

Indonesian *Mirabilis jalapa* Linn. : A Pharmacognostical and Preliminary Phytochemical Investigations

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ABSTRACT

Introduction: *Mirabilis jalapa* Linn. is an important medicinal plant and used extensively by the people from different countries for the treatment of several disorders. The plant was the raw material for the herb-drug product, so some parameters identified were needed to ensure the safety, quality and efficacy of the product. **Objective:** The aim of this study was to undertake pharmacognostical studies to fulfill the work required for the identification the *M. jalapa* plant, which is collected from the Bogor area, Indonesia. **Methods:** Macroscopic and microscopic evaluation, fluorescence standards, phytochemical screening and physicochemical parameters were carried out on the above plant. **Results:** The parameters values of total ash, water soluble and acid insoluble ash were obtained 11.81, 5.06 and 0.41%, respectively. Moisture content, alcohol, water and ether soluble extractive were found to be 12.41, 11.02, 18.63 and 7.17% respectively. The results of preliminary phytochemical analysis of aqueous ethanolic extract of this drug were positive for alkaloids, tannins, flavonoids, steroid, triterpenoids, saponin, phenols, glycosides and carbohydrate. Thin layer chromatography (TLC) of alcoholic, chloroform and aqueous extracts showed 9, 7 and 4 spots respectively. **Conclusion:** The present study on botanical pharmacognosy and TLC profile of this plant above thus provides useful information for correct identification and quality control parameters for the crude drugs, and also will be useful in making monograph of the plant. **Key words:** Chromatography profile, Fluorescence character, Microscopic, Nyctaginaceae, Physicochemical.

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INTRODUCTION

Mirabilis jalapa Linn. (Nyctaginaceae) is a perennial herb and is has been called by various vernacular names around the world, like Gulambasa' in Ayurveda, 'Gul-abbas' in Hindi, 'Four o' clock' in English,¹ and "pukul 4" in Indonesia.² 'Four o' clock' received the name because of habit of opening in the late afternoon. This plant is a widely used traditional medicine in many parts of the world for the treatment of various diseases viz. virus inhibitory activity,³ diarrhea,⁴ inflammation treatment,^{5,6} anti-bacterial^{7,8} and anti-spasmodic activity.⁹ The root is believed to be an aphrodisiac as well as diuretic and purgative, and antioxidant activity.^{1,10} *Mirabilis jalapa* has been extensively used in almost all folklore remedies around the world included Indonesia for treating a variety of conditions. The Indonesia Traditional medicinal ("Jamu") this plant is widely for the treatment of diarrhea, dysentery, muscularpain, and abdominal colics, inflammation, antibacterial, antiviral, and antifungal functions.² To ensure reproducible quality of herbal products, authentication of the starting material is essential.¹¹ This present study is concerned to characterization of different pharmacognostical parameters, has included here, botanical identification, microscopic study, powder characteristics, analytical standardization, florescence study and preliminary phytochemical screening. This preliminary study helped for standardization of the crude drug as well as further processing of the sample with

some indication regarding the nature of chemical compounds present in it. The pharmaceutical use of traditionally used medicinal plants is hampered due to the lack of standards of quality, safety and efficacy.¹² The pharmacognostical and preliminary phytochemistry description of a medicinal plant is the first step towards establishing the identity and the degree of purity of such materials and should be carried out before any tests are undertaken.¹³ This study is comprised the Indonesian *M. jalapa* Linn.

MATERIALS AND METHODS

Collection and Authentication

The whole fresh plants material of *Mirabilis jalapa* Linn. were collected in the month of August – September 2016 (from Bogor area, Indonesia), and authenticated by the Research center for Biology, Indonesian Institute of Sciences, Cibinong, Bogor, Indonesia. A voucher specimen has been preserved in the Pharmacognosy laboratory, Faculty of Pharmacy and Sciences, University of Muhammadiyah Prof. Dr. HAMKA, for further references.

Preparation of plant material

The collected *M. jalapa* whole plant was washed with tap water. The plant was cut in to small pieces and air-dried thoroughly under shade (at room temperature) for 1 week to avoid direct loss

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of phytoconstituents from sunlight. The shade dried materials were powdered using the pulverizer and sieved up to 60 meshes, and stored in a well closed glass bottle.

Pharmacognostical Evaluation

Macroscopic characteristics

Macroscopical examination was carried out to the freshly plants material, and to the powder. Morphological studies of plants material such as color, size, odor, taste, surface characteristic and fracture were observed based on the description given in Evans WC¹⁴ and Indonesian Herb Pharmacopoeia.¹⁵ Organoleptic characters were observed, noted and photographs were taken in the original environment.

Microscopic characteristics

Mature, fresh and healthy the different plant parts as leaf, stem, root, etc. and the powder were used for microscopic evaluation. The transverse sections of the different plant parts were prepared by free hand, it was put between the pith and fine sections were cut with the help of a sharp razor. The sections so obtained were cleared using chloral hydrate solution. Preparing for powder microscopy, small amount of whole plant material powder was placed on a glass slide containing one to two drops of chloral hydrate solution. After placing the cover slip, it was warmed gently on spirit lamp to remove air bubbles and then observed under the microscope. Different tissues were observed under the microscope and were photographed.^{16,17}

Physicochemical Evaluation

Physicochemical characteristics such as moisture content, total ash, water soluble and acid insoluble ash tests were performed according to the official methods and the WHO guidelines on the quality control methods for medicinal plant materials.^{13,1} The determination of the extractive values (alcohol, water and ether soluble) of the powdered drug was carried out according to the procedure described in Indonesian Herb Pharmacopoeia, 2008.¹⁵ Fluorescence characters of powdered and extracts material with different chemical reagents were determined under ordinary and ultraviolet light.

Moisture content

About 2 g of the air dried powdered drug was weighed in a watch glass, kept in hot air oven at 105°C and dried for a period (about 30 minutes) until constant weight was obtained. Weight loss on drying was noted and difference in weight gives the moisture content of powdered drug. Total moisture content of the drug was noted.

Determination of total ash value

Two grams of the powdered material was placed in a silica crucible which previously was ignited and tarred crucible accurately weighed. The powdered material was spread as a fine even layer at the bottom of the crucible. The crucible was incinerated until the temperature about 450°C, and a white material was obtained, that is indicating the absence of carbon. The crucible was cooled and weighed. The procedure was repeated until the constant weights. The percentage of the total ash was calculated with reference to the air dried powdered sample.

Determination of water soluble ash value

The total ash obtained was boiled with 25 ml of water for five min. The insoluble matter was collected on a ash less filter paper & washed with hot water. The insoluble ash was transferred into pre-weighed silica crucible, ignited for 15 min at a temperature not exceeding 450°C, cooled and weighed. The procedure was repeated to get the constant weight. The weight of the insoluble matter was subtracted from the weight of total ash. The percentage of water soluble ash was calculated with reference to the air-dried sample drug.

Determination of acid-insoluble ash value

Twenty-five (25) ml of 2N hydrochloric acid was added to the crucible containing the total ash, covered with a watch-glass and boiled gently for 5 minutes. The watch-glass was rinsed with 5ml of hot water and this liquid added to the crucible. The insoluble matter was collected on an ash less filter-paper and washed with hot water until the filtrate was neutral. The filter-paper containing the insoluble matter was transferred to the original crucible, ignited by gradually increasing the heat to 450°C until the constant weight was obtained. The percentage of acid-insoluble ash was calculated with reference to the air dried drugs.

Water extractive value

Separately place about 10.0 g (accurately weigh) of whole plant powder of the *M. jalapa*, in an accurately weighed glass stopper conical flask. Then 100 ml of distilled water was added to the flask and weighed to obtain the total weight including the flask. The contents was macerated, shaken well during the first 6 hrs and allowed to stand for 18 hours. The solution filtered rapidly through a dry filter. The flask was readjusted to the original total weight with distilled water. Twenty-five ml of the filtrate was transferred to an accurately weighed, tarred flat-bottomed dish and evaporated to dryness on a water-bath. Finally, it was dried at 105°C until the constant weight was obtained. The percentage of the value of water extractive was calculated with reference to the air-dried drug.

Ethanol extractive value

Same procedure as water extractive value was followed using ethanol instead of distilled water to determine extractable matter in ethanol. The ethanol extractive value was calculated with reference to the air-dried drug.

Ether extractive value

Same procedure as water extractive value was followed using ether to determine extractable matter in ether. The ether extractive value was calculated with reference to the air-dried drug.

Fluorescence Characters

Fluorescence characters of powdered drug material were sieved through 60 mesh and observations with different chemical reagents. These were observed under visible light, short and long ultraviolet (254 and 366 nm). The reagents were methanol, 2N hydrochloric acid, 50% sulphuric acid, 50% nitric acid and 5% potassium and sodium hydroxide.^{18,19} The intensity of the coloration determines the abundance of the compound present.

Phytochemical Screening

Weighed 25 g of plant material powdered was extracted in a reflux apparatus with 250 ml methanol for 30 minutes. The extracts were filtered and evaporated to dryness under reduced pressure and controlled temperature (40-50°C). The extracts were subjected to preliminary phytochemical investigation for the detection of following compounds; carbohydrates, protein, steroids and terpenoids, glycosides, flavonoids, alkaloids, tannins, phenolic compounds anthraquinones and saponins, as per the standard procedures described by Indonesian Herb Pharmacopoeia and Harborne.^{15,20} The presence of phyto-constituents from the above plants was presented in the Table 3.

Chromatographic Profile

The powder material (10 g) was subjected it to successive Soxhlet extraction with 100 ml solvents: hexane, dichloromethane (DCM) and methanol respectively. The extraction was continued until the solvent became colorless. The 3 extracts were concentrated using a rotary evaporator then analyzed by TLC using silica gel 60 F254 TLC plates for the chromatographic profile. Each extract was faintly dissolved in methanol and

capillary tubes were used to uniformly apply the dissolved samples on the plates and allowed to dry. The mobile phases for the plates developed were hexane –DCM (6:4), chloroform- methanol (9:1), ethyl acetate – methanol (7:3). The plates were dried and observed under visible light and ultraviolet light 366nm, and by spraying with 10% sulfuric acid followed by heating at 105°C for 5-10minutes in an oven.²¹The retention factor (Rf) value was calculated by using this formula:

$$R_f = \frac{\text{Distance moved by the solute (compound)}}{\text{Distance moved by the solvent front}}$$

RESULTS

Macroscopic Studies

Mirabilis jalapa Linn. (Nyctaginaceae) is a perennial herb that reaches a height of 50-100 cm, and a popular ornamental plant grown worldwide for the beauty of its flowers. They have numerous branches and opposite, and the flowers are borne singly or in clusters, and can be red, magenta, pink or yellowish, have light fragrance characteristic taste. Individual flowers are trumpet shaped, about an inch across at the end and

2.5-3.5 cm length. Leaves are green, slightly bitter and having characteristic odor. These are ovate shape, pinnatifid, acuminate apex, crenate, cordate base and 5–11cm in length, 4–7 cm in width. The plants have black carrot shaped tubers that can be a foot or more length, and wrinkled black obovoid fruits (Figure 1).

Microscopic Studies

Transverse section of *M. jalapa* Linn leaf shows presence of multicellular trichomes on both surfaces. The upper and lower epidermis consists of oval shaped parenchyma cells in single layer and also absence of stomata. The vascular bundles are composed of both xylem and phloem in collateral open arrangements. *M. jalapa* Linn. leaf also show the presence of cuboidal and raphides calcium oxalate crystal (Figure 2A, 3A). Transverse section of *M. jalapa* steam show much trichomes, single cell of epidermis, collenchyma and some layer of parenchyma cells (Figure 2B). Transverse section of the root show the cuboidal calcium oxalate crystal and raphides calcium oxalate crystal (Figure 2C), and Figure 3A showed also the cuboidal and raphides calcium oxalate crystal. Fragment of stigma, anther and vascular bundles were showed in Figure 3B, 3C and 3D respectively.

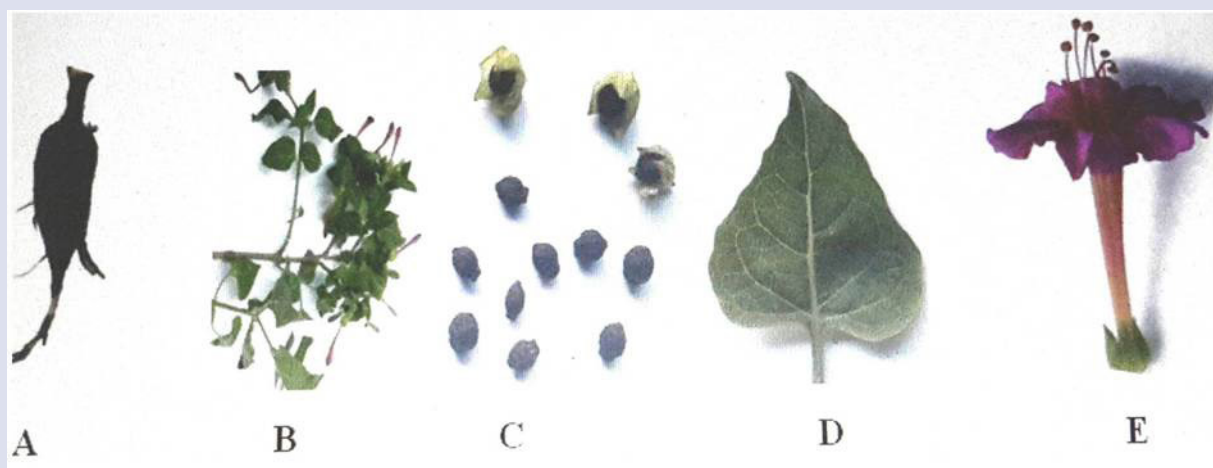


Figure 1: A. Tuber, B. Branch, C. Seeds, D. Fresh leaf, and E. Flower of *M. jalapa*.

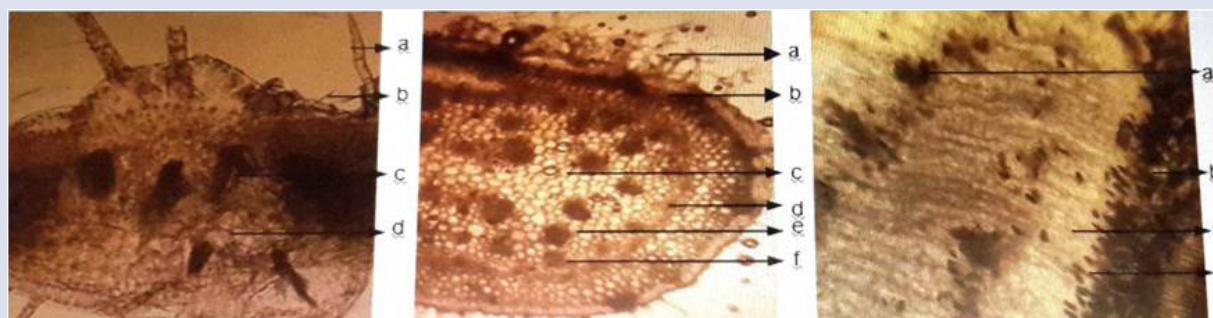


Figure 2: A. Transverse section of leaf: a. trichome; b. raphides calcium oxalate crystal, c. sclereid cell, d. cuboidal calcium oxalate crystal. B. Transverse section of stem: a. epidermis hair, b. collenchyma, c. pith, d. endodermis, e. phloem, f. xylem, C. Transverse section of root: a. endodermis, b. cuboidal calcium oxalate crystal, c. cambium, d. raphides calcium oxalate crystal.



Figure 3: A. Raphides and cuboidal calcium oxalate crystal, B. Portion of stigma; C. Fragment of anthers with oil gland, D. Vascular bundles

Physicochemical characteristics

The physico-chemical characteristics such loss on drying, ash values, water, alcohol and ether soluble extractive, were given in the Table 1.

Fluorescence Characters

Fluorescence characters of powdered material were analyzed under visible and ultraviolet light (254 and 366 nm), which signifies their characteristics (Table 2).

Table 1: Physicochemical Parameter of Powder of *M. jalapa*

Sl.No	Parameters	Average (%w/w)
1	Moisture content	
	Loss on drying	12.41% ± 0.005
2	Ash Value	
	a. Total ash	11.81% ± 0.001
	b. Water soluble ash	5.06% ± 0.001
	c. Acid insoluble ash	0.41% ± 0.001
3	Extractive values	
	a. Alcohol soluble	11.02% ± 0.007
	b. Water soluble	18.63% ± 0.007
	c. Ether soluble	7.17% ± 0.007

Table 2: Fluorescence Analysis of Powder and Ethanolic Extract of *M. jalapa*

Treatment	Normal light	UV (254 nm)	UV (366 nm)
Powder			
+Water	Green	Light yellow	Light yellow
+KOH	Brown	Dark brown	Brown
+NaOH	Greenish brown	Yellowish brown	Brown
+H ₂ SO ₄	Light brown	Bright yellow	Light brown
+HCl	Light brown	Bright yellow	Light yellow
+HNO ₃	Yellowish brown	Greenish brown	Greenish brown
Alcohol Extract 70%			
+Water	Brown	Yellow	Yellow
+KOH	Yellowish brown	Brown	Light Brown
+NaOH	Greenish brown	Yellowish brown	Greenish brown
+H ₂ SO ₄	Light brown	Bright yellow	Yellow
+HCl	Brown	Bright yellow	Yellow
+HNO ₃	Light brown	Brown	Light brown

Phytochemical Screening

Preliminary Phytochemical screening determination of ethanolic extract by some chemical test was carried out. The results were positive for alkaloids, tannins, flavonoids, glycosides, phenols, steroid, triterpenoids and saponin, carbohydrate (Table 3).

Chromatographic Profile

Thin layer chromatographic profile evaluated of *M. jalapa* leaves extracts (hexane, DCM, ethanol) constituted different colored phytochemical compounds with different R_f value. The R_f values were calculated for the optimum solvent system revealed the presence of promising spots as shown in Table 4.

DISCUSSION

Macroscopical and microscopical characters will help in the identification of right variety and search for adulterants. The morphological characters of different parts of the plant can serve as diagnostic parameters. Microscopic evaluation is one of the simplest and cheapest methods for establishing the correct identification of the source of the materials. In this microscopical character of *M. jalapa* leaves show the presence of

Table 3: Phytochemical Screening of Plant Extracts of *M. jalapa*

Phytoconstituens	Test Performed /reagents	Results
Carbohydrates	Fehling	+
	Molisch	+
Proteins	Biuret	-
	Ninhydrin	-
Alkaloids	Dragendorff	+
	Mayer	+
	Bouchardat	+
Anthraquinones	Borntrager	-
Flavonoids	Shinoda	+
	Ammonia	+
Glycosides	Molisch	+
	Fehling	+
Phenols	Ferric chloride	+
	Foam	+
Saponins	Liebermann	+
	BurchardSalkowski	+
Steroids - terpenoids	Gelatin	+
	Lead acetate	+

Description: (+) = present; (-) = Absent

Table 4: Chromatographic Profile of Different Extracts of *M.jalapa* Leaves

Extract	Solvent System	Total Spots	Spraying reagent (10% H ₂ SO ₄)	Rf Value
Hexane	Hexane - DCM 6:4.	9	Light brown, light grey, yellow, light grey, light yellow, light grey, light yellow, light yellow, yellow	0.08, 0.17, 0.28, 0.35, 0.50, 0.60, 0.75, 0.86, 0.92
DCM	Choroform – methanol 9:1.	7	Light brown, light grey, grey, light yellow, yellow, light blue, light yellow.	0.05, 0.15, 0.46, 0.60, 0.68, 0.75, 0.90
Ethanol	Ethyl acetate – ethanol 7:3	4	Light brown, light yellow, yellow, light yellow	0.05, 0.31, 0.41, 0.90.

cuboidal^{22,23} and raphides needle in shape of calcium oxalate crystal in Indonesian drug. Drying plays a very important role in the quality as well as purity of the material. Moisture was not so high as to facilitate activation of enzymes and gives suitable condition, to the proliferation of microorganisms, and is an inevitable component of crude drugs, which must be eliminated as far as practicable. The ash values are useful in determining authenticity and purity of drug and also important quantitative standards. Total ash values of drug give an idea of earthy matter or the inorganic composition and other impurities present along with drug. The extractive values are useful for determination of crude drugs and it gives an idea about the nature of the chemical constituents present. Water-soluble indicated the presence of sugar, acids, and inorganic compounds. The alcohol soluble indicated the presence of polar constituents, and ether soluble indicated the presence of non-polar constituents. Physical constants like ash and extractive values help in establishing the pharmacopoeia standards of drug. The physicochemical parameter of this Indonesian drug is almost same with the Indian drug,^{1,22,23} although there were many factors can affected the quality of herbal medicines, such as light exposure, temperature, water availability, nutrients, period and time of collection, method of collecting, drying, packing, storage and transportation of raw material, age and part of the plant collected. Fluorescence study of the drug powder and the extracts helps in the qualitative evaluation which can be used as a reference data for the identification of adulterations. Thin layer chromatographic profile of hexane, DCM and ethanol extracts were carried out with the different mobile phase system to determine how many compounds in the 3 kinds of these extract, the each compound has a different colored and different Rf value.

CONCLUSION

The present study on pharmacognosy of different parts of the *M.jalapa* Linn. provides useful information for quality control parameters for the crude drugs. Macro- and microscopic powder, quantitative and fluorescence standards discussed here can be considered as identifying parameters to substantiate and authenticate the drug. This preliminary information is necessary for standardization of the plant material used in the formulation of drugs. The results of the present investigation are significant and encouraging towards the goal for future utilization and standardization of above plants. *M.jalapao* rigin from Indonesia, India and South India showed the same phytoconstituens compounds such as alkaloids, tannins, flavonoids, terpenoids, glycosides, saponins and carbohydrates.

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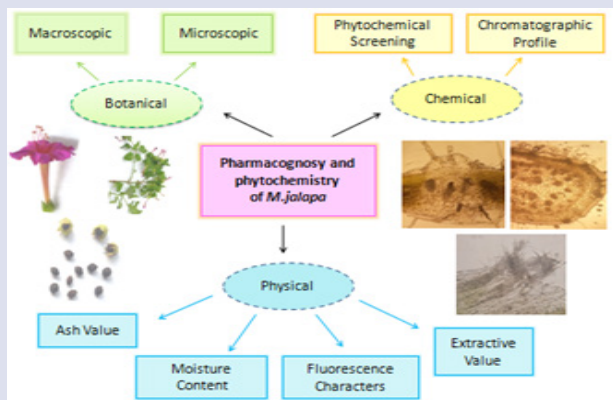
CONFLICT OF INTEREST

Authors declare no conflict of interest.

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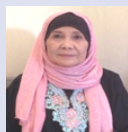
GRAPHICAL ABSTRACT



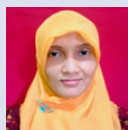
HIGHLIGHTS OF PAPER

- *Mirabilis jalapa* Linn. has been called by various vernacular names around the world, like Gulambasa' in Ayurveda, 'Gul-abbas' in Hindi, 'Four o' clock' in English,¹ and "pukul 4" in Indonesia.
- This plant is used for treatment of various diseases viz. virus inhibitory activity, diarrhea, inflammation treatment, anti-bacterial and anti-spasmodic activity.
- *M.jalapa* origin from Indonesia, India and South India showed the same phytoconstituens compounds.
- This is the first report of Indonesian *M. jalapa* Linn. on the pharmacognostical studies, and provides useful information for quality control parameters for the crude drugs.

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