. While previous studies mainly focused on the biological activities or phytochemical constituents of *G. amygdalina* from Africa or other regions, this research provides the first detailed morphological, microscopic, and chromatographic profiles of Indonesian-grown *G. amygdalina*. These findings contribute valuable baseline data for the standardization, authentication, and future pharmacological utilization of *G. amygdalina* as a potential medicinal plant in Indonesia.

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INTRODUCTION

Gymnanthemum amygdalina (Delile) Sch.Bip. is a medicinal plant belonging to the family Asteraceae, originally from Africa. This plant is commonly known as "bitter leaf," while in Indonesia it has the local name "African leaf." Traditionally, *G. amygdalina* has been widely used as a vegetable and as herbal medicine due to its therapeutic effects. One of its notable pharmacological properties is its ability to reduce blood glucose levels, which has been utilized by the people of Talang Kelapa, Palembang, as a traditional therapy for diabetes mellitus (Habisukan & Suhertini, 2024).

Several pharmacological studies have reported significant bioactivities of *G. amygdalina*. Putri (2019) demonstrated that treatment with 400 mg/kg BW African leaf extract in diabetic mice reduced fasting blood glucose levels by 32.1%. Similarly, Gumilar (2022) reported that administration of 250 mg/kg BW *G. amygdalina* leaf fraction decreased blood glucose in diabetic mice by 61.9%. In addition, Daturara et al. (2024) found that 20% ethanol extract of *G. amygdalina* leaves promoted wound healing in rabbits, with complete closure observed on the eleventh day. Beyond antidiabetic and wound-healing activities, *G. amygdalina* has been scientifically validated as an antioxidant, antibacterial, anticholesterol, anticancer, and antidiabetic agent (Wahyudi et al., 2024).

The pharmacological potential of *G. amygdalina* is closely associated with its diverse secondary metabolite profile. According to Bestari (2021), the plant's activity is attributed to the presence of bioactive compounds such as flavonoids, saponins, alkaloids, tannins, phenols, and terpenoids. Several secondary metabolites have been successfully isolated, including vernonioside A1, vernonioside A2, vernonioside B1, vernonioside B2, vernodalin, vernolepin, vernomygdin, vernodalol, vernodalinol, vernoamyoside, luteolin, luteolin 7-O-β-glucoside, and luteolin 7-O-glucuronide (Alara et al., 2017). Kaur et al. (2019) further emphasized that flavonoid compounds from *G. amygdalina* can significantly reduce blood glucose levels and regenerate pancreatic beta cells in diabetic rats. Moreover, research by Fermin et al. (2024) revealed that leaf extracts at concentrations of 500–4000 ppm from Nueva Ecija, Philippines, exhibited cytotoxic activity against human hepatocarcinoma cell lines (HepG2).

Given its wide range of pharmacological activities, *G. amygdalina* has been commercialized in the form of dried simplicia and powders as raw materials for herbal and pharmaceutical preparations. However, the increasing demand has raised concerns about adulteration and substitution, which can compromise safety and efficacy. Without proper authentication, counterfeit or misidentified raw materials may fail to provide the intended medicinal benefits and could even pose health risks. Thus, generating basic scientific data on the identity of *G. amygdalina* leaves is essential to support its safe utilization in the pharmaceutical industry.

One of the fundamental approaches to ensure authenticity is through botanical, pharmacognostic, and phytochemical studies. The botanical study involves identifying morphological characteristics such as leaves, stems, and roots. The pharmacognostic evaluation focuses on microscopic powder analysis to identify diagnostic fragments, while the phytochemical investigation includes preliminary screening of secondary metabolites and chromatographic profiling, such as Thin Layer Chromatography (TLC). The novelty of the present study lies in the sampling of *G. amygdalina* leaves from Bandung City, West Java, Indonesia, where scientific data are still very limited compared to studies conducted in Africa or other regions. The findings from this study are expected to provide basic but essential information on the botanical, pharmacognostic, and phytochemical characteristics of *G. amygdalina*. Furthermore, the data can serve as a reference for future pharmacological studies and educational resources in plant biology, pharmacognosy, and natural product chemistry.

MATERIALS AND METHOD

This research tool includes a light microscope (Olympus CX23LEDDRFSI), TLC chamber (Camag), UV lamps, silica gel plate 60 F254 (10 cm × 2 cm), rotary evaporator (IKA-RC 2). The meterials used are *G. amygdalina* obtained in Cipongkor, Bandung, West Java, Indonesia, reagents dragendroff, Mayer, Wagner, methanol, anhydrous acetic acid, HCl 2N, $FeCl_3$ 5% and 10%, ammonium hydroxide, anhydrous

acetic acid, sulfuric acid, aquadestilata, chloralhydrate, HCl(p), chloroform, magnesium powder, dichloromethane, and ethyl acetate.

Sample preparation. Sample preparation includes plant determination, washing, wet sorting, drying, dry sorting, and powder making (Perwitasari et al., 2023)

Macroscopic and microscopic powder examination. Macroscopic examination of *G. amygdalina* leaf powder includes colour, odour, and taste. Microscopic examination was performed by placing simplicia powder on top of the object glass, dripping it with chloralhydrate, covering it with cover glass, fixing it on a bunsen burner, and then observing it with a 10x,40x, and 100x magnification microscope.

Extraction. Extraction was carried out for 3 days by putting 150 grams of simplicia powder in a container containing 1.5 L ethanol 70% solvent (stirring every 1 day). The liquid extract is filtered. The liquid filtrate is evaporated with a rotary evaporator (temperature 40℃, 60 rpm) to obtain a viscous extract (Anindita et al., 2022a). The results of the viscous extract are then weighed and calculated as a percentage (%) of the yield with the formula;

$$\% \text{ yield } \frac{\text{viscous extract weight (gr)}}{\text{Simplicial powder weiht (gr)}} \times 100\%$$

Phytochemical screening. Phytochemical screening with colour reagents includes testing for alkaloids, flavonoids, saponins, tannins, and terpenoids/steroids. Alkaloid tests are performed with Meyer, dragendorf, and Wagner reagents. Flavonoid test with Mg and HCL reagents. Test Tannins with $FeCl_3$ reagents. Saponin test with Aquades reagent and HCl. Terpenoid/steroid test with Chloroform reagent, H_2SO_4 anhydrous acetic acid. Standard alkaloid solutions use caffeine; flavonoids use quercetin, and tannins use tea. The test sample solution and the comparison standard were prepared by dissolving 500 mg of the sample solution in 50 mL of 70% ethanol. Positive results of alkaloids with Meyer reagent if there is a white precipitate, dragendorf reagent orange precipitate, brown precipitate Wagner reagent, flavonoid positive if red/orange is formed, tannin positive if blackish-blue or blackish-green is formed, saponin positive if stable foam is formed for 5 minutes, terpenoid/steroid positive if bluish-green is formed (Anindita et al., 2024a).

Thin Layer Chromatography (TLC). The TLC method was carried out by preparing 60 GF254 silica gel plate, capillary tube, and the mobile phase of chloroform: dichloromethane: ethyl acetate (7:4:1). Before use, the silica gel plate is heated for 10 minutes at a temperature of 100 ◦C and saturated with a chamber containing a mobile phase for 30 minutes. The test solution sample was prepared by dissolving ethanol extracts of *G. amygdalina* leaves in a 1 mg/mL methanol concentration. Standard quesertin is dissolved in methanol at a concentration of 10µg/ml.

The sample and quesertin standard solution are dripped on the TLC plate using a capillary tube 1 cm from the bottom and top. TLC plates are inserted in the chamber that has been saturated. The phase of mobile is observed until it is almost close to the upper limit of the TLC plate. The plates are lifted and allowed to dry. TLC plates were subsequently illuminated under short-wave UV (254 nm), long-wave UV (366 nm), and white light by using TLC-Visualizer (Kartini et al., 2020). The results of the spots were marked using a pencil, and then the sample Retention factor (Rf) value was calculated and compared with the standard Rf value of the comparator. The formula used to calculate the Rf value is:

$$Rf = \frac{distance\ moved\ by\ solute}{distance\ moved\ by\ solvent}$$

RESULTS AND DISCUSSION

The plant determination in this study was carried out at the National Innovation Research Agency, Cibinong, West Java, Indonesia. Based on the results of the determination test Number: B-777/II.6.2./IR.01.02/3/2024 shows that the sample used has the Latin name *Gymnanthemum amygdalina* (Delile) Sch.Bip with the Asteraceae family. The results of identifying the morphology of the leaves, flowers, stems, and roots of *Gymnanthemum amygdalina* plant can be seen in **Figure 1.**

Figure 1. A. Morphology plant *Gymnanthemum amygdalina.*B. Leaf. C. Stem. D. Root

Based on **Figure 1**, it can be seen that *G. amygdalina* has leaf morphological characteristics with a length of 10-22 cm, a width of 2-8 cm, oblongus shape, acuminate tips and acute bases, pinnate leaf bones, serrated leaf edges, rough leaf surfaces, woody stems, and taproots. According to Kaur et al. (2019),, the

G. amygdalina plant has a tree habitus above 10 meters. Leaf size 10-15 cm x 4-5 cm, dark green, acuminate tip, acute base, smooth serrated leaf edges, short stalk 1-2 cm. The plants that have been identified are then made leaf simplicia powder. The results of examining the macroscopic powder of *G. amygdalina* leaves can be seen in **Figure 2.**

Figure 2. Macroscopic powder leaves of *G. amygdalina.*

Figure 2 shows that both samples have a bitter taste. The characteristics of macroscopic powder are that the leaf powder of *G. amygdalina* is dark green. Sample was then observed for microscopic powder. The results of the microscopic powder examination can be seen in **Figure 3.**

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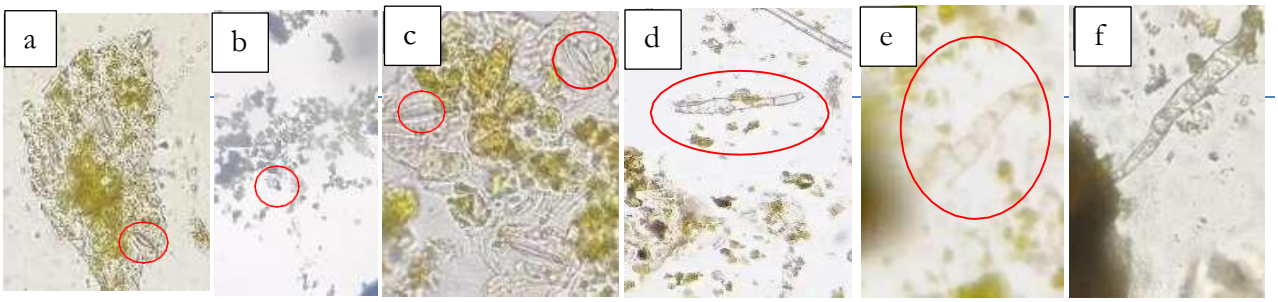


Figure 3. Microscopic powder marker fragments of *G. amygdalina* leaves. a. stomata (10x magnification). b. stomata (40x magnification).c. stomata (100x magnification). d. trichome (10x magnification). e. Tricome (40x magnification).f. Trichome (100x magnification).

Based on **Figure 3,** it can be seen that the characteristic of the microscopic powder marker fragment of *G. amygdalina* leaf is trichome and stomata. The results of this study in accordance with (Karlina et al., 2023), microscopic powder marker fragments of *G. amygdalina* simplicia leaves showed transport bundles with mesh-type thickening, parenchyma with rosette-shaped, calcium oxalate crystals, covering hairs, sclerenchyma, lower epidermis with stomata, and leaf mesophyll, and scale hairs. The powder was then extracted using a 70% ethanol solvent and evaporated to obtain a viscous extract. The results of the viscous extract can be seen in **Figure 4**, while the percentage of condensed extract yield can be seen in **Table 1.**

Figure 4. viscous extract. *G. amygdalina*

Table 1. Percentage of viscous extrac

Sample weight	Extract weight	Rendemen (%)	Standard (Farmakope Herbal Indonesia
150 g	26.49 g	17.66 %	No less than 11.8 %

Based on **Figure 4**, it can be seen that percentage of viscous extract yield of *G. amygdalina* in Table1 was 17.66 %. The percentage of yields of *G. amygdalina* according to the standard of herbal pharmacopoeia, less than 11.8%. The results of the percentage of yield in this study complement the research of (Kartikawati et al., 2021), samples with a weight of 250 grams given a can produce a percentage of yield of 19,6%, while (Rahmadani et al., 2021), use 200 grams of powdered leaves, stems, bark of *G. amygdalina* from East Kutai using methanol solvent can produce a percentage of yield of 2.66%, 4.15%, 3.70%. Other screening results were carried out by (Sukmawati et al., 2017) using 100 grams of powdered leaves of *G. amygdalina* from Ternate, North Maluku.

According to Anindita et al. (2022), ethanol has semi-polar properties that can dissolve secondary metabolite compounds with high, medium and low polarity. Polarity compatibility makes it easier for penetrating ethanol to enter the membrane of the simplicia cell and bind to secondary metabolite compounds, thus affecting the yield value produced during maceration extraction.

The results of the viscous extract were then qualitatively screened for phytochemicals using colour reagents to test for the presence of alkaloids, flavonoids, tannins, saponins, and terpenoids/steroids. The phytochemical screening test in this study used standard (positive control), caffeine as an alkaloid, quercetin as a flavonoid, and tea as a tannin. The results of the phytochemical screening test with colour reagents can be seen visually in **Figure 5**, while the qualitative results can be seen in **Table 2.**

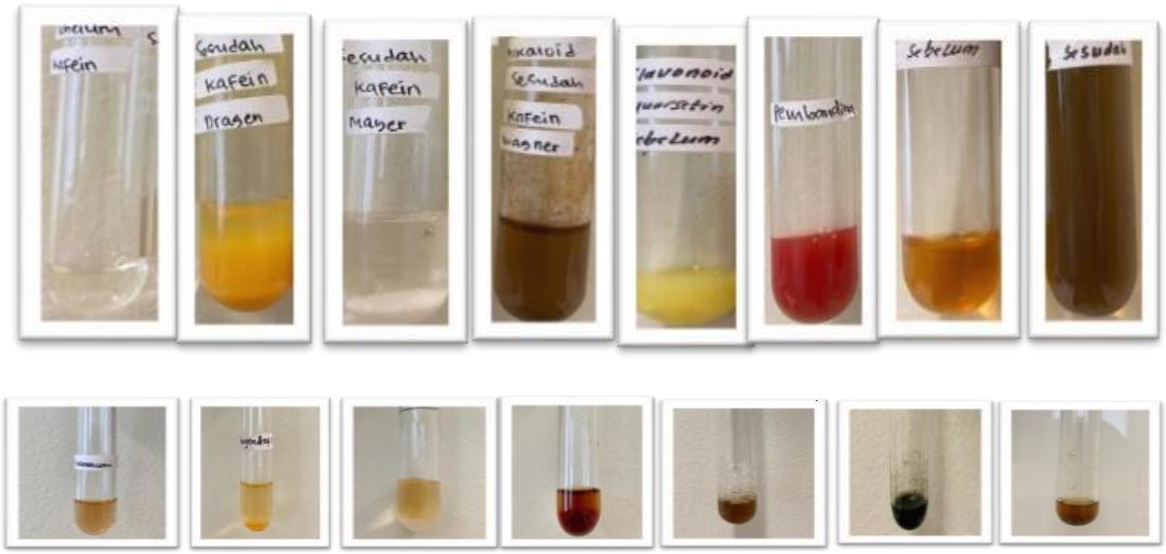


Figure 5. Phytochemical screening test standard. a. caffeine (alkaloid). b. dragendorff (orange precipitate) test. c. Meyer test (White precipitate), d. Wagner (brown precipitate). e. quesertin (flavonoid). f. Flavonoid (orange) test. g. Tea (tannins). H Tannin test (Blackish green). 1. leaf extract *G. amygdalina* before being dripped with the colour reagent. 2. Dragendorff test. 3. Meyer test. 4. Wagner test. 5. Saponin test. 6. tannin test. 7. terpenoid/steroid test

Table 2. Phytochemical screening test

Compound	Reagen	Indicator
Alkaloid	Mayer	Whit e precipitate
	Dragendorf	Oran ge precipitate
	Wagner	brown precipitate
Flavonoid	HCL +Mg	Orange
Saponin	Aquadest	Stable foam 1-2 minutes
Tannin	$FeCl_3$ 1%	Blackish green
Terpenoid/steroid	Chloroform+anhydrous acetic acid +H_2SO_4	Browning ring at the solvent boundary

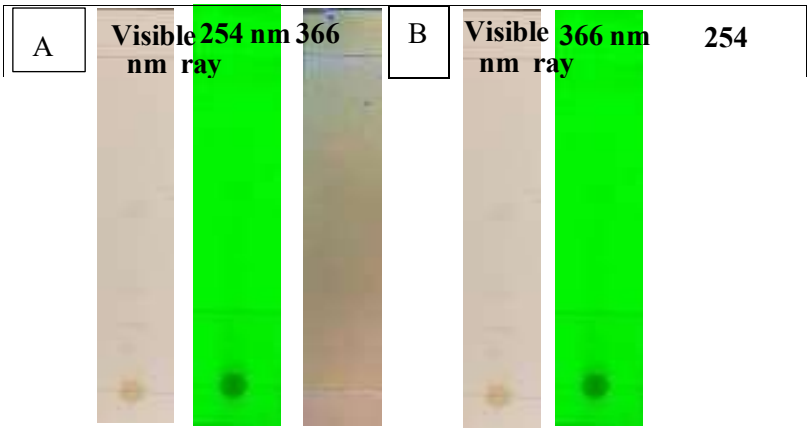
Phytochemical screening is a procedure to detect the presence of secondary metabolite compounds in natural materials. The detection is shown through a colour reaction between the reagent and the test

compound. The existence of a colour reaction will provide an overview of the initial prediction of a natural material compound (Anindita et al.,2024). Based on Figure 5 and table 2. Phytochemical screening tests for alkaloids were carried out with a caffeine standard after the dragendorff test was positive to produce an orange precipitate, the Meyer test was positive to produce a white precipitate, the Wagner test to produce a brown precipitate, and the flavonoid test was carried out using a quercetin standard and positively produced orange. In contrast, the tannin test used a tea standard and positively produced blackish green; regarding control results, phytochemical screening tests were carried out for *G. amygdalina* ethanol extracts.

The results of this study are in accordance with (Meilani & Kusumastuti, 2019), (Kartikawati et al., 2021), (Pratiwi & Elsye, 2018), *G. amygdalina* positive test samples contained alkaloids, flavonoids, tannins, saponins, and terpenoids/triterpenoids. However, phytochemical screening of *G. amygdalina* leaf extract purified with n-hexane: water (1:1) showed negative results for alkaloids ((Karlina et al., 2023). (Rahmadani et al., 2021) research with *G. amygdalina* leaves from East Kutai resulted in negative alkaloids and saponins, the dominance of positive results for secondary metabolite compounds was found in stem simplicia. Phytochemical screening by Fermin et al. (2024) using *G. amygdalina* leaves from Ecija Baru, Philippines, was positive for all secondary metabolites. Based on the review of Alara et al. (2017) several bioactive compounds isolated from *G. amygdalina* leaves include flavonoids, saponins, alkaloids, tannins, phenolics, terpenes, steroidal glycosides, triterpenoids, and several types of sesquiterpene lactones. Sesquiterpene lactones (vernodalinol, vernolepin, vernomygdin, hydroxyvernolide, vernolide and vernodalol) have been reported to be able to inhibit the growth of cancer cells, tumors, and bacteria. Flavonoids, tannins, saponins, and triterpenoids have the potential as antioxidants and hypolipidaemic. The results of the study by (Vonia et al., 2022) proved that luteolin as one of the flavonoids in *G. amygdalina* extract from Bandung was able to inhibit alpha-glucosidase activity by 6.02% -8.18%. This study also conducted a thin-layer chromatographic test using a quercetin standard as a control for flavonoid compounds. The results of the thin-layer chromatography test observed in this study were band distance and Rf value using UV 254 nm and UV 366 nm. The mobile phase is chloroform dichloromethane ethyl acetate (7: 4: 1) with 3x replication tested. The results of the thin-layer chromatography test can be seen in **Table 3**.

Table 3. Thin-layer chromatographic test *of G. amygdalina leaf*

Spots	Avg. Rf	Visible rays	254 nm	366 nm
1	0.06	Green	Attenuation	Red
2	0.12	Green	Attenuation	Red
3	0.21	-	-	Red
4	0.32	-	-	Red
5	0.58	-	-	Red



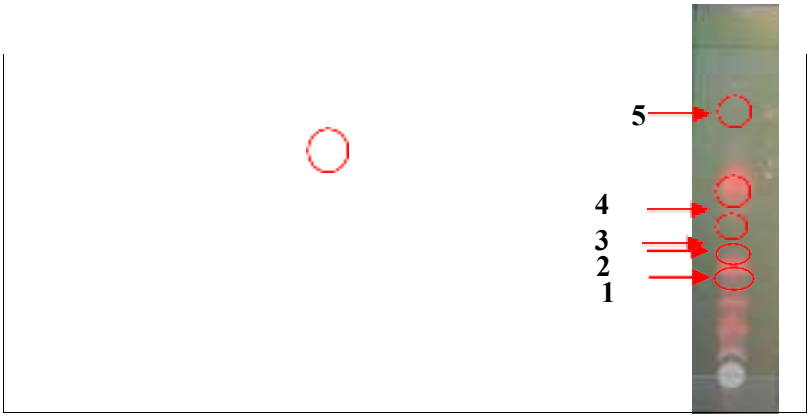


Figure 6. A. Standard (quersetin). B. *G. amygdalina*

Based on **table 3** and **figure 6**. The results of the TLC of *G. amygdalina* leaf of ethanol extract with obtained five spots with red colours. The average Rf value of *G. amygdalina* of ethanol extract was 0.06-0.58. The average Rf value is close to the quercetin standard Rf value : 0.71 at UV 366 nm. Quercetin is a flavonoid compound that can form colour complexes. The flavonoid spot produced on the TLC plate are red with a range of Rf values of 0.69-0.81. The result of spot no 5 with an Rf value of 0.58 is likely an alkaloid. This is in accordance with the research of (Monoarfa, 2016) using n-hexane:ethyl acetate (1:3) to produce an orange stain (Rf=0.56) which indicates positive alkaloids. The results of this study did not produce flavonoid spot from TLC plates. This indicator is based on evidence from Karlina (2023) which uses the n-butanol: acetic acid: water (4:1:5) mobile phase which is non-polar, semi-polar, and polar and produces an Rf value of 0.68 in accordance with the quercetin standard.

The novelty of this study lies in the comprehensive pharmacognostic characterization of *G. amygdalina* collected from Indonesia, particularly West Java, which has never been previously reported. While previous studies mainly focused on the biological activities or phytochemical constituents of *G. amygdalina* from Africa or other regions, this research provides the first detailed morphological, microscopic, and chromatographic profiles of Indonesian-grown *G. amygdalina*. These findings contribute valuable baseline data for the standardization, authentication, and future pharmacological utilization of *G. amygdalina* as a potential medicinal plant in Indonesia.

CONCLUSION

This study concludes that the main morphological characteristics of *G. amygdalina* be found in length of 10-22 cm, a width of 5-8 cm, oblongus shape, acuminate tips and acute bases, pinnate leaf bones, serrated leaf edges, rough leaf surfaces with powder microscopic identification fragments in the form of trichome. Qualitative phytochemical screening contains all classes of secondary metabolite compounds with identity compounds resulting from thin-layer chromatography with five spot (red) and Rf values of 0.06-0.58 at a wavelength of 366 nm.

AUTHORS CONTRIBUTION

R.A designed and conducted the research, analysed and interpretation the data, and wrote the draft of manuscript. M.E.T designed the research, analysed and interpretation the data, reviewed the draft of manuscript, and supervised all the process. I.K.P & M.U.B designed the research, reviewed the draft of manuscript, and supervised all the process.

CONFLICT OF INTEREST

The authors declare that there are no competing interests.

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