Pharmacogn J. 2019; 11(5):1100-1105.
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
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Research Article

Pharmacognostical and Preliminary Phytochemical Evaluation of *Cordia sebestena* L.

Endang Hanani[1,2,*], Sri Harsodjo Wijono Soewandi[1,2], Hayati[1,2], Nellysa Revita[2]

ABSTRACT

Background: *Cordia sebestena* L. belongs to family Boraginaceae is commonly known as "Geiger Tree" in Indonesia, it is generally found in Indonesia included Jakarta, and many other countries. Some researchers have revealed the presence of several pharmacology activities. Thus this study is an attempt to present an overview of pharmacognostic and phytochemical evaluation reported on this plant. Objective: In the present investigation, various pharmacognostic standards have been investigated to prove the authenticity of the plant for the claimed traditional uses. This will help in the identification of powder drug prior to using in medicine. **Methods**: Macroscopic, microscopic and physicochemical evaluation, of the drug was performed using standardized procedures as mentioned in the WHO and Indonesian Herb Pharmacopoeia guidelines.Thin layer chromatography profile was done using some different mobile phase system to the identification of the drug. **Results**: The physicochemical parameters such as moisture content, ethanol- and water-soluble extractive, total ash, acid-insoluble ash value, were determined. Phytochemical screening showed the presence of alkaloids, flavonoids, phenols, saponins, tannins, steroids, terpenoids in the ethanolic extracts of leaf of this plant. The TLC profile of *n*-hexane, dichloromethane and methanol extract were carried out 5, 7 and 2 spots respectively. **Conclusion**: The present study on pharmacognostical, physicochemical and phytochemical standards could be useful information for authentication and preparation monograph for *C.sebestena*.

Key words: Boraginaceae, Chromatography Profile, Fluorescence, Microscopic, Physicochemical.

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History

- Submission Date: 14-05-2019;
- Review completed: 27-05-2019;
- Accepted Date: 04-06-2019.

DOI : 10.5530/pj.2019.11.172

Article Available online

http://www.phcogj.com/v11/i5

INTRODUCTION

Cordia sebestena L. is also known as Geiger tree, belong to family Boraginaceae. The family Boraginaceae consists of about 2.700 species which are distributed in tropical, sub-tropical and warmer regions around the world. It grows up to 25 feet in tropical as well as subtropical countries. Hawaiians refer to the plant *C. sebestena* as Kou Haole though, which means a "foreign plant". This plant is an evergreen tree that flowers throughout much of the year, but is at its best in June and July. In Hawaiian Islands the plants are used for many traditional items ranging from canoes to food vessels.[1] The plants were planted in tropical and sub-tropical countries, included Jakarta, Indonesia. *Cordia sebestena* plant indicated also possess various pharmacological activities such as hepatoprotective activity[2], hypoglycemic, hypolipidemic and potent antioxidant[3], anti-inflammatory and analgesic activity[4], antibacterial activity against *Bacillus cereus* and *Staphylococcus aureus*[5], antiulcer[6], antidiabetic activity[7], and the essential oil showed a potential antioxidant in the *in-vitro* analysis.[8] Sebestenoids A-D (1-4) were isolated from bioassay-guided fractionation prepared from the *Cordia sebestena* fruit extract.[9] The authentication of the starting material is essential to ensure the reproducible quality of herbal products.[10] The World Health Organization (WHO,1998)[11], has described guiding principles for the standardization of medicinal plants with regard to their macroscopic and microscopic description. Although the *C. sebestena* plant widely studies, especially in the pharmacology activities, there is no studies characterizing the botanical (macros- and microscopic) have been published. The correct species material identification is an important step for researches reproducibility and the standardization is one of the parameters required for quality control of the drug. The present study was undertaken with the objective to study the anatomical characteristics of the stem, leaf, and preliminary phytochemical screening of *C. sebestena*.

MATERIALS AND METHODS

Plant collection and authentication

The whole fresh plants material of *Cordia sebestena* L. were collected in the month of October 2018, from Bogor area, Indonesia. The botanical identification of the plant was done by the Research center for Biology, Indonesian Institute of Sciences, Cibinong, Bogor, Indonesia. A voucher specimen was deposed in the Pharmacognosy Laboratory, Faculty of Pharmacy and Sciences, Universitas Muhammadiyah Prof. DR. HAMKA, for further references. The plant material was washed under running tap water, air-dried and powdered using a blender and kept it in glassware container for analysis.

Cite this article: Hanani E, Soewandi SHW, Hayati, Revita N. Pharmacognostical and Preliminary Phytochemical Evaluation of *Cordia sebestena* L.. Pharmacog J. 2019;11(5):1100-5.

Pharmacognostical evaluation

Macroscopic study

Morphological characteristics were carried out to the freshly plants material, such as color, size, odor, taste, surface characteristic and fracture were examined using the terms and outlines given in Evans WC[12] and Indonesian Herb Pharmacopoeia.[13] The organoleptic characters were observed, noted and photographs were taken in the original environment.

Microscopic study

Microscopical characteristics were an evaluation to the mature, fresh and healthy the different plant parts as leaf, stem, and the powder. The plant material was put between the pith, and several fine sections were cut with the help of a sharp razor. The sections obtained were cleared using chloral hydrate solution. The powder microscopical were prepared using the small amount of the leaves powder were placed on a glass slide containing one to two drops of chloral hydrate solution. After placing the cover slip, it was warmed gently on a spirit lamp to remove air bubbles and then examined under the microscope. Different tissues were observed under the microscope and photographed.[14,15]

Physicochemical evaluation

Physicochemical parameters of samples for loss on drying, moisture content, total ash, water- soluble and acid-insoluble ash value were performed according to the official methods prescribed in Indonesian Herb Pharmacopoeia[13] and the WHO methods on quality control for medicinal plants materials.[11] The extractive values (alcohol, water, and ether soluble) of the powdered drug were determined according to the methods described in Indonesian Herb Pharmacopoeia.[13]

Fluorescence character

The fluorescence character of leaves powder was carried out using Kokoski[16] standard method. The powdered leaves were extracted with different solvents like *n*-hexane, dichloromethane (DCM), ethanol in cold maceration technique for 24 hr; the sample was filtered, and filtrate was observing any specific fluorescence. A small quantity of the powder and extracts were examined by treating the leaves powder with acidic, basic, organic and inorganic solvents viz. 50% sodium hydroxide, 50 % sulphuric acid, 50% HCl, ammonium hydroxide. After the treatment the color change was observed in day-light, long UV (365 nm), short UV (256 nm) sprayed by 5% H_2SO_4/ethanol.[17]

Phytochemical Screening

Preliminary phytochemical screening was carried out using 25 g powdered air-dried material and and subjecting it in a reflux apparatus, was extracted with 250 ml ethanolic for 30 minutes.[18] The extract was filtered and concentrated using a rotary evaporator. The ethanol extract of *C.sebestena* leaves was performed for the detection of various phytoconstituents such as steroids, alkaloids, phenols, tannins, flavonoids, triterpenoids and saponins using standard procedures, described by Indonesian Herb Pharmacopoeia[13] and Harborne.[19]

Chromatographic Profile

The chromatographic profile was carried out using10 g powdered material and subjecting it in a Soxhlet apparatus, was extracted with 150 ml solvents *n*-hexane, DCM and ethanol 70%, respectively.[18] The extracts were concentrated using a vacuum rotary evaporator. The TLC profile of *n*-hexane, DCM, ethanolic extract was performed using standard method,[20] and the Rf values were determined. TLC profiling was carried out to confirm the presence of bioactive compounds in the extract. The three extracts were prepared on a stationary phase (silica gel 60 F_{254} TLC plate), and as mobile phase, different solvents based on their polarity were use, for observing the chromatographic profile. These solvent systems were *n*-hexane-DCM (7:3), *n*-hexane-DCM (1:9) and chloroform-methanol (2:8). The dried plates were observed under ultraviolet and visible light, and spraying with 10% sulfuric acid followed by heating at 105° C for 5-10 minutes, and the Rf values were determined.[21]

RESULTS

Macroscopic and microscopic analysis

Cordia sebestena L. (Boraginaceae family) is a small shapely tree which grows up to be 25 feet tall and as wide and can develop a trunk 12 inches thick. The leaves type is simple, alternate, cordate; ovate, pinnate, dark-green color, thick, rough and hairy on the upper surface, to 9 inches long and 4.5 inches wide, feeling much like sandpaper (Figure 1A). Flowers are bright orange, tube-form, with a narrowly crinkly tube, short yellow-orange stamens in the throat, up to 1 1/2 inches long, with 5 to 7 creepy lobes (Figure 1B). Flowers occur in small clusters located at the branch tips. Fruits shape are oval, drupes, egg-shaped, up to 2 inches long, dry or hard covering, the color changes from green to white, in persistent calyces, sticky when ripe, 1 to 4 seeds (Figure 1C&1D).

The transverse section of *C.sebestena* leaf showed calcium oxalate crystal and trichome (Figure 2A), and the transverse of the stem has shown parenchyma cells with and without chloroplast (Figure 2B).

The characters of powder microscopy showed the presence of fiber, calcium oxalate crystals, anomocytic stomata, trichomes, transverse section fragment of the leaf (Figure 3).

Physicochemical evaluation

The physicochemical constants such as ash values, loss on drying, water- and alcohol-soluble extractive were given in Table 1.

Fluorescence character

Fluorescence studies of powdered drug

The fluorescence characteristics of powdered and extracts drug were studied in visible light and UV light (254 and 365 nm) after treatment with various reagents.[22] The results are represented in Table 2.

Figure 1: *Cordia sebestena*: A: leaves; B: flowers; C: fruits; D: fruit with seeds inside.

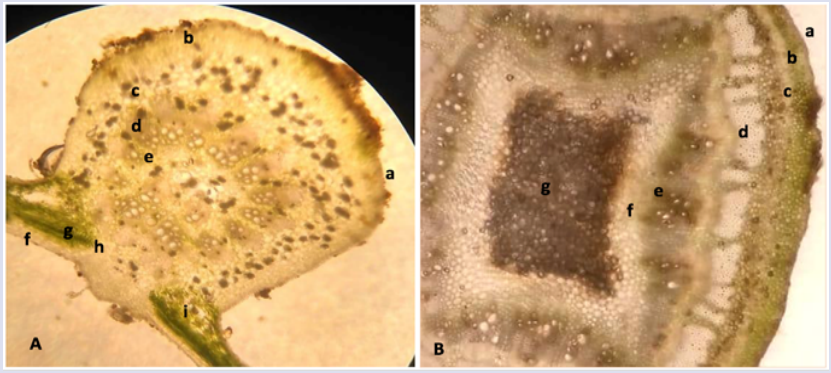

Figure 2: A) Transverse section of *C.sebestena* leaf: a: lower epidermis; b: sclerenchyma; c: phloem; d: xylem; e: colenchym; f: upper epidermis; g: palisade; h: spongy tissue; i: calsium oxalate crystal. **B)** Transverse section of the stem of *C. sebestena*: a: epidermis; b: parenchyma cells with chloroplast; c: parenchyma cells; d: collenchyma; e: phloem; f: xylem; g: pith.

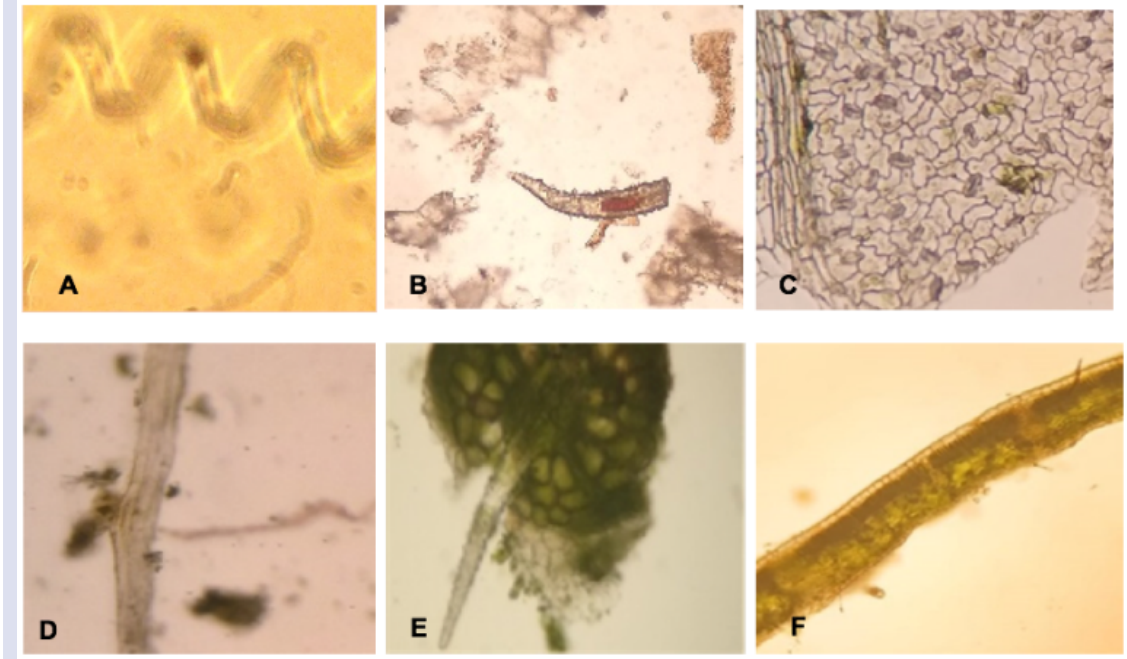

Figure 3: A: spiral fiber; B: epidermis hair; C: anomocytic stomata; D: fiber; E: epidermis hair; F: transverse section fragment of the leaf.

Table 1: Physicochemical parameter of the leaf of *Cordia sebestena*.

No.	Parameters	Average (% w/w)
1.	Moisture conten Loss on drying	2.29
2.	Ash Value a) Total ash b) Acid insoluble ash	 13.6 0.46
3.	Extractive values a) Alcohol-soluble b) Water-soluble c) n-Hexane-soluble	 13.19 11.10 8.71

Preliminary phytochemical screening of ethanolic extract of the leaf of *C. sebestena* showed alkaloids, flavonoids, phenols, saponin, tannins, steroids, terpenoids. The presence of the constituents from the *C. sebestena* plants was presented in Table 3.

Chromatographic profile

TLC profile was done for all the extracts (*n*-hexane, dichlormethane and 70% ethanolic) which showed several spots indicating the presence of the number of chemical constituents (Table 4). TLC pattern of *n*-hexane extract showed five spots with Rf value 0.12; 0.29; 0.60; 0.75 and 0.80 using the solvent system *n*-hexane-DCM (7:3).The DCM extract indicating seven spots with Rf value: 0.03; 0.06; 0.22; 0.33; 0.38; 0.55 and 0.73 using the mobile phase *n*-hexane-DCM (1:9), while the ethanol extract showed two spots with Rf value 0.36 and 0.84 using the solvent chloroform-methanol (2:8).

DISCUSSION

The macroscopic and microscopic description of a medicinal plant is the first step towards identification and determination of purity of such materials and should be carried out before any test are undertaken.[11] Ash values are helpful to determine of the quality and purity of a crude drug, especially in the powdered form. The objective of the ash values of plant material is to determine the inorganic matter present in the drug. On incineration, the crude drugs leave ash usually consisting of sulfates, phosphates, carbonates, and silicates of sodium, potassium, calcium, and magnesium.[23] Ash values of powdered leaves of *C. sebestena* were found 13.6% which indicates that crude drug is clean and free from dirt and sandy material.[11]The extracts of crude drug are indicative of approximate measures of their chemical constituents. Many solvents are used for the extraction of the chemical compounds in a particular amount.[24] Some compounds i.e., coumarin, flavonoids, produce

Table 2: Fluorescent analysis of various extracts of *Cordia sebestena*.

Extract/ Treatment	Normal light	UV (254 nm)	UV (366 nm)
Powder			
+ Water	Green	Brown	Dark brown
+ 50% NaOH	Green	Yellowish brown	Brown
+ 50% H2SO4	Light brown	Bright yellow	Dark brown
+ 50% HCl	Light brown	Bright yellow	Dark brown
+ 50% HNO3	Yellowish brown	Greenish brown	Brown
+ NH_4OH	Brown	Brown	Dark brown
***n*-Hexane**			
+ Water	Green	Yellow	Yellow
+ 50% NaOH	Green	Yellow	Yellow
+ 50% H2SO4	Dark green	Brown	Dark brown
+ 50% HCL	Green	Yellow	Green
+ 50% HNO3	Orange	Brown	Brown
+ NH_4OH	Green	Yellowish brown	Brown
DCM			
+Water	Red	Red	Red
+ 50% NaOH	Yellowish brown	Bright yellow	Bright yellow
+ 50% H2SO4	Greenish brown	Yellow	Yellow
+ 50% HCl	Greenish brown	Green	Dark green
+ 50% HNO3	Brown	Bright yellow	Bright yellow
+ NH_4OH	Green	yellow	yellow
70% Ethanol			
+Water	Brown	Yellow	Yellow
+ 50% NaOH	Dark brown	Brown	Dark brown
+ 50% H2SO4	Dark brown	Brown	Dark brown
+ 50% HCl	Brown	Yellow	Yellow
+ 50% HNO3	Light brown	Brown	Brown
+ NH_4OH	Brown	Brown	Brown

Table 3: Phytochemical screening of leaf extracts of *Cordia sebestena*.

Constituents	Test Performed /reagents	Results
Alkaloids	Dragendorff	+
	Mayer	+
	Bouchardat	+
Flavonoids	Shinoda	+
	Ammonia	+
Phenols	Ferric chloride	+
Saponins	Foam	+
Steroids and terpenoids	Liebermann Burchard Salkowski	+
		+
Tannins	Gelatin	+
	Lead acetate	+

+ = indicates present; - = indicates absent;

Table 4: Chromatographic profile of different extract of *Cordia sebestena*.

Extract	Solvent System	No.of spots	Rf (UV 366 nm)	Spraying reagent (H_2SO_4)
n-Hexane	*n*-hexane - DCM (7 : 3)	5	0.12 0.29 0.60 0.75 0.80	Light brown Light brown Light gray Light gray Light gray
DCM	*n*-hexane - DCM (1 : 9)	7	0.03 0.06 0.22 0.33 0.38 0.55 0.73	Light brown Light brown Light yellow Light yellow Light yellow Light brown Light brown
Ethanol (70%)	chloroform - methanol (2 : 8)	2	0.36 0.84	Light brown Light yellow

specific fluorescence characteristics which are helpful for preliminary chemical components study as well as for standardization of the plant materials. The phytochemical screening of *Cordia sebestena* confirm the presence of alkaloids, saponins flavonoids, phenols, tannins, steroids, terpenoids which may be responsible substances for its pharmacology activities. TLC profiling of hydroethanolic extract has been carried out to achieve some chromatographic studies to confirm the presence of active components in *Cordia sebestena*. TLC profiling shows different spots with different Rf values. The separation of phytochemicals in the TLC plate gives different Rf values with different mobile phase. The difference in the Rf values depend upon the polarity of the compound so that the solvent system which analyzed appropriate is used for the further isolation from the plant extract.[25] Phytochemical screening and TLC profile of crude extract can be very useful for developing the quality parameters. The *C. sebestena* is one of the therapeutically important plants. Since long time, it has been used for treatment of variety of ailments but the details of its quality attributes like pharmacognostic, phyto- and physicochemical characteristics were not available. There are no reports of pharmacognostic studies and phytochemical analysis of *C.sebestena*, so it was envisaged that above mentioned studies will be worth and useful for the scientific community as well as commercial purpose.

CONCLUSIONS

The pharmacognostic character, physicochemical constant, the presence of several bioactive compounds could be a useful tool for identification, authentication, and preparation of suitable monograph of *Cordia sebestena* L. These data will serve as a reference for the quality control of the preparation from this plant. From some researcher reports, it is found that the *Cordia sebestena* is used widely to cure various diseases. Thus the present study also helps to check the falsification of this important medicinal plant and to be significant and encouraging towards the goal for standardization.

ACKNOWLEDGEMENTS

The authors are thankful to Dr. Hadi Sunaryo, Apt. Dean of Faculty of Pharmacy and Siences, Universitas Muhammadiyah Prof. Dr. HAMKA Jakarta Indonesia, for providing facilities to carry out the present research work.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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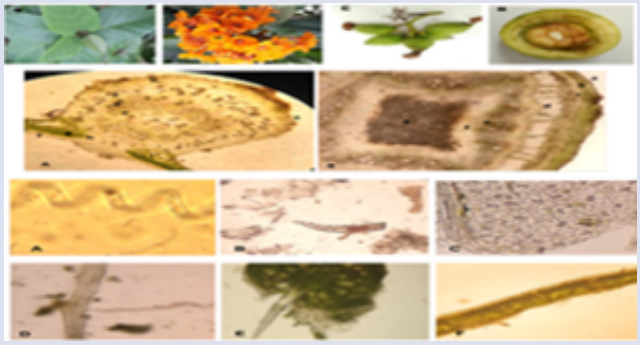
GRAPHICAL ABSTRACT



SUMMARY

- The *Cordia sebestena* Linn is commonly known as "Geiger Tree" in Indonesia.
- This plant has some pharmacological activities such as hepatoprotective, hypoglycemic, analgesic, hypolipidemic, anti-inflammatory, antidiabetic, antiulcer, and antibacterial activity.
- The present study is helped to check the adulteration of this medical plant and to be significant and encouraging towards the goal for standardization.
- The pharmacognosy, phytochemistry screening could be a useful tool for identification, authentication and preparation of suitable monograph of *Cordia sebestena.*

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Cite this article: Hanani E, Soewandi SHW, Hayati, Revita N. Pharmacognostical and Preliminary Phytochemical Evaluation of *Cordia sebestena* L.. Pharmacog J. 2019;11(5):1100-5.