Pharmacognostic Study of *Morinda citrifolia* L. Leaf

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Abstract

A Study has been undertaken on *Morinda citrifolia* L. belonging to Rubiaceae family. The anatomical, physicochemical and phytochemical of leaves were investigated. In anatomical study, paracytic type stomata were present on lower and absent on the upper surface. Trichomes were absent on both surfaces. Upper epidermis has thick cuticle and lower does not have. The spongy mesophyll cells were 5-8 layered and the palisade parenchyma cells were one layered. Midrib was semicircular shaped with slightly convex at axial side in outline. There were collenchyma cells forming the hypodermis and below containing parenchyma cells. Raphids and starch grains mixed with resin clots were found in mesophyll and cortex regions. There was a crescent - shaped vascular bundles having 21 to 40 rows of xylem and 3 to 8 layers of phloem. In physicochemical determination such as total ash value, water soluble ash value and acid insoluble ash value were 10 %, 3 % and 4% on leaves respectively. The preliminary phytochemical tests were carried out for Alkaloids, Flavonoids, Phenol, Saponins, Glycosides, Lipophilic compounds, Tannins, Reducing sugars, Carbohydrates and Cyanogenic glycosides. All studied compounds were found except the cyanogenic glycosides. The pharmacognostics studies of leaf were helpful in sample identification, standardization and authentication in medicinal purposes.

Keywords : *Morinda citrifolia*, anatomical, physicochemical, phytochemical

Introduction

Human beings have been utilizing plants for basic preventive and curative healthcare since immemorial time. Medicinal plants are precious part of the world flora. Before the introduction of modern medicines, disease treatment was entirely managed by herbal remedies. It is estimated that about 80% of the world population residing in the vast rural areas of the developing and under developing countries still rely mainly on medicinal plants.

The plants from which wonderful chemical compounds extracted are able to cure several diseases and are very much popular in medicinal world. Phytochemicals are secondary metabolites of plants having protective or disease preventing properties.

The medicinal value of the drug plant is due to the presence of chemical substances. Recently, a rich source of promising chemical compounds from medicinal plants, are important for health care of many people. Active phytocompounds with a wide range of therapeutic effects are required for the development of herbal drugs

Morinda citrifolis L. (Ye yo) is a perennial, evergreen and small tree. It is a well- known plant in indigenous system of medicine for its beneficial effects. It may help to prevent cancer and other diseases, and maintaining overall good health.

Morinda citrifolia, known as Noni, grows widely throught the pacific and is one of the most significant sources of traditional medicines among Pacific island societies. It is originated in tropical Asia or Polynesia. Due to its medicinal and nutritional value, it is considered as the "Queen" of the other 80 species belonging to the Rubiaceae family.The roots, barks, leaves, fruits and flowers of *Morinda citrifolia* have been traditionally used as a folk remedy to treat diseases such as diabetes, hypertension and cancer (Mathew and Britto 2016).

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There are hundreds of plants used all over the world, which are used in herbal medicines as treatments for many diseases. These plant extracts contain chemical compound that produces a definite pharmacological action on the human body.

The phytochemical screening on *Morinda citrifolia* water extracts and reportsthe presence of alkaloids, carbohydrate, saponin, phytosterols, phenols, flavonoids, amino acid, tannins, coumarins and triterpenoids (Hui *et al.* 2018).

These phytochemical have potential for using as drugs, and the content and known pharmacological activity of these substances in medicinal plants is the scientific basis for their use in modern medicine, if scientifically confirmed (Fabricant & Farnsworth 2001).

In rural area where western medicines are not available, people are still using plant based traditional medicines. Today, by using Western medicines, people can get side effects and they are very expensive. So they have now been replaced with Myanmar traditional medicines that have less side effects and very cheap. Moreover, the practitioners had traditionally used plant parts for the treatment of diseases. However, there are still many plants required to be studied and to be recorded scientifically. So the extensive research works had been done to know the efficacy of medicinal plants.

The aims and objectives of this research paper are to examine the morphological, anatomical characters and dried powder of leaves for standardization of crude drug and to investigate the physicochemical and phytochemical activies of leaves for the detection of various plant constituents in medicinal purposes.

Materials and Methods

Morphological Study

Morinda citrifolia L. (ye yo) used in traditional medicine growing as wild in Monywa Township collected during the flowering period from January to March in the year 2018. The color photograph of leaves was recorded. Identification of the collected specimen was carried out by referring to the book which was written by Dassanayake (1998) and Kress *et al.* (2003).

Anatomical Study

The leaves were cut by using a new razor blade to obtain thin sections for microscopic study. These sections were killed and fixed in 70% ethyl alcohol solution. Then about five sections were placed on slide. Chloralhydrate solution was dropped onto the sections for 10 minute and then washed by water. The sections were stained with standard safranin for 15 minute and then washed by water. The stained sections were mounted in glycerin enclosed with a cover slip.

Powdered samples were also examined to get diagnostic characters for the standardization of traditional medicine. For powdered microscopical study, the leaves *Morinda citrifolia* were cut into small pieces and air dried at room temperature for 4 weeks. Small pieces were ground to get powder by using a blender or grinding machine and then, sieved through 60 μm sieve size by sieve shaker. All powdered form specimens were stored in air tight container for further research work. The diagnostic characteristics of the powdered leaves were examined on a glass slide and under the light microscope. These were cleared with chloralhydrate solution before microscopical examination. The safranin solution was used to stain the lignified cell walls. The presence of calcium oxalate crystals was tested by using 80% sulphuric acid.

In the present study, the cells were studied and the distinct characters were recorded by the microscope. Then, photo micrographic figures of the free- hand sections were also prepared by the use of a microscope with digital camera. The terminology was used for microscopic description as it had given by Chattaway(1932) and Wheeler *et al.* (1989).

Physicochemical study

The physicochemical analysis such as total ash value, water soluable ash value and acid insoluable ash value were determined according to method described in WHO (2002) guidelines. Physicochemical examination was carried out in the University Research Center, Department of Botany, Monywa University.

Determination of Total Ash

5 gm of sample powdered were weighed in a crucible and placed a muffle-furnace at 500°C until the substance turned into ash. After ashing, the crucible was cooled in dessicator and weighed. This procedure was repeated until a constant weignt was obtained and the percentage of total ash was calculated by the following formula.

$$\%\,\text{ash} = \frac{\text{wt.ash}}{\text{wt.sample}} \times 100$$

Determination of Water Soluable Ash

The total ash was boiled with 10ml of water for 5 minutes. The insoluable matters were collected on an ashless filter paper and wash with water. Then, insoluble matters were ignited to constant weight. The weight of insoluble matter was deducted from the weight of total ash, the difference in weight was represented the water soluable ash. The percentage of water soluable ash was calculated.

Determination of Acid Insoluable Ash

The total ash was boiled gently with 10 ml of 6% dilute hydrochloric acid for 5 minutes. The insoluble matters were collected on an ashless filter paper and wash with hot water until free of acid testing with P^H paper. The acid insoluble ash residue together with the crucible were dried in an oven at 110°C and weighed again.The percentage of the acid insoluble ash was calculated.

Phytochemical Study

The leaves of *Morinda citrifolia* were cleaned thoroughly. Then, they were cut into small pieces and were dried in shady place at room temperature for 2-3 weeks to get stable dried products. Then the dried leaves were pulverized by blender to get fine powder and stored in air tight container.

The powdered leaves were extracted with 1% diluted hydrochloric acid (HCl), 95 % ethanol (EtOH) and distilled water (H_2O) and then tested for the presence or absence of alkaloids, flavonoids, phenolic compound, saponins, glycosides, lipophelic compound, tannins, reducing sugars, carbohydrate, cyanogenic goycosides by Harbones (1984) method. Phytochemical examination was carried out in the University Research Center, Department of Botany, Monywa University.

Results

Morphological Study *(Morinda citrifolia* L.,Ye yo)

Distinct characters - Perennial, small tree;leaves simple, opposite and decussate, interpetiolar stipulate; inflorescence terminal or axillary globose head with many folwer; flower actinomorphic; ebracteate , sessile; calyx tubular, 4-6 partite; corolla infundibuliform; 4 to 6-lobed; anther dithecous; filament short; ovary tetralocular; axile placentae; baccate, sorosis, the distinctive grenade like yellow fruit.

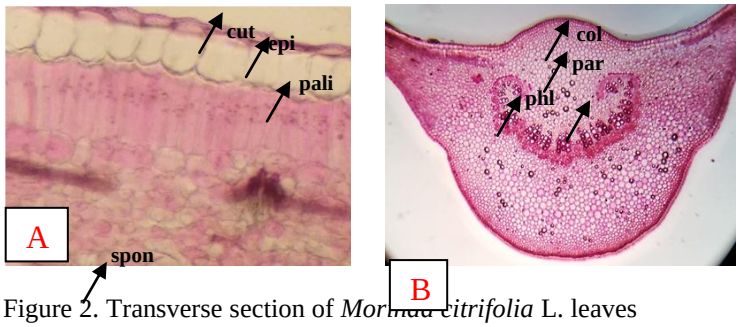
Figure 1. Morphological characters of *Morinda citrifolia* L. leaves
(A) Inflorescences with leaves (C) Close up portion of an inflorescence (C) various size of leaves

Anatomical Study

Leaf was dorsiventral. In surface view, paracytic types of stomata were abundantly present on the lower surface and absent on the upper one. Stomata are rubiceous. The stomata consisted of oval-shaped in outline with very small pores and guard cells were reniform-shaped. Trichomes were absent on both surfaces.

In transverse section, below the epidermis was the mesophyll tissue consisting of upper compactly arranged palisade parenchyma cells which were one-layered thick vertically elongated and lower loosely arranged spongy parenchyma cells which were 5 to 8-layered of oval, circular and tubular in shaped. These two were photosynthetic as they contained chloroplast. Raphids were present in mesophyll cells and also resin mixed with starch grains were found in mesophyll regions.

In the mid-rib, the ground tissue was made of outer collenchyma and an inner parenchyma tissue. Under the epidermis, collenchyma cells were found forming hypodermis in both sides.There was a single crescent-shaped vascular bundle having prominent xylem and comparatively thin layer of phloem.Phloem was found 3 to 8-layered in thickness and the cells were irregular-shaped. Phloem composed of sieve tube, companion cells, and phloem fibres and phloem parenchyma.The xylem were found 21 to 40 rows. Each row 2 to 6 celled and the cells were polygonal-shaped. Xylem was composed of vessels, tracheids, xylem fibres and xylem parenchyma.



cut = cuticle
epi = epidermal
pali = palisade
spon = spongy
col = collenchyma
par = parenchyma
xyl = xylem
phl = phloem

Figure 2. Transverse section of *Morinda citrifolia* L. leaves
(A) T.S of Lamina (B) T.S of midrib

Figure 3. Powdered microscopy of leaves of *Morinda citrifolia* L.
(A) fiber (B) vessel (C) tracheid (D) paracytic stomata

Sensory characters study

The colour, odour, taste and the texture of powdered leaves of *Morinda citrifolia* L were shown in Table 1.

Table 1. Sensory characters of the powdered leaves of *Morinda citrifolia* L

No	Sensory characters	
1	Colour	dark green
2	Odour	Astringent
3	Texture	Grain
4	Taste	bitter and pleasant

Physicochemical Study

The physicochemical parameters like total ash, water soluble ash and acid insoluble ash were observed as shown in Table 2.

Table 2. Physicochemical Characteristics on Leaves of *Morinda citrifolia* L.

Parameters	Value
Total ash	10 %
Water soluble ash	3 %
Acid insoluble ash	4 %

Phytochemical Study

The preliminary phytochemical screening was performed to find out the phytoconstituent present in the leaves of *Morinda citrifolia* L.as shown in Table 3.

Table 3. Phytochemical Test on Leaves of *Morinda citrifolia* L.

No	Test	Extract	Test reagent	Observation	Results
1	Alkaloids	1 % HCl	Dragendroff reagent Mayer's reagent Wegner's reagent	Orange ppt White ppt Reddish ppt	+ + +
2	Flavonoids	EtOH	Conc: HCl + Mg	Blue black color	+
3	Phenolic compounds	EtOH	10% $FeCl_3$ solution	Black ppt	+
4	Saponins	H_2O	Distilled water (or) $NaHCO_3$ solution	Frothing	+
5	Glycosides	H_2O	10 % lead aceteate solution	White ppt	+
6	Lipophelic compounds	H_2O	KOH solution	Yellow color Reddish ring	+
7	Tannins	H_2O	1 % Gelatin + 1 % $FeCl_3$ solution	Yellow ppt	+
8	Reducing sugars	H_2O	Benedict's solution	Dark brown color	+
9	Carbohydrates	H_2O	Benedict's solution + Conc: H_2SO_4	Dark green color	+
10	Cyanogenic glycosides	H_2O	Conc:H_2SO_4 + Sodium Picrate solution	No color change, no ppt	–

– = Absent , + = Present , Conc: = Concentrated , ppt = precipitate

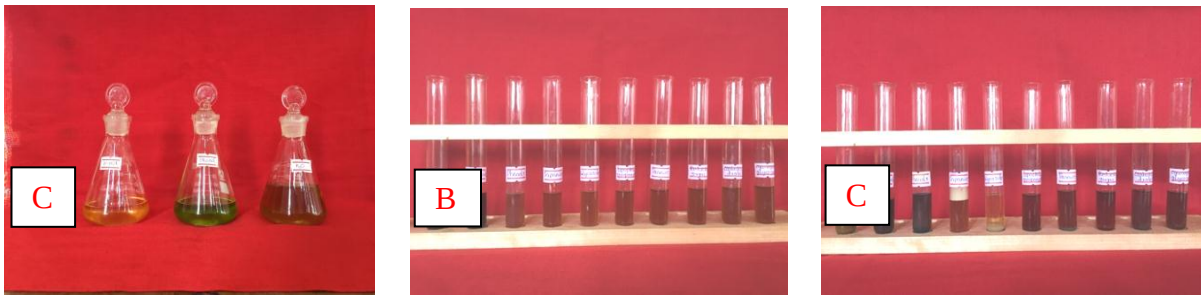


Figure 3. Phytochemical test on powdered leaves of *Morinda citrifolia* L.
(A) Various extracts
(B) Various extracts before phytochemical test
(C) Various compounds after phytochemical test

Discussion and Conclusion

Morinda citrifolia L., Ye yo (Noni) is included in the family Rubiaceae. It is one of the most important traditional medicinal plants. The noni tree has been used in traditional medicine. The leaves are dark green above and pale green beneath in color, astringent odour, tase is bitter and pleasant, size is 9-20 cm long, and shape is broadly elliptic to obovate.

The leaves have been studied extensively in the past decade. The leaves of this plant are directly used on skin for ulcerations and for minor infections.Several animal studies suggested noni may have anti- cancer (Subramanian *et al*. 2017)

Morinda citrifolia is cultivated plant and also grows wild in many places of Myanmar. According to Kyaw Soe and Tin Myo Ngwe (2004), the leaves are applied to treat arthritis which agrees with Manjula *et al*. (2016). Myint Myint Swe *et al*. (2005) recorded that the leaves of this plant can be used as traditional remedy for diabetes which is in agreement with Akihisa *et al*. (2007), Kakad *et al*. (2015) and Manjula *et al*. (2016). Besides, this plant is also used in the treatment of hypertension and cancer (Akihisa *et al*. 2007) and it agrees with the statement of Kakad *et al*. (2015). *Morinda. citrifolia* is used as antioxidant as it had been described by Kakad *et al*. (2015). Trease and Evants (2002) reported that the *Morinda citrifolia* was employed in traditional medicine.

In this study, leaf was dorsiventral. In surface view, paracytic types of stomata were abundantly present on the lower surface and absent on the upper one. Stomata were rubaceous. In transverse section, below the epidermis was the mesophyll tissue consisting of upper compactly arranged palisade cells and lower spongy parenchyma cells. These two were photosynthetic as they contained chloroplast. Resin mixed with starch grains were found in mesophyll regions which agreed with those described by the research of Shikerkar and Shrikanth (2015)

In the mid-rib, there was a U-shaped vascular bundle. The ground tissue was made of parenchyma cells as it had been described by Shikerkar and Shrikanth (2015) which did not agree with study. In this study, there was a single crescent-shaped vascular bundle.The ground tissue was made of outer collenchyma and an inner parenchyma tissue. Under the epidermis, collenchyma cells were found forming hypodermis in both side.

The preliminary phytochemical screening revealed the presence of alkaloid, flavonoids, glycosides, phenol, tannins, saponins, reducing sugars, carbohydrates and lipophilic compounds were found but cyanogenic glycosides compound was not found in leaves of *Morinda citrifolia* .which agreed with those stated by the Hui *et al*. (2018) and Kakad *et al*. (2015). But, Subramania (2017) described that the gylcosides

were absent in leaves which were not agreed with this research. In the present study, glycosides were present in leaves which were agreed with Kakad *et al*.(2015). According to the preliminary phytochemical investigation, the absence of cyanogenic glycosides was observed in leaves.Thus, it can be assumed that this medicinal plant may be potentially safe and harmless for home remedies.

Since early time, human must have searched plant substance that would relieve their suffering. Poor people in our country cannot afford modern allopathic drugs due to their high cost. So there is an urgent need for alternative treatment for different types of diseases. Moreover, active phytocompounds with a wide range of therapeutic effects can be provided for the development of new herbal drugs.

The present study describes the morphological, anatomical, physicochemical and phytochemical screening of *M. citrifolia* . The morphological and anatomical characters of *Morinda citrifolia* . are very useful and informative for their proper utilization in identification and helpful to differentiate from closely related other species of *Morinda*. Moreover, sensory characters of this plant will provide for confirmation of its identity and detection of nature of adulteration. So the present findings may support to identify the correct species and would serve as a reference for identification of these plant species commercially available and to differentiate them from their substitute and adulterant. The information obtained from preliminary photochemical screening will be useful in finding out the genuity of the drug.

The present research may provide information for medicinal properties of *Morinda citrifolia* leaves for other researchers and pharmaceutical industry.

Further studies are needed to isolate the active chemical constituents as well as detailed pharmacological activity will be carried out by proper scientific way. Moreover, an acute toxicity test by mice or rabbit is needed in order to assure its safety.

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