

Pharmacognostic Studies on the Leaves of *Annona muricata* Linn.

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ABSTRACT

Introduction: *Annona muricata* Linn. (Family: Annonaceae) is a well-known traditional and natural medicine over the world; in Malaysia it serves as a treatment for many kinds of diseases. Studies have been reported that *A. muricata* can be used to treat diseases due to its antibacterial, antiviral, antifungal, antitumor, anthelmintic, analgesic, hypotensive, anti-inflammatory, and has immune enhancing properties. Despite having several medicinal functions and properties, however there is no standardization parameters have been reported in the literature for the leaves of *A. muricata*. **Methods:** Therefore, through this research study, the macroscopical and microscopical characteristics, physicochemical parameters such as ash values, extractive values, fluorescence analysis and preliminary phytochemical analysis of the leaves were investigated. **Results:** Based on the observation of the transverse section of the leaves, the presence of upper cuticle, upper epidermis, palisade cells, vascular bundle, spongy mesophyll, phloem fibers, lignified vessels, xylem vessels, collenchyma, lower epidermis, lower cuticle and parenchyma served as important key differentiating features for the studied plant. The powder microscopy revealed the presence of pieces of trichome, collapsed uniseriate multicellular covering trichome, spongy mesophyll, phloem fibres, xylem vessels, paracytic stomata and fragment of epidermis showing cell and palisade cell. Calcium oxalate crystals were also observed even though the captured image was slightly unclear. The phytochemical screening of the leaves was carried out using four different extracts which showed the presence of steroids, saponins, flavonoids, tannins carbohydrates and proteins, respectively. **Conclusion:** Based on this research finding, the pharmacognostic standardization of the plant can be established thus, providing ease in identifying and determining the purity and quality of the investigated plant.

Key words: *Annona muricata*, Fluorescence analysis, Macroscopy, Microscopy, Physicochemical parameters, Preliminary physicochemical screening.

INTRODUCTION

Medicinal plants have always retained their therapy in several traditional systems of medicines due to their effectiveness, low cost and easy availability. In spite of recent advances of modern medicines, traditional medicinal plants are still used in different communities across the world for their primary health care needs.¹ Unfortunately, crude drugs from natural origin are susceptible towards adulteration and substitution due to the absence of standards relating to the genuineness of drugs. This, in turn will affect the potency, quality and purity of the drugs. In order to ensure the reliability of herbal medicine, a pharmacognostic study needs to be conducted. Since there is no pharmacognostic work reported on the leaf of this plant, thus the current study will aim to develop certain quality standards on the leaf of *A. muricata*.

Annona muricata L., a member of Annonaceae family, commonly known as “Soursop” or “Gaviola” is a medium-sized evergreen tree with exotic fruits, growing up to 5 to 6 meters in height and features with large, glossy, dark green leaves.² The plant is originated from the South and North America, and now has been distributed in several countries, including in Malaysia, Nigeria, Australia and Africa.³ The plant is popularly known

as “Lakshmana phala” in Ayurvedic medicine⁴ and recommended to treat illnesses ranging from stomach ailments to cancer. The plant pacifies vitiated conditions of pitta, constipation, burning sensation, bacterial infections, parasites, hypertension, depression, fever, viral infections, malignancy and general tonic. Root decoction is a powerful purgative. Powdered seed kills body and hair lice on external application.⁵ The leaves and seeds are mostly used traditionally in different countries for the treatment of countless diseases and ailments such as insecticide and parasiticide, antipyretic, sedative, antidiabetic, antihypertensive, in the treatment of respiratory illness, malaria, gastrointestinal problems, liver, heart and kidney affections and cancer.⁶ In Malaysia, the plant is known as “Durian belanda” where the decoction of the leaves are used as a remedy for the treatment of cough⁷, used to sponge down high fever⁸, used to treat scabies, head lice and other skin disease.⁹ More than 200 chemical compounds, primarily alkaloids, phenols, flavonoids, sterols and acetogenins have been identified in this plant¹⁰ and a good number of pharmacological activities such as antimicrobial, anti-inflammatory, anti-protozoal, antioxidant, insecticide, larvicide, anxiolytic, antitumoral, antiulceric, wound healing, hepatoprotective and hypoglycemic activities have been reported.¹¹

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MATERIALS AND METHODS

Collection and preparation of plant material

Fresh leaves of *A. muricata* leaves were collected from Jalan Pipit 2, Off Jalan Sultanah Alor Setar Kedah, during February 2019 and authenticated by the botanist at Kompleks Pertanian Industri Telok Cengai Kuala Kedah. After confirmation, fresh leaves were collected in bulk, cleaned with water to remove dirt, shade dried and powdered using a mechanical grinder. The dried leaf powder was passed through a sieve of 40 mesh to obtain coarse powder and stored in an airtight container until further use.

Macroscopic study

Fresh plant materials are used for the macroscopic studies such as shape, size, color, odor, taste and leaf structure such as margin, apex, base surface, venation and inflorescence other aspects.¹²

Microscopic study

Transverse section of plant leaves

The thinnest section of fresh leaves was taken stained with phloroglucinol and concentrated hydrochloric acid at the ratio of 1:1. Then, the stained section was mounted on microscopic slide. The sample is covered by using glycerine and cover slip before it can be examined under binocular compound optical microscope. To get a clear picture, the images were taken by using a microscope (Leica DM750 Germany) fitted with a digital camera.¹³

Powder microscopy study

Dried powder is taken instead of a section of the plant that is similar to microscopic study. Powder study used staining agent such as phloroglucinol N/20 iodine solution, concentrated hydrochloric acid at ratio 1:1. To identify the presence of cellular components distilled water is applied to the sample.¹³

Proximate Analysis

The Ash value such as total ash, water soluble ash and acid insoluble ash were determined as per procedure mention in the British Pharmacopoeia.¹⁴

Extraction

About 3 g of the dried powdered leaves was extracted with various solvents with increasing polarity. The solvents used were petroleum ether, chloroform, methanol, and distilled water. The extraction was performed using ultrasonic extraction bath for 30 minutes for each solvent. Preliminary phytochemical analysis was performed in different extract to study the class of phytochemical contained.¹⁵

Qualitative preliminary phytochemical analysis

Preliminary phytochemical analysis was determined on various extracts of *A. muricata* L., leaves. Following identification tests were performed to detect presence of alkaloids, carbohydrates, proteins, tannins, steroids, saponins and flavonoids.¹⁶

Fluorescence analysis

The fluorescence characteristics of all liquid extracts were studied under ultraviolet light at short (254 nm) and long (365 nm) wavelengths.¹⁷

RESULTS

Macroscopic characteristics

The macroscopic characteristics of the leaves were observed and recorded in the Figure 1.

Macroscopic evaluation is a method used to determine the morphological of the plant part which are observed by the naked eye or magnifying lens. Macroscopic evaluation is important in distinguishing the characters and determining the correct identification of the crude drugs. Based on the results of the macroscopic studies of the leaves of *A. muricata* were observed to be compound and odd-pinnate leaflet arrangement. The diameter of the leaves is about 8-16 cm long and the width is about 3-7 cm long while the petiole is about 3-7 mm long. The leaflets consist of 5 to 11 nearly opposite in manner. Leaflets are oblong-ovate in shape and fleshy. The top sides of the leaves are smooth and dark green with glossy. The undersides are finely hairy and pale green in color. It shows glabrous surface, acute apex, entire margin and oblique base with characteristic odor and taste.

Microscopic characteristics

Transverse section of the leaves of *A. muricata* L. Transverse section of the leaf was shown in Figure 2.

Transverse section of the leaf revealed presence of upper and lower epidermis covered with thin cuticle. The epidermal cells consisted of small polygonal parenchymatous cells, compactly arranged with no intercellular space between them. Below the upper epidermis a single layer of parenchymatous cells is seen. Uniseriate, multicellular and unbranched covering trichomes with a blunt surface are seen on both surfaces, more along the lamina and less along the midrib region. Numerous paracytic stomata are seen on the epidermis.

Mesophyll revealed section of lamina shows dorsiventral nature of the leaf. The mesophyll is clearly differentiated into palisade and spongy parenchyma. Palisade cells are elongated, compactly arranged in 2-3 rows without any intercellular space and filled with chloroplasts. Palisade cells are present just below the upper epidermis, continue on the upper-part of the midrib and is arranged in a plane at right angle to the upper epidermis. The spongy parenchyma region is present just below the palisade layer and extends up to the lower epidermis. The cells of spongy parenchyma are loosely arranged with intercellular spaces between them. Few lignified vessels are also seen in the mesophyll region.

The midrib region consists of vascular bundle, parenchymatous cells and collenchyma. The vascular bundle is ovoid in shape and consists of xylem vessels (lignified) and phloem fibres (non-lignified). Vascular bundle is leptocentric where the xylem vessels are seen towards the outer side that surrounds the phloem. The phloem consisted of sieve tubes, companion cells and phloem parenchyma. Vascular bundle is surrounded by a bundle sheath with the help of thin-walled parenchymatous cells. Both parenchymatous and collenchymatous cells are present below the large vascular bundle.

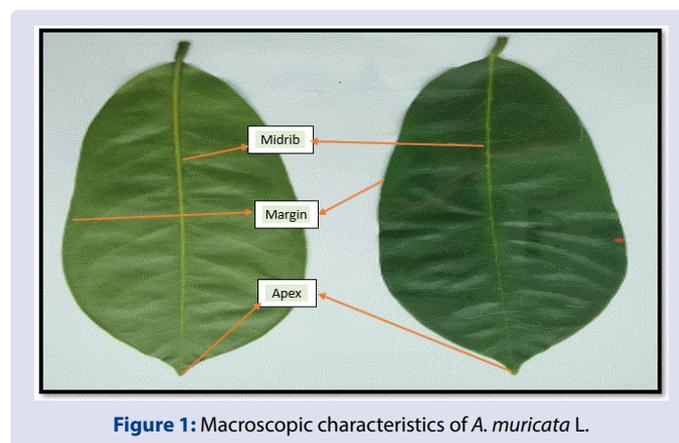


Figure 1: Macroscopic characteristics of *A. muricata* L.

Powder microscopy of the plant specimens

Powder microscopy of *A. muricata* leaves revealed presence of uniseriate multicellular covering trichomes with few collapsed trichomes, spongy mesophyll, paracytic stomata, xylem vessels and non-lignified phloem fibres, fragments of epidermis showing palisade cells and calcium oxalate crystals. Powder microscopy of the leaf powder was observed and recorded in Figure 3.

Preliminary phytochemical screening

The results of the preliminary phytochemical screening are presented in Table 1 by using four different extracts showed the presence of carbohydrates and proteins (in aqueous extract), steroids (in petroleum ether and chloroform extracts), tannin, saponin and flavonoids (in methanol and aqueous extracts).

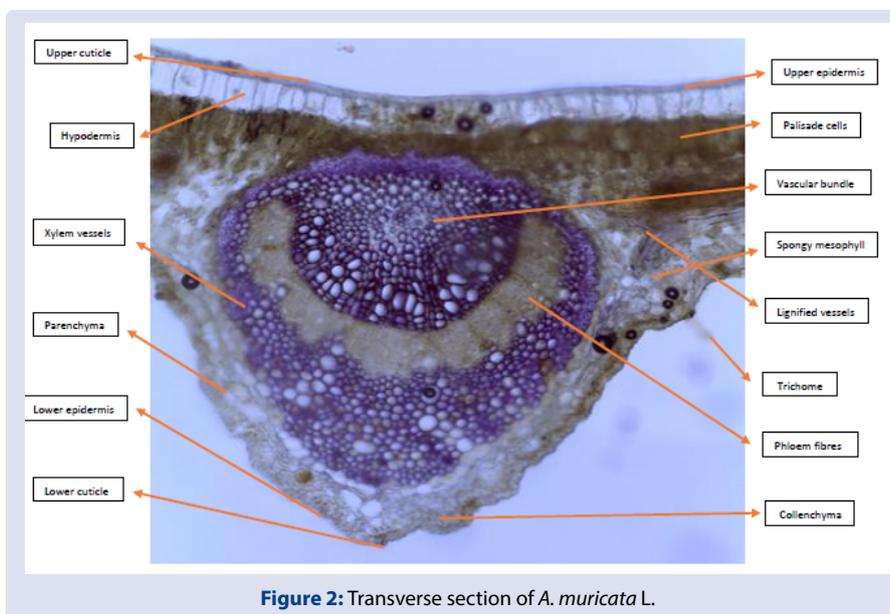


Figure 2: Transverse section of *A. muricata* L.

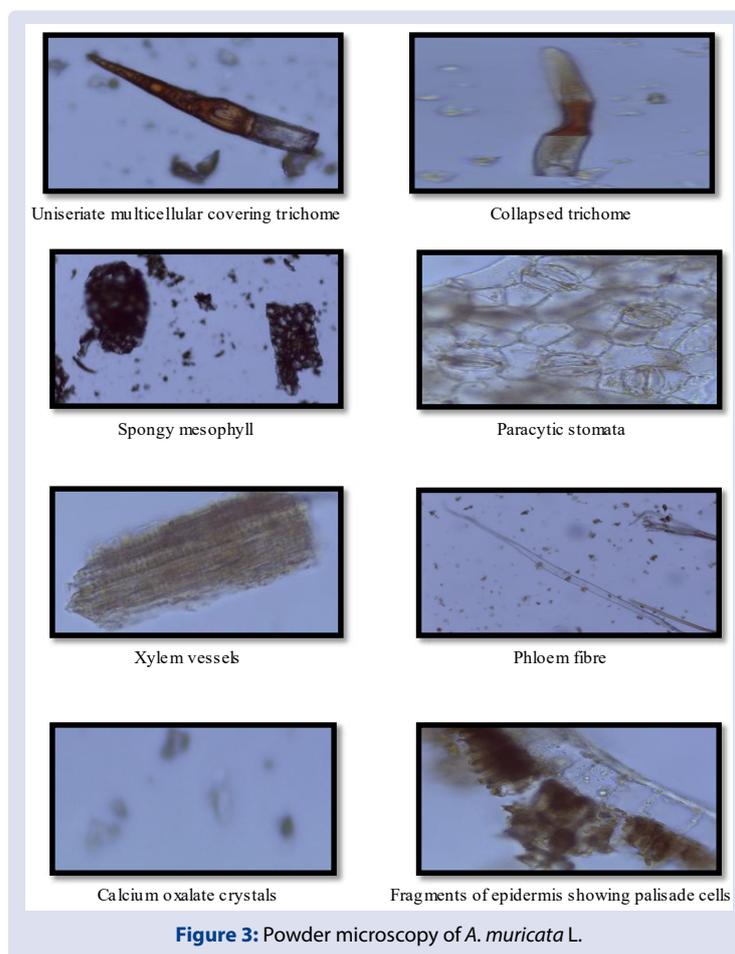


Figure 3: Powder microscopy of *A. muricata* L.

Physicochemical parameters

Ash values & extractive value

The percentage of total ash, acid-insoluble ash, water soluble ash, water soluble extractive and ethanol soluble extractive are presented in Table 2. The total ash, acid insoluble ash and water soluble ash values were observed to be (5.7) % w/w, (5.3) % w/w, and (3.9) % w/w respectively on dry basis where extractive value of ethanol and water were found to be (12) % w/w and (16) % w/w respectively.

Fluorescence analysis of the plant extracts

Fluorescence characteristic of powdered leaves can be used when there is doubtful specimen that need to be confirmed. By using with four extracts of *A. muricata* leaf powder in three conditions such as in visible light, short and long ultra-violet light has shown different colors. The results of the fluorescence analysis of the drug powder were revealed in Table 3 and the image in Figure 4.

DISCUSSION

The finding from macroscopic study revealed the characteristic of the leaves such as shape, color and size which can be used as a guide to identify important features of a plant. The, preliminary phytochemical analysis could be used as standardization applied to identify, verify and detect falsification to ensure quality control of crude drugs available in the market. As we know the leaves of *A. muricata* have been widely used since ancient times to treat a variety of diseases traditionally, but there are so many species of *A. muricata* species found throughout the world. Therefore, based on this finding, it can help to classify each type of genus members based on physicochemical parameter obtain.

Ash values could be used to determine the quality and purity of crude drug and also these values are important as a qualitative standard parameter. Based on the ash value, it can provide information about the mineral content found in a crude drug like the example carbonate, oxalate and silicate. In general, water soluble ash is used to estimate the

Table 1: Results of phytochemical analysis of various extracts of *A. muricata* L.

Chemical Constituents	Chemical tests	Petroleum ether extract	Chloroform extract	Methanol extract	Aqueous extract
Alkaloids	Dragendroff's test	-	-	-	-
Carbohydrates	Molisch's test	-	-	-	+
Proteins	Biuret test	-	-	-	+
Tannins	Ferric chloride test	-	-	+	+
Steroids	Liebermann-Burchard test	+	+	-	-
Saponins	Foam test	-	-	-	+
Flavonoids	Shinoda test	-	-	+	+

'+' = present; '-' = absent

Table 2: Physico-chemical analysis of *A. muricata* L.

Parameter	Mean (% w/w)
Total ash	5.7
Acid-insoluble ash	5.3
Water-soluble ash	3.9
Ethanol soluble extractive	12
Water soluble extractive	16

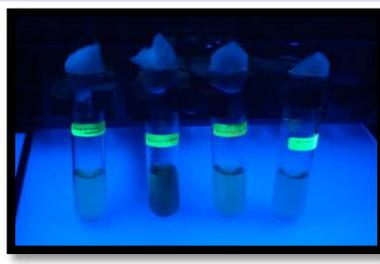
Table 3: Results of fluorescence analysis on various extracts of *A. muricata* L.

Reagents	Colour observed in day light	Colour observed under ultraviolet light	
		Short (254 nm)	Long (365 nm)
Petroleum ether	Pale green	Pale green	Light green (F)
Chloroform	Pale green	Pale green	Deep red (F)
Methanol	Pale green	Deep green	Pale brown (F)
Aqueous	Pale yellow	Pale yellow	Colourless

(F) = Fluorescence



Visible / Day light



Short (254 nm)



Long (365 nm)

Figure 4: Fluorescence analysis of different extracts of *A. muricata* L. (petroleum ether, chloroform, methanol and distilled water) under day light and ultraviolet light (254 nm & 365 nm).

amount of inorganic compound present in crude drugs and important criteria to identify either the crude drug is exhausted by the water or not while the acid insoluble ash which mainly consist of silica may indicate contamination with earthy material. From the result obtain show that total ash value, acid insoluble ash and water-soluble ash was found to be 5.7 %, 5.3 % and 3.9 % respectively.

The water soluble extractive value revealed important parameter in evaluation of crude drugs. Based on the less extractive value it can be caused by certain factor such as exhausted of crude drugs, adulteration, and due to the incorrect method during drying and storage. The alcohol soluble extractive value was also revealed the same purpose as the water soluble extractive. The water soluble extractive value proved to be higher than an alcohol soluble extractive value which found to be 16% and 12% for alcohol soluble extractive respectively.

Fluorescence analysis was carried out in U.V light at wavelength of 254 nm and 366 nm. Exhibition of various chemical constituents in the crude plants can be detected by using fluorescence analysis. Certain constituents in the plant have fluorescence characteristics at the visible light or when tested with ultraviolet radiation. The shifting of colour from visible light to ultraviolet light can be served as an indicator for the presence or absence of chemical constituents as organic molecules absorb light over a specific range of wavelength and reemit radiations. Hence, fluorescence analysis becomes one of the important parameter in determining the purity and quality of the crude drugs.

CONCLUSION

The study concludes, pharmacognostic standardization and preliminary phytochemical evaluation of *A. muricata* leaves are critically important to provide beneficial information about standardization for particular plants. In addition, this standardization is useful for solving any adulterant specimens and compiling appropriate monographs for proper identification purposes to overcome problems from irresponsible parties by trying to gain more profits due to lack or absence of standardization for particular plants. Detailed study of the morphological and microscopical characters could be helpful in terms of determining the distinction of anatomy in adulterated drugs and providing ease in differentiating plants of the same or different species.

SOURCES OF FUNDING

Self.

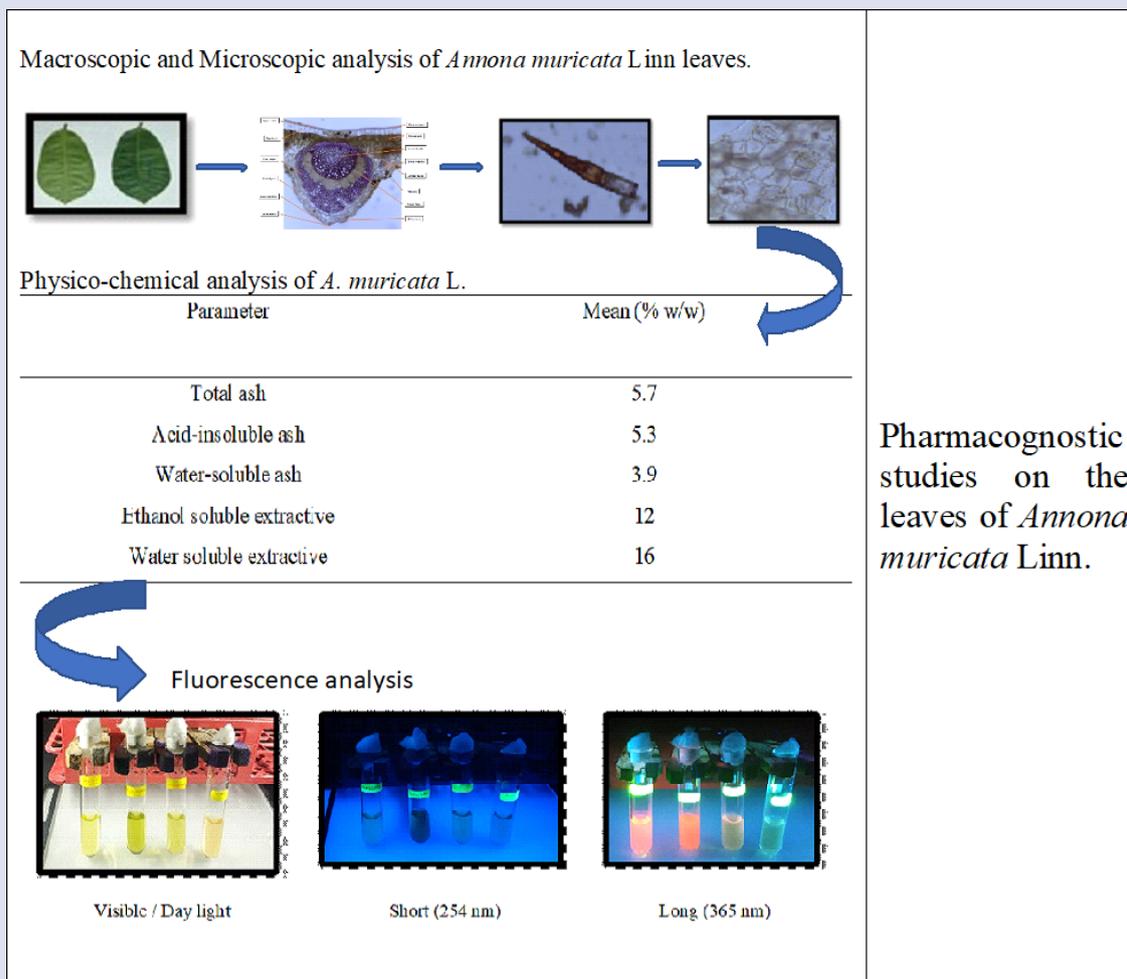
CONFLICTS OF INTEREST

None.

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GRAPHICAL ABSTRACT



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