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Article

Pharmacognostic and Toxicity Evaluation of Tabar Kedayan (*Aristolochia papilifolia* Ding Hou) Rootstock

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Abstract

Tabar kedayan (*Aristolochia papillifolia* Ding Hou.) is an endemic plant from northern Kalimantan and is widely used as traditional medicine by the Dayak people. This study aims to determine the pharmacognostic profile and toxicity data of Tabar Kedayan rootstock. The results showed macroscopically woody stem type, round stem shape, grooved or serrated stem surface, yellow-brown stem color, weak characteristic odor, bitter taste, microscopically found starch grains, calcium oxalate crystals, stone cells, cork cells, tracheids, and wood vessels. Fluorescence analysis under visible and ultraviolet light for Tabar Kedayan rootstock powder with different chemical reagents showed different fluorescence effects. Water content (0.466 ± 0.01%), ethanol content (0.325 ± 0.014%), moisture content (12.40 ± 0.15%), total ash content (1.438 ± 0.15%), and acid insoluble ash content (0.423 ± 0.06%). Phytochemical screening of the chemical compounds of the ethanolic extract showed the presence of alkaloids, terpenoids, saponins, flavonoids, and tannins. The levels of total polyphenols (15.095 ± 1.11 µg GAE/mg sample), total flavonoids (0.184 ± 0.02 µg QE/mg sample), and total alkaloids (0.056 ± 0.02 µg QAE/mg sample) were found. The brine shrimp lethality (BSLT) assay results showed the highest LC_{50} value by the n-hexane fraction with an LC_{50} value of 17.660 µg/ml.

Keywords: *Aristolochia papilifolia* Ding Hou; brine shrimp lethality test; pharmacognostic profiles; toxicity;

1. INTRODUCTION

The island of Kalimantan has a high biodiversity potential, including medicinal forest plants that have long been known and used by various indigenous ethnic groups in Kalimantan to treat a variety of ailments. The Dayak tribe is one of the

Indigenous peoples of the Indonesian island, found in all corners of Kalimantan, even as far as Serawak, Sabah, and Brunei Darussalam. The Dayak tribe lives around forested areas that contain plants with medicinal properties [1]. One of the endemic plants found in North Kalimantan is Tabar Kedayan (*Aristolochia papillifolia* Ding Hou.). Tabar kedayan is a Malinau endemic plant that is traditionally used by the Dayak people as a traditional medicine for the detoxification of poisons (especially insects and snakes) and all kinds of poisonous animal bites, food poisoning, diarrhea, toothache and can also be used to neutralize alcohol in the human body (alcoholics). However, scientific data on this species (especially secondary metabolites and potential activities) have not been reported [2].

The roots of Tabar Kedayan contain several groups of compounds that have potential as drugs. Studies have reported that the roots contain alkaloids, flavonoids, saponins, tannins, terpenoids, steroids, and phenolics [1]. Another study stated that the ethyl acetate fraction of the roots contained alkaloids, flavonoids, steroids, and phenolics [3]. Another study mentioned that the roots showed analgesic effects at doses of 25 and 50 mg/20 gBB, which had analgesic power equivalent to 0.05% tramadol [4]. Another study reported that the ethyl acetate fraction of tabar kedayan has antioxidant activity [3].

Aristolochic acid is an active ingredient in herbal medicines from the plant family Aristolochiaceae. Aristolochic acid can inhibit inflammation caused by immune complexes as well as immunological and non-immunological agents. Its activity is mainly due to its inhibitory effect on phospholipase A2 (PLA2), which regulates the synthesis of arachidonic acid, an intermediate in the production of prostaglandins [5,6]. Given the many uses of this plant, there is a need to scientifically investigate the properties and safety of Tabar Kedayan rootstock.

Adulteration can be defined as the deliberate substitution of the original plant material with other plant materials or the deliberate addition of foreign substances to increase the weight of the plant for financial gain. The therapeutic efficacy of medicinal plants is contingent on the quality and quantity of chemical elements. Erroneous identification of herbal medicines or natural products is a primary cause of their misuse, underscoring the necessity for pharmacognostic studies of medicinal plants. It is imperative to establish the pharmacognostic profile of medicinal plants utilized as herbal medicines [7].

Research on Tabar Kedayan is still in its infancy. The potential of tabar kedayan plants to be used as medicinal plants is significant, and it is for this reason that research is being conducted on their pharmacognostic profiles. The pharmacognostic profile of tabar kedayan rootstock was studied to determine various pharmacognostic standard parameters such as macroscopic characteristics, microscopic characteristics of stem powder, fluorescence evaluation, ash and acid insoluble ash content, moisture content, juice content in certain solvents, secondary metabolite assay and total secondary metabolite content. In addition to the toxicity testing of Tabar kedayan plants in Kalimantan, this study utilized the Brine Shrimp Lethality Test (BSLT) method to assess the safety of their utilization. This underscores the necessity for further exploration of knowledge concerning plant species that offer potential benefits to health and medicine.

This study offers the prospect of a valuable reference for the future development of pharmaceutical drugs derived from natural ingredients, with particular reference to Tabar Kedayan rootstocks. Further testing is recommended to ascertain the content of compounds present and the plant's pharmacological activity.

2. MATERIALS AND METHODS

2.1. Material

2.1.1 Plant Collection and Authentication

Rootstock of Tabar Kedayan (*A. papilifolia*) was collected from Malinau District, Nort Kalimantan, Indonesia, the voucher specimen was deposited at the Laboratory of Research and Development, PT. Borneo Riseta Naturafarm. Moreover, this plant was identified at Laboratorium Ekologi dan Konservasi Biodiversitas Hutan Tropis, Faculty of Forestry, Universitas Mulawarman (by Dr. Ir. Paulus Matius, M.Sc). The dried samples employed in this study are stems that have undergone a drying process and have been subjected to rigorous analysis. The samples are then subjected to a process of purification,

involving the removal of any extraneous matter such as dirt and soil, and the peeling of the bark. The resultant powder is then ground into a fine consistency using a grinder.

2.1.2 Chemical Materials

The chemical materials used in this study include aluminum chloride, ammonia, acetic acid, glacial acetic acid, chloride acid, nitric acid, citric acid, sulphuric acid, bromocresol green, chloroform, dimethyl sulphoxide, ethyl acetate, n-hexane, ethanol, Ferrum chloride, phloroglucinol, gelatine, iodine, potassium iodide, potassium hydroxide, chloralhydrate, gallic acid, quercetin, quinine alkaloid, magnesium, mercuric chloride, sodium acetate, sodium hydroxide, sodium carbonate, sodium chloride, Folin-Ciocalteau, yeast and *Artemia salina* Leach eggs.

2.2. Instrument

The main instruments and supporting tools used in this study included Magnetic Hotplate Stirrer (Thermo Fisher Scientific, USA), Microscope binocular (Olympus Corporation, Japan), Rotary Evaporator (Buchi, Germany), UV-Vis Spectrophotometer (Thermo Fisher Scientific, USA), Muffle Furnace (PT. Cakrawala Bima Instrument, Indonesia), Vortex Mixer (DLAB MX-S, Indonesia).

2.3. Method

2.3.1. Extraction and Fractionation Processes

The dry powder of Tabar Kedayan rootstock obtained weighed as much as 250 grams as the initial weight. The extraction process employs the maceration method, utilizing 2.5 L of 96% ethanol as the solvent. The container is securely closed and left to submerse for a period of 3 x 24 hours. The extraction process is continued until the result of the maceration solution approaches a state of near colorlessness. The filtrate obtained is then concentrated using a rotary evaporator at 50°C until a thick extract is obtained [2].

The dried ethanol extract of 3 grams was dissolved in a mixture of water and ethanol, followed by liquid-liquid partitioning using 150 mL of n-hexane as the solvent. This process was carried out in a separatory funnel, with the contents shaken on occasion and the funnel cover opened after each shake. The mixture was then left to stand for 30 minutes, after which two layers had formed. This process was repeated three times. The water-ethanol layer was then re-extracted with ethyl acetate solvent, using 3 x 150 mL of the same treatment. The results of the fraction were concentrated using a rotary evaporator.

2.3.2. Macroscopic and Fluorescence Evaluation

An observation study of Tabar Kedayan rootstocks was conducted to ascertain their characteristics, which included color, shape, odor, taste, size, and other surface characteristics (Chanda, 2014). The evaluation of fluorescence involved the addition of sample powders to a solution comprising 1N sodium hydroxide, 1N hydrochloric acid, iron (III) chloride 10%, sulfuric acid, chloroform, anhydrous acetic acid, ammonia 10%, potassium hydroxide 10%, nitric acid 25% and iodine solution. The observation of color change was conducted under visible light (visible), and UV 254 & 366 nm [8,9].

2.3.3. Microscopic Evaluation

In the experiment, a minimal quantity of specimen powder was applied to a glass slide. Then, 2-3 drops of chloralhydrate, distilled water, and fluoroglucinol-acid chloride were added to each glass slide. Thereafter, each slide was covered with a cover glass, and examined under a microscope at a suitable magnification setting [9,10].

2.3.4. Physicochemical Profile

1. Determination of Total Ash Content and Acid-Insoluble Ash Content

   A precise quantity of approximately 1 gram of dry powder from the sample is placed into a crucible that has been incinerated and tared, followed by a process of flattening. The incineration process is then carried out in a furnace at a temperature of ± 600°C until the material has fully combusted. The resultant ash is then cooled in a desiccator and weighed to a fixed weight. The total ash content is then calculated and expressed as a percentage of the weight of the dried sample [8].

To calculate the ash content, 25 mL of a dilute hydrochloric acid solution must be added to the original sample and left to cook for a period of five minutes. Following this, the acid-insoluble part is collected, filtered using filter paper, and then ignited until it has reached a constant weight. The resulting ash content can thus be determined as a percentage of the initial sample weight.

2. Determination of Water Content

Determination of water content is done by gravimetric method [9]. Briefly, 5 grams of sample powder was weighed in a crucible that had been dried at 105°C and known by weight. The crucible was then dried in an oven at 105°C for 5 hours, after which it was cooled in a desiccator and weighed to a fixed weight. The content was then calculated as a percentage of the air-dried material. The drying and weighing processes were conducted at one-hour intervals, with the condition that the difference between two consecutive weighings was no greater than 0.25%. It is widely accepted that the achievement of consistency in weight is indicated when two consecutive weighings following a drying period of 30 minutes and a cooling period of 30 minutes in a desiccator demonstrate a maximum discrepancy of 0.01 grams.

3. Determination of Water and Ethanol Soluble Extract Content

The dried powder (5 g) was weighed and transferred to an Erlenmeyer flask, followed by the addition of 100 mL of 96% ethanol and 100 mL of a water-chloroform mixture. The contents were then subjected to repeated stirring for a duration of six hours. The mixture is then left for 18 hours. 20 mL of the mixture is then evaporated to dryness in a porcelain cup that has been heated to 105 °C and calibrated. The residue was then subjected to heating at 105°C. The residue should then be removed, transferred to a desiccator, and weighed. This process is repeated until a constant weight is obtained. The calculated content in percent of water and ethanol soluble compounds to the weight of the initial extract was then determined [9].

2.3.5. Secondary Metabolite Identification

The identification of secondary metabolite compounds is achieved through the utilisation of specific reagents, which are selected based on the targeted secondary metabolites. This approach encompasses the identification of flavonoids, alkaloids, saponins, tannins, and terpenoids/steroids. The methodology employed in this study aligns with the findings reported in previous research [9,11], which is outlined as follows:

1. Flavonoid

The extract was dissolved in ethanol and then boiled for 5 minutes. 0.05 mg of magnesium powder and 1 mL of concentrated hydrochloric acid were added and the mixture was shaken vigorously. The red color produced indicates the presence of flavonoid compounds.

2. Alkaloid

The extract was added to a solution of 2 mL of chloroform and 2 mL of ammonia, and the mixture was then filtered. The filtrate was then added 3-5 drops of concentrated sulfuric acid and shaken until two layers were formed. The chloroform fraction was then collected. The filtrate was then divided into two parts in a test tube and added Mayer and Dragendorff reagents. The presence of a white or yellow clumpy precipitate was indicative of a positive Mayer reaction. Conversely, the addition of Dragendorff yielded a positive result, manifesting as a brick-red precipitate.

3. Saponins

The extract was added to 10 mL of water and vigorously shaken for 10 seconds. The resultant mixture was found to be stable and frothy. Following the addition of a single drop of 2 N hydrochloric acid, the froth remained intact.

4. Tannins

The extract is added to hot water, followed by the addition of a few drops of 10% iron (III) chloride reagent. A positive test result is indicated by the formation of red or blue-black. The aqueous extract was dissolved in hot distilled water, followed by thorough shaking until homogeneity was achieved. The filtrate was then divided into two parts, the first

of which was added to a 10% sodium chloride solution in 1% gelatin solution. The occurrence of a white colloidal precipitate was then observed. The second was added with 10% iron (III) chloride, showing a positive result if a red or blue-black solution was formed.

5. Terpenoids/Steroids

The extract was dissolved in chloroform and then added with 10 drops of anhydrous acetic acid and 2 drops of concentrated sulfuric acid. The solution was then subjected to gentle shaking and left for a period of time. A positive test for steroids produces a blue or green color, while triterpenoids produce a red or brown ring.

2.3.6. Determination of Total Secondary Metabolite Content

1. Total Polyphenols Content

The total polyphenol content value was determined according to our previous studies [12,13], with some modifications. In Brief, a total of 10 mg of the test sample was dissolved in 10 mL of ethanol to obtain a sample solution of 1000 µg/mL. Subsequently, 0.1 mL of the test sample solution was added to 5 mL of distilled water, along with 0.5 mL of Folin-Cioceltaeu reagent and 2 mL of 20% sodium carbonate. The mixture was left for 30 minutes, then the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 761 nm. This process was repeated on three separate occasions. The equation of $Y = 0.0027X + 0.0317$ with $R^2 = 0.9983$ was utilized to express the yield value of the total polyphenols content (µg GAE/mg DW extract) with several different concentrations, with gallic acid serving as the standard. In this equation, X denotes the yield value of the total polyphenols content and Y denotes the absorption.

2. Total Flavonoid Content

The total flavonoid content value was determined according to the previous studies [12,14,15], with adjusted modifications. Briefly, a total of 10 mg of sample was weighed and dissolved in 10 mL of ethanol to obtain a concentration of 1000 µg/mL. Then, 0.5 mL of the test sample was added with 0.1 mL of 10% aluminum (III) chloride, 0.1 mL of 1M sodium acetate, and 2.8 mL of distilled water. The mixture was then left to incubate for a period of 30 minutes. The next step was to measure the absorbance of the extract solution using a UV-Vis spectrophotometer at a wavelength of 430 nm. The value of total flavonoid content was calculated using the equation $Y = 0.0047X + 0.279$, with a value of $R^2 = 0.9995$ expressed in units of µg QE/mg DW extract, with quercetin as the standard. In this equation, X is the value of total flavonoid content and Y is the value of the measured light absorption.

3. Total Alkaloid Content

The determination of total alkaloid content was conducted according to the literature [14,16]. Briefly, a volume of 1 mL of the extract was transferred into a separating funnel, followed by the addition of 5 mL of phosphate buffer (pH 4.7) and 5 mL of BCG solution. The extraction process was then initiated by the addition of 1, 2, 3, and 4 mL of chloroform. The chloroform phase was then homogenized using a vortex. The resulting mixture was then subjected to a series of analytical procedures, the first of which was the measurement of its optical density at a wavelength of 422 nm. The total alkaloid content was then calculated using the following equation: $Y = 0.0039X + 0.1883$ with $R^2 = 0.9531$ in ug QAE/mg DW extract and quinine as standard.

2.3.7. In Vitro Toxicity Activity Assay

The toxicity tests were conducted using the brine shrimp lethality test (BSLT), as described in the literature [9,17,18] with modifications. Each extract was evaluated at concentrations of 40, 20, 10, 5, 2.5, 1.25, and 0.75 µg per ml (in seawater). These concentrations were achieved by transferring appropriate volumes of stock solution; this was done by placing ten 48-hour-old brine shrimp (mainly nauplii at instar III/IV) into a vessel containing the test extract solution and incubating at 28-30°C with strong aeration, under a continuous light regime. The number of survivors was then enumerated and calculated, subsequently analyzed using statistical methods (regression linearity) to determine the LC_{50}.

3. RESULT AND DISCUSSION

3.1. Pharmacognostics Profile

The pharmacognostic profile of Tabar Kedayan rootstock was identified to determine various pharmacognostic standard parameters and secondary metabolite testing. The objective of conducting a pharmacognostic profile study is to ascertain the distinctive characteristics of Tabar Kedayan rootstock or specific plants. This approach can also be utilized for the identification of simplicia and extracts from these plants, thereby preventing the proliferation of herbal plant counterfeiting. Furthermore, pharmacognostic profiles can function as a reference point in the development of pharmaceuticals derived from natural ingredients.

3.1.1. Macroscopic and Fluorescence Profile

The observation of macroscopic characteristics was conducted through the implementation of organoleptic and fluorescent tests. The organoleptic observations drew upon the senses of sight, smell, taste, and touch. In contrast, the fluorescent test entailed the use of several reagents, including 1N sodium hydroxide, on the dried powder of Tabar Kedayan rootstock. The following reagents were utilized: 1N hydrochloric acid, iron (III) chloride 10%, sulfuric acid, chloroform, anhydrous acetic acid, ammonia 10%, potassium hydroxide 10%, nitric acid 25%, and iodine solution. Subsequently, the color changes that occur visually were observed, and assistance was provided by UV 254 nm and UV 366 nm. A comprehensive analysis was conducted on the dried sample of the Tabar Kedayan rootstock to ascertain its distinguishing characteristics. Macroscopic observations were made on the sample. The findings of the observations are presented in Tables 1 and 2.

Table 1 Observation Results of Macroscopic Characteristics

Observation	Results	Figure
Macroscopic Characteristics:		
Shape	Round rootstock shape (*teres*)	
Types	Woody rootstock (*lignosus*)	
Size	Old stems are 1.5 – 2.5 cm in size	
Surface Texture	Grooved or serrated bark	
Organoleptic:		
Colors	Brownish-yellow	
Smell	Weak characteristic odor	
Taste	Bitter	

An observational study was conducted to ascertain the macroscopic characteristics of Tabar Kedayan rootstock. The rootstocks were observed under a microscope to ascertain their morphology, size, and organoleptic properties. The objective of macroscopic observations is to ascertain the inherent characteristics of a given plant part, in addition to its preliminary description through the five senses. This process entails the meticulous documentation of its physical characteristics, including its morphology, pigmentation, olfactory properties, and gustatory characteristics. These attributes are delineated in Table 1.

The evaluation of fluorescence was conducted to identify medicinal plants in powdered simplified form based on their fluorescence characteristics. It has been demonstrated that the fluorescence of sample powder of various plant species is subject to variation when exposed to ultraviolet (UV) radiation. The study proceeded with the observation of the fluorescence characteristics of each sample under both visible and UV light, with the distinction of varying wavelengths. Consequently, the evaluation of fluorescence is employed in the identification and authentication of plant materials. Moreover, it can serve as a reference point in the formulation of plant monographs [7].

Tabel 2 Flourescence Evaluation Results

Treatment	Visible	Under Ultra Violet at	
		254 nm	366 nm
Tabar Kedayan rootstock powder	Brown	Brown	Brown
Powder + 1N hydrochloric acid	Brown	Brown	Brown
Powder + Chloroform	Brown	Greenish brown	Greenish brown
Powder + Sulfuric Acid	Black	Black	Black
Powder + 10% Iron (III) chloride	Yellowish brown	Dark brown	Dark brown
Powder + 1N Sodium Hydroxide	Reddish brown	Dark brown	Dark brown
Powder + Anhydrous Acetic Acid	Blackish brown	Greenish Yellow	Dark green
Powder + 10% Ammonia	Brown	Dark brown	Dark brown
Powder + 10% Potassium Hydroxide	Blackish brown	Black	Reddish brown
Powder + 25% Nitric acid	Reddish brown	Brown	Dark brown
Powder+ Iodine solution	Yellow	Bluish-green	Dark brown

The assessment of certain crude drugs is frequently conducted in this manner, and they serve as a critical component of pharmacognostic evaluation. The results obtained from the evaluation of the fluorescence of specific Tabar kedayan in the treatment of chloroform, reagent, anhydrous acetic acid, potassium hydroxide, and iodine solution are presented in **Table 2**. These results indicate a color change, which can be attributed to the specific interactions between the reagents and the dried powder of Tabar Kedayan. Iodine has been utilized in the identification of starch and alkaloid compounds. Color changes indicative of positive outcomes include purple-black complexes and blue. The reaction that occurs is the formation of a complex between starch and iodine, while in alkaloids, a complex is formed between iodine and nitrogen groups. Anhydrous acetic acid has been demonstrated to be a useful tool in the identification of terpenoid and steroid compounds [19]. The presence of terpenoids is indicated by the occurrence of brown color changes, while the presence of steroids is indicated by green color changes. This phenomenon can be attributed to the acetylation process of hydroxyl groups catalyzed by anhydrous acetic acid. The presence of hydroxy groups is confirmed with sodium hydroxide or potassium hydroxide diagnostic reagents. Flavonol compounds, which are characterized by the presence of hydroxyl groups, undergo a bathochromic shift. Sodium hydroxide has been demonstrated to react by ionizing all free hydroxyl groups contained in flavonoid compounds. Furthermore, potassium hydroxide has been shown to identify the presence of possible quinone compounds by producing a single yellowish stain that is predicted to be an anthraquinone derivative compound [20]. The results obtained from this study suggest the presence of alkaloid, terpenoid, and flavonoid compounds, as evidenced by the analysis of fluorescence.

3.1.2. Microscopic Profile

Microscopic characteristics were observed using a microscope to study the anatomy and histology of the stem. Microscopic observations are conducted to identify fragments that are characteristic of specific components of the plant. The rootstock powder was subjected to heating with chloralhydrate, followed by the addition of a floroglusin-HCL stain. The incorporation of chloralhydrate, in conjunction with heating, is intended to facilitate the dissolution of the cell cytoplasm, thereby enhancing the visibility of the cell walls, the inclusion of substances, and the arrangement of cells and tissues. Floroglusin-HCl staining is a staining method used to identify the presence of lignin in cells. In this staining procedure, lignin-positive cells are observed to exhibit a red coloration. The process of powder identification entails the microscopic observation of stem fragments. A series of observations were meticulously conducted on the dried powder fragments to ascertain the specific characteristics of tissue forms that are instrumental in facilitating the identification of samples [21].

The results of microscopic observations of Tabar Kedayan rootstock powder are shown in Figure 1. A thorough observation of rootstock powder reveals the presence of fragments in the form of brachisclerid-type stone cell groups characterized by thick cell walls. These fragments also exhibit trichomes, which are hair-like structures and are non-glandular in nature. The trichomes are multi-cellular in shape and resemble needles. The presence of prism-shaped calcium oxalate crystals, the existence of wood vessels with vessels (xylem), and the observation of tracheid fibers with spiral vessels were also noted. Parenchyma exhibits a red hue as a result of its reaction with phloroglucinol in hydrochloric acid. It contains prism-shaped calcium oxalate crystals and round starch grains.

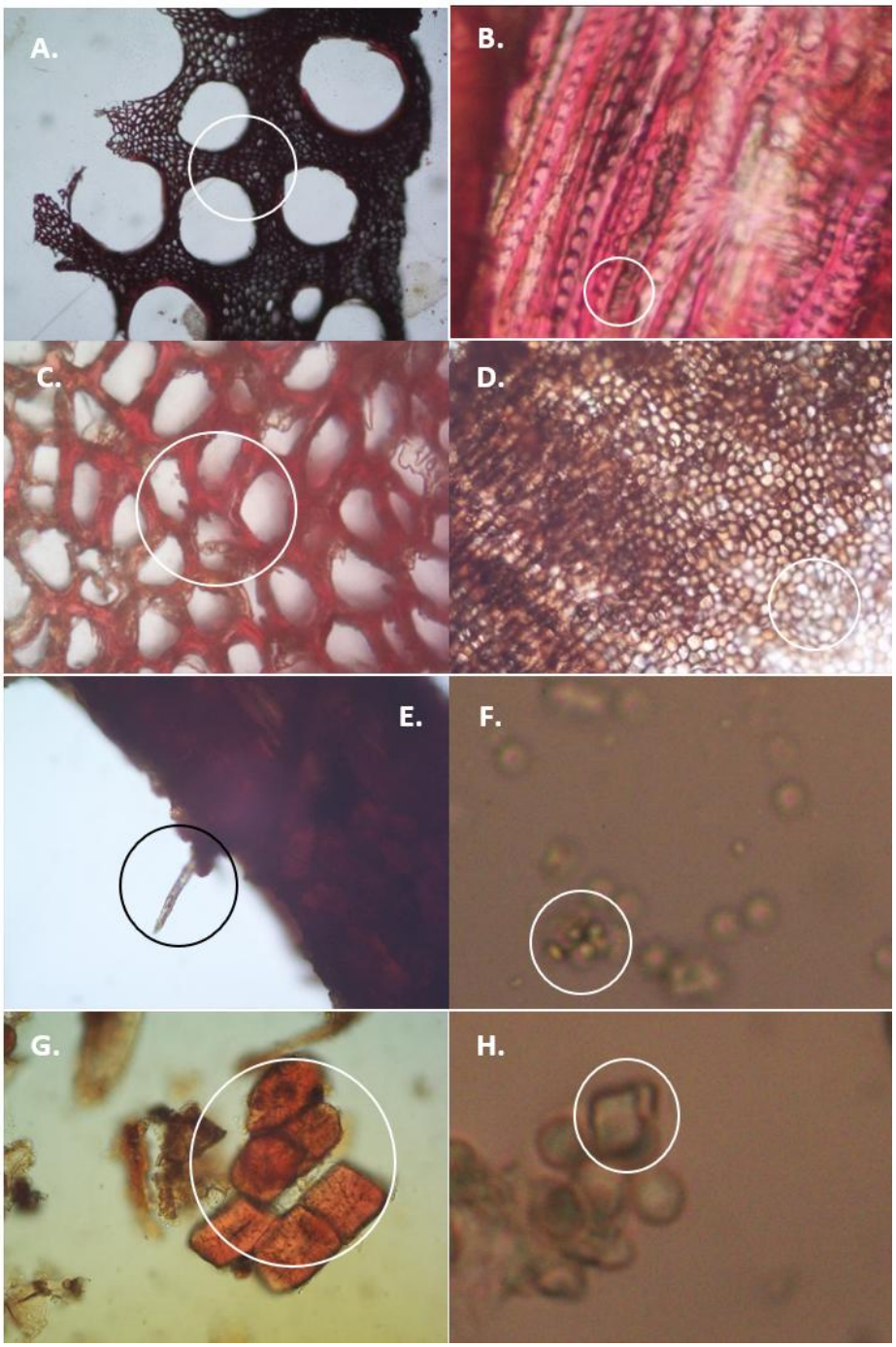

Figure 1. Microscopic photograph. Description: Stem powder microscopy at 40x and 100x magnification (A) xylem vessels with vessels, (B) tracheids with spiral vessels, (C) parenchyma tissue, (D) cork cells, (E) trichomes, (F) starch grains, (G) stone cells, (H) prism-shaped oxalate crystals.

3.1.3. Physicochemical Characteristics

As demonstrated in Table 3, the physicochemical characteristics under consideration are as follows: total ash content, acid-insoluble ash content, water content, water-soluble and ethanol-soluble extract.

Table 3 Physicochemical Characteristic of Tabar Kedayan rootstock

Parameter	Results (%b/b)
Total Ash Content	: 1.438 ± 0.147
Acid Insoluble Ash Content	: 0.423 ± 0.063
Water Content	: 12.40 ± 0.15
Water-Soluble Extract	: 0.466 ± 0.011
Ethanol-Soluble Extract	: 0.325 ± 0.014

The ash content of the dried sample of Tabar Kedayan rootstock can be determined by measuring 1 gram of the material. The ash content is determined by incinerating rootstock powder in a furnace at a temperature at which organic compounds and their derivatives are decomposed and evaporated, leaving mineral and inorganic elements. Determining the ash content of a specimen is pivotal in ascertaining the total amount of material remaining after the process of incineration. This ash can be categorized into two distinct types: physiological ash, which is derived from the plant tissue itself, and non-physiological ash, which constitutes a residue of extraneous compounds (such as sand and soil) attached to the surface of the plant. In this study, the ash content of the sample was found to be 1.438%, while the acid-insoluble ash content was determined to be 0.423%. Under the stipulations set out in Kepmenkes RI Number 261/MENKES/SK/IV/2009, the ash content should not exceed 10.2%. The term "acid insoluble ash content" refers to the residue obtained following the dissolution of total ash with dilute hydrochloric acid, thereby releasing the remaining insoluble material. The primary function of the index is to quantify the quantity of silica present, with the majority of this silica being found in the form of silica-containing sand and soil [21].

Determination of water content aims to provide a minimum limit or range of the amount of water content in the dried powder of the sample. The value of water content is 12.40% and exceeds the allowable standard. This can happen if the storage process of the dried sample of Tabar Kedayan rootstock is not carried out in the right place because the dried powder can absorb water in the air. The water content in traditional medicinal preparations including extracts should not exceed the 10% limit. The high water content of the sample can cause instability during storage due to the growth of microorganisms and allows a medium for the growth of fungi and molds which allows enzymatic reactions to occur that can decompose active substances in dried samples [22,23].

The water and ethanol solubility of juice content is a reliable indicator of the estimated level of active compounds that can be extracted by water and ethanol solvents. The levels of active compounds in a sample are influenced by factors such as plant age, harvest time climate, and place of growth. The findings of the study indicate that the extract content of the Tabar Kedayan rootstock sample in water soluble content is 0.466%, while the ethanol soluble content is 0.325%. This finding indicates that the content of secondary metabolite compounds is more soluble in water than in ethanol. This result suggests that the active compounds present in the dried powder of Tabar Kedayan rootstock can be readily absorbed into water and ethanol.

3.1.4. Secondary Metabolite

Secondary metabolite identification is conducted through qualitative and quantitative analysis. Qualitative identification is carried out by chemical methods using typical reagents for each class of secondary metabolites. The reagent will react with the core framework of certain secondary metabolite groups, resulting in the manifestation of typical physical properties,

including discoloration and precipitate formation. Concurrently, the quantitative testing process entails the measurement of compound levels through a spectrophotometer instrument.

1. Qualitative Identification

The identification of the compounds contained within plants is achieved by employing qualitative metabolite testing. The fundamental principle underlying this process is to observe alterations in the reaction that occur upon the addition of a reagent that is specific to a given content. The results of secondary metabolite testing of the ethanol extract of Tabar Kedayan rootstock are presented in Table 4.

Table 4 Secondary metabolite from Tabar Kedayan Rootstock

Secondary Metabolite	Reagent	Result	Description
Alkaloid	Mayer	+	Yellow precipitate
	Dragendoff		Brick-red precipitate
Flavonoid	Concentrated hydrochloric acid Mg Powder	+	Red
Tannin	10% Iron(III) chloride	+	Blackness
Terpenoid	Liebermann - Burchard	+	dusky brown ring
Saponin	Chloride acid	+	Forms stable foam

As a consequence of the process of identification of secondary metabolites, it was determined that the given extract contains alkaloids, flavonoids, saponins, tannins, and triterpenoids. In the process of identifying alkaloids, the formation of white precipitates with Meyer and orange to red-brown precipitates with Dragendorff is indicative of the formation of insoluble complex compounds. In the identification of flavonoids, the reduction process with magnesium and concentrated hydrochloric acid produces a red color. It is evident from the identity results obtained that froth is formed due to the fundamental properties of saponins. These properties include the ability to form colloidal solutions in water and to form froth when the solution is agitated. In the identification of tannins, the formation of a blue-black color after the addition of iron (III) chloride is attributable to the phenol compounds contained within the tannins. These phenol compounds are capable of forming complex compounds with Fe_3^+ ions [24]. The identification of triterpenoids is achieved through the implementation of the Liebermann-Buchard test. The hydration of triterpenoid/steroid compounds is achieved through the addition of strong acids, which subsequently leads to the formation of salts. These salts, in turn, result in a series of color reactions.

2. Quantitative Identification

The subsequent secondary metabolite identification test involves the determination of total polyphenols, total flavonoids, and total alkaloids, as illustrated in Table 5.

Table 5 Total Secondary Metabolite Contents

Secondary Metabolite Content	Results
Total Polyphenols	15.095 ± 0.0007 µg GAE/mg sample
Total Flavonoid	0.184 ± 0.02 µg QE/mg sample
Total Alkaloid	0.056 ± 0.02 µg QAE/mg sample

The determination of the total polyphenol content was achieved through the implementation of the Folin-Ciocalteu method, utilizing gallic acid as a standard reference. The results obtained included the total polyphenol content (15.095

± 1.11 μg GAE/mg sample). The determination of total flavonoid content was achieved through the utilization of the aluminum chloride colorimetric method, employing standard quinine as the standard reference. The results obtained included the quantification of total flavonoid levels (0.184 ± 0.02 μg QE/mg sample) and the determination of total alkaloids using the Bromocresol Green (BCG) method with anhydrous quinine as the standard. The results obtained indicated the presence of total alkaloid levels of 0.056 ± 0.02 μg QAE/mg sample. Equivalent value is defined as the value of equivalence between the concentration of the comparator and the content of each milligram of the extracted sample. This enables the detection of the amount of flavonoid, alkaloid, and polyphenol compounds from the extract of Tabar Kedayan rootstock. This value is obtained from the linear regression equation derived from the standard used [25]. It is hypothesized that the results of the fluorescence evaluation are indicative of the presence of alkaloid, terpenoid, and flavonoid compounds. The results of the qualitative secondary metabolite identification, which included alkaloids, flavonoids, saponins, tannins, and triterpenoids, were corroborated by quantitative analysis, which confirmed the presence of polyphenols, flavonoids, and alkaloids.

3.2. Toxicity Evaluation

The Brine Shrimp Lethality Test (BSLT) is a method of determining the acute toxicity of a compound or extract, using Artemia salina experimental animals as test subjects. The toxicity of a compound can be determined by calculating the number of Artemia salina larvae that succumb to the parameter 50% lethal concentration (LC_{50}). The BSLT method is employed to determine whether a material is toxic, with an LC_{50} of less than 1000 μg/mL indicating toxicity. The results of the test are set out in Table 6.

Table 6. Toxicity Test Results of Extracts and Fractions of Kedayan Tabar Rootstock

Sample	Concentration (μg/mL)	Mortality Percentage (%)	LC_{50} Value (μg/mL)
Ethanol Extract	10	26.6	20.989
	20	43.3	
	30	60	
	40	73.3	
	50	83.3	
N-Heksane Fraction	5	20	17.660
	10	30	
	15	43.3	
	20	53.3	
	25	83.3	
Ethyl Acetate Fraction	50	30	95.499
	100	50	
	150	60	
	200	76.6	
	250	83.3	

These concentrations were obtained subsequent to orientation with a range of increasing constant levels. Following a rigorous testing process, the number of dead larvae was recorded. This data was then utilized to calculate the percentage of larval mortality. From the data on the percentage of mortality, the concentration that gives the percentage value of larval mortality between 20% and 80% is taken as the lowest and highest concentrations, respectively. The percentage of larval mortality ranging from 20% to 80% was utilized because the mortality percentage can already provide a more linear curve, thereby enabling the LC_{50} obtained in this BSLT test to better describe the actual results.

The Artemia larvae utilized in the experimental procedure were 48 hours after hatching. The 48-hour-old larvae are in the most sensitive state due to the softness of their cell walls at this point, meaning that only a small concentration of the sample is required to elicit the observed effect. Subsequently, a yeast suspension was introduced into each tube, thereby providing a nutritional source. It is imperative to incorporate food as a supplement to ensure that larval mortality is not attributable to a deficiency in nutrition. The concentration was determined to be 3 mg of yeast dissolved in 5 mL of seawater. Following 24 hours, the number of live larvae was enumerated. The vitality of the organism is said to be indicated by the presence of movement in the larvae, regardless of the amplitude of this movement. The larvae cannot remain motionless, as the second antenna serves not only as a means of movement but also as a means of respiration. After ascertaining the quantity of living larvae, the number of deceased larvae can be enumerated. The percentage mortality of each treatment concentration and control was subsequently calculated. The control was utilized to rectify larval mortality that was not attributable to the impact of extracts and fractions of Tabar Kedayan rootstock. The experimental results are displayed in Table 6. As illustrated in Table 6, the percentage of larval mortality ranges from 20% to 80%.

Following analysis by probit analysis, the linear equations of each of the ethanol extract, N-hexane fraction, and ethyl acetate fraction were found to be y = 2.244x + 2.0334, y = 1.6801 + 2.9051 and y = 2.1105x + 0.8217 respectively. The relationship curve between the probit value and the log concentration of the extract and fraction can be drawn. The results of the probit analysis indicated that the LC_{50} values were, respectively, 20.989, 17.660, and 95.499 μg/mL.

4. CONCLUSION

The results obtained from the study should be considered as preliminary data for future studies and may be used to establish a pharmacognosic parameter. This parameter has been documented for the first time concerning this particular plant. In addition to its function in establishing parameters for the identification of raw materials and the preparation of a plant monograph, the project will also assist in the development of future studies.

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