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RESEARCH ARTICLE

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Pharmacognostical, Preliminary Phytochemical Evaluation and Flavonoids Content of *Paederia foetida* Linn.

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ABSTRACT:

Paederia foetida, locally (Indonesia) known as “sembukan” and “skunkvine” (English), is one of Rubiaceae family member that has a diverse pharmacological and phytochemical importance. The name derives from the distinct odor when the leaves are crushed. The species name “foetida” is a Latin word for “stinky” or “foul smelling”. The current work was investigated to perform the morphoanatomical, physicochemical, phytochemical analysis and flavonoids content of *Paederia foetida* Linn. Pharmacognostical studies were carried out for different parameters include macroscopic, microscopic, and fluorescence. Physicochemical parameters, like the loss on drying, ash value, extractive values, etc. were measured as per WHO guidelines. Preliminary phytochemical screening was also performed for major groups of compounds and the flavonoid content. The TLC profile of the leaves extracts (n-hexane, DCM and ethanolic) of *P. foetida* showed 9, 7 and 3 spots respectively in the three different solvents. The total flavonoid concentration was 1.32 mg/g, expressed as quercetin equivalents. The various macroscopic, microscopic, physical and phytochemical parameters listed here for *P. foetida*, and the present work can be used with respect to its identification, authentication, and standardization.

KEYWORDS: Chromatographic Profile, Microscopic, *Paederia foetida*, Physicochemical, Rubiaceae.

INTRODUCTION:

Paederia foetida is an important medicinal plant for “jamu” (Indonesian traditional system). The name is from the Greek word ‘paederos’ meaning opals, and ‘foetida’ means stinking. This plant is commonly known by The Indonesian as “sembukan”, meaning it spreads bad smell. In Malaysia, the local name for *P. foetida* is “akar sekuntut” and in India is called “Gandhavadulia”¹. This plant belongs to Rubiaceae family and one of among 30 species in *Paederia* genus. The origin of this plant is considered to be Eastern and Southern Asian.

This aromatic climbing plant is a leafy vegetable that can be eaten raw or steamed. It has also been reported for antinociceptive, antiviral², antidiarrheal³, the antiinflammatory⁴, antitussive⁵, hepatoprotective⁶, and antioxidant activity^{7,8}. The leaf extract of *P. foetida* also showed remarkable antihyperglycemic⁸ activity due to its possible systematic effect involving in the pancreatic and extrapancreatic mechanism. Iridoid glycosides, paederolone, paederone, paederine, and paederenine are the phytochemicals identified in this plant⁹. Previous studies (Raj RS)¹⁰ also identified a number of steroids and terpenoids and other 77 constituents in the volatile oils of the leaves, stems, and flowers of *P. foetida*, some high levels. They constitute an effective source of traditional and modern medicines and play an important role in health care programs^{11,12}. Therefore, it becomes extremely important to make an effort toward

standardization of the plant material as medicine. The process of standardization can be achieved by stepwise pharmacognostic studies. Pharmacognosy is an essential measure of authentication and quality. The macroscopic microscopic description of a medicinal plant is the simplest and cheapest method to start with, to establish the correct identification and determination of the purity of the source materials¹³. The present study was undertaken with the objectives to delineate the pharmacognostical profile of stem, root, and leaves of *P. foetida*. It may assist in standardization of whole, cut or powdered plant material samples and be able to guarantee the accurateness, means of identifying crude drugs, and also fill the lacuna of our understanding about botanical pharmacognosy of *P. foetida*.

MATERIALS AND METHODS:

Collection and Authentication:

The whole fresh plants material of *Paederia foetida* Linn. were collected in the month of September - October 2018, from Bogor area, Indonesia. The taxonomical identification and authentication of the plant were done 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, Universitas Muhammadiyah Prof. Dr. HAMKA, for further references. Disease-free plant parts (root, stem, and leaf) were shade dried, powdered and stored in airtight containers.

Pharmacognostical Evaluation:

Macroscopic study:

Morphological studies was carried out to the freshly plants material, such as color, size, odor, taste, surface characteristic and fracture. They were examined using the terms and outlines given in Indonesian Herb Pharmacopoeia¹⁴ and Evans WC¹⁵. The organoleptic characters were observed, noted and photographs were taken in the original environment.

Microscopic study:

Microscopic of transverse sections of fresh leaf, stem, and root was performed. For this purpose, a transverse section by free hand was prepared. The leaf, stem, and root were 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. A small amount of different powder material was macerated with chloral hydrate suspension. One drop of solution was taken on a slide, then it was heated on spirit lamp and examined under a microscope. Different tissues were observed under the microscope and photographed^{16,17}.

Physicochemical Evaluation:

Physicochemical evaluation of samples for loss on drying, moisture content, total ash, water soluble and acid insoluble ash value was performed according to the official methods as per WHO guidelines¹⁸ on the quality control of the medicinal plant material. The extractive values (alcohol, water, and ether soluble) of the powdered drug were determined according to the methods described in Indonesian Herb Pharmacopoeia¹⁴. Fluorescence characters of powdered and extracts material were carried out with and without chemical treatment and to their color observed/determined under ordinary and ultraviolet light¹⁹.

Moisture content:

As many as 2 g of the air dried powdered material was placed in a watch glass, kept in oven at 105°C and dried for a period until constant weight was obtained. The moisture content of powdered material was the difference in the weight before and after the material dried in oven.

Total ash value:

As many as 2 g of powdered material was spread in a fine even layer at the bottom of the constant weights crucible. It was ignited by gradually increasing the heat 400°C until it became white, that indicating it is free from carbon. The crucible was cooled and weighed. The procedure was repeated until the constant weights. The total ash value was calculated with reference to the air dried powdered sample.

Water-soluble ash value:

The total ash obtained was boiled with 25 ml of chloroform water for 5 minutes. The insoluble matter collected on an ash less filter paper and washed with hot water. The insoluble ash was transferred into pre-weighed silica crucible, ignited for 15 minutes at a temperature (not exceeding 400°C), cooled and weighed until getting the constant weight. The percentage of water soluble ash was calculated with reference to the air dried drugs.

Acid insoluble ash value:

The obtained total ash was boiled with 25 ml of dilute hydrochloric acid (2N) for 5 minutes. The insoluble ash was collected on ash-free filter and washed with hot water, then transferred into pre-weighed silica crucible, ignited, cold and weighed until the constant weight was obtained. The percentage of acid insoluble ash was calculated with reference to the air-dried powder sample.

Ethanol-soluble extractive value:

As many as 10 g of air-dried coarse powdered material was weighed accurately and then was macerated with 100 ml of 90% ethanol for 24 hours. The next step, it was be shaken frequently during 6 hours, and it was set

40 le for 18 hours. The solution was filtered rapidly
9 ough a dry filter. As much as 25.0 ml of the filtrate
was 45 porated to dryness in a tarred flat bottomed dish
and was dried at 105°C in the oven and being weighed.
The value of extractable matter was calculated with the
reference to the air-dried material.

26 Water-soluble extractive value:

As many as 10 g of air-dried coarse powdered material
was weighed accurately, in a glass-stopper conical flask,
9 100 ml of chloroform water macerated for 24 hr
34 king frequently during the first 6 hours, then allowed
30 stand for 18 hours. Filtered rapidly taking precautions
5 ml of the filtrate and evaporated to dryness in a tarred
11 t bottomed dish, dried at 105°C and weighed. The
1 percentage of the value of water-soluble extractive was
calculated with reference to the air-dried drug.

42 Ether soluble extractive value:

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

28 Fluorescence character:

Fluorescence study of leaves powder was performed as
per reported procedure by Kokhasi¹⁹. A small quantity of
the powder was observed with or without chemical,
under visible and ultraviolet (366 nm) light. The
50 micals were methanol, 2N hydrochloric acid, 50%
sulphuric acid, 50% nitric acid, 50% sodium hydroxide
and ammonium hydroxide.

3 Phytochemical Screening:

Preliminary phytochemical screening was carried out
using 25 g powdered material and subjecting it to
successive extraction in a reflux apparatus with 250 ml
ethanolic for 30 minutes²⁰. The extraction was filtered
and concentrated using a rotary evaporator. The
ethanolic extract was used to analyze qualitatively
43 ous phytoconstituents such as alkaloids, glycosides,
steroids, phenolic compounds, tannins, flavonoids and
carotenoids using standard procedures, described by
Indonesian Herb Pharmacopoeia¹⁴ and Harborne²¹.

Chromatographic Profile:

The chromatographic profile was carried out using 10 g
powdered material and subjecting it to successive
extraction in a Soxhlet apparatus with 150 ml solvents
hexane, dichloromethane (DCM) and ethanol 70%,
respectively²². The extracts were concentrated using a
rotary evaporator. Thin layer chromatography profile of
n-hexane, DCM, ethanolic extracts was performed using
standard method²³ and the RF values were determined.
Those three extracts were prepared on silica gel 60 F254
TLC plate for observing the chromatographic profile.
The plates were developed using various solvent systems

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as chloroform-ethyl-acetate (6: 4), *n*-hexane-ethyl-
acetate (2: 8), ethyl-acetate - formic acid-acetic acid
glacial--water (5: 0.5: 0.5: 1). The dried plates were
1 served under ultraviolet and visible light after
spraying with 10% sulfuric acid followed by heating at
105° C for 5-10 minutes. Next, the Rf values were
determined²⁴.

51 Total Flavonoid Content:

The total flavonoid content in the extracts was
determined using the aluminum chloride colorimetry
method described by Chang^{25,26} with slight
modifications. The extract sample was diluted with
methanol until 100 µg/mL and the quercetin standard
was also diluted in methanol (0-100 µg/mL), to make
11 calibration curve. The diluted extract or quercetin
(2.0 mL) was mixed with 0.1 mL of 10% (w/v)
aluminum chlo 55 solution and 0.1 mL of 0.1 mM
11 ium acetate solution. The mixture was incubated at
15 m temperature for 30 minutes. Then the maximum
52 orbance of the mixture was measured at 415 nm
using a uv-33 spectrophotometer. The total flavonoid
content was expressed as mg of quercetin/g of extract
and counted using the equation of the standard curve.

RESULTS:

44 Macroscopic and microscopic analysis:

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4 *Pederia foetida* belonging to family Rubiaceae is a
per 4 nial twining vine from woody rootstock; stems to 7
m or more, climbing, or prostrate and rooting at the
nodes; leaves are gre-4 in color, opposite, oval to linear-
lanceolate 2-11 cm, with conspicuous stipules; petioles
commonly to 6 cm long. The leaf surface can be hairy or
glabrous, often lobed at the base.

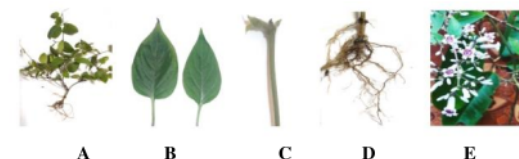


Fig. 1: The plant of *P. foetida* (A); Leaves (B); Petiole (C); roots (D) and Flowers (E)

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Leaves and stems have a disagreeable odor, especially
when crushed (Fig. 1A, B, C). The taproot has the same
4 color as the stem, brown (Fig. 1D). Flowers are small,
13 ish pink or lilac, in broad or long, "leafy," curving
13 sters, terminal or at leaf axils; corolla densely hairy,
tub 13 with 5 (usually) spreading lobes (Fig. 1E). Fruits
4 e a shiny brown, nearly globose capsule, to 0.7 cm
wide, with 2 black roundish seeds, often dotted with
white raphides⁶. In this studied we did not find the fruit,
because it usually emerges on the dry season. Transverse
section of *P. foetida* was shown in Fig. 2.

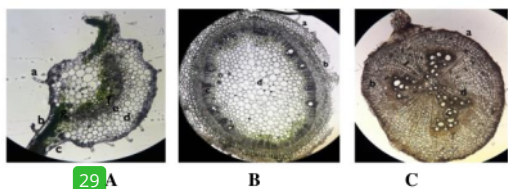


Fig. 2: A. TS of *P. foetida* leaf; a. trichomes; b. upper epidermis; c. lower epidermis; d. parenchyma; e. collenchyma; f. vascular bundle (phloem, xylem); g. mesophyll; B. TS of *P. foetida* stem; a. epidermis; b. cortex; c. vascular bundle; d. pith and C.TS of *P. foetida* root; a. epidermis; b. cortex; c. xylem; d. phloem

Transverse section of *P. foetida* leaf shows trichomes are present on both on the upper and lower epidermis. The upper and lower epidermis consists of oval-shaped paracytic cells in a single layer. Mesophyll composed of single layer palisade cells and 3-4 layered spongy tissues. Epidermis covered with cuticle, and vascular bundle is composed of both xylem (upper side) and phloem (lower side) (Fig. 2A). Transverse section of *P. foetida* stem showed trichomes, epidermis, parenchyma, vascular bundle, xylem and phloem composed of thin-walled cells (Fig. 2B). Transverse section of the root showed epidermis; cortex; phloem and xylem (Fig. 2C). The microscopical of the plant powder were shown in Figure 3.

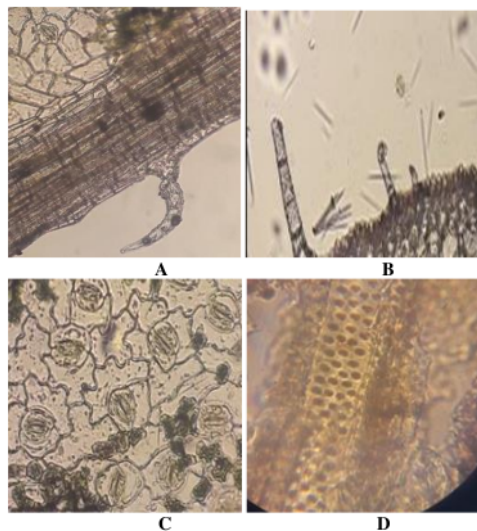


Fig. 3: Microscopy of *Paederia foetida* leaves powder; A, B: trichomes and raphides calcium oxalate crystals; C: stomata type is paracytic which is the character of Rubiaceae; and D: vascular bundle (spiral type)

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

Table 1: Physicochemical parameter of powder of *P. foetida*.

No.	Parameters	Average (% w/w)
1	Moisture content	
1	Loss on drying	4.42
2	Ash Value	
	a. total ash	6.93
	b. water soluble ash	2.16
	acid insoluble ash	1.81
3	Extractive values	
	a) Alcohol soluble Extractive	11.58
	b) Water soluble extractive	24.34
	c) Ether	4.21

Fluorescence observation:

The extract of *P. foetida* leaf was treated with different acid reagents of various concentrations observed for the color under daylight and ultraviolet rays. The results of the fluorescence of the leaves extract of *P. foetida* are summarized in Table 2. Fluorescence provided by a drug is one of the several methods used for analyzing crude drugs. The different compounds produce specific fluorescence characteristics which are helpful for preliminary chemical study as well as for standardization of specific plant materials²⁰.

Table 2: Fluorescent analysis of various extracts of *P. foetida*.

Extracts Treatment	Day light	UV (366 nm)
Hexane		
+ 2N HCl	Greenish yellow	Light yellow
+ 50% H ₂ SO ₄	Greenish yellow	Light yellow
+ 50% HNO ₃	Light Brown	Yellow
+ 50% NaOH	Brownish yellow	Brownish green
+ NH ₄ OH	Brownish yellow	Yellow
DCM		
+ 2N HCl	Green	Light yellow
+ 50% H ₂ SO ₄	Green	Yellow
+ 50% HNO ₃	Orange	Brownish yellow
+ 50% NaOH	Brownish yellow	Brownish yellow
+ NH ₄ OH	Yellowish brown	Brownish yellow
Ethanol (70%)		
+ 2N HCl	Light yellow	Yellow
+ 50% H ₂ SO ₄	Light yellow	Yellow
+ 50% HNO ₃	Yellow	Light yellow
+ 50% NaOH	Yellowish brown	Brownish yellow
+ NH ₄ OH	Yellow	Brownish yellow

The color was seen in the daylight and ultra-violet rays at 366 nm for observing any specific fluorescence. Colors visible in leaf extract in the visible range are green, light yellow and brownish yellow. Main colors observed in ultraviolet light in leaf extract are light yellow and brownish yellow.

Physicochemical evaluation:

The physicochemical constants such as loss on drying, ash values, water and alcohol soluble extractive were given in Table 3.

Table 3: Physicochemical parameter of powder of *P. foetida*.

No.	Parameters	Average (% w/w)
1	Moisture content Loss on drying	4.42
2	Ash Value a. total ash b. water soluble ash c. acid insoluble ash	6.93 2.16 1.81
3	Extractive values a) Alcohol soluble Extractive b) Water soluble extractive c) Ether	11.58 24.34 4.21

Chromatographic Evaluation:

The chromatographic studies were performed using 3 various solvent systems to confirm the phytochemical studies. Prepared silica gel TLC plates were used for the chromatographic evaluation^{16,23,24}. Usually, Rf values and colored were calculated and shown in Table 4.

Table 4: Chromatographic evaluation of different extract of *P. foetida*.

Extract	Solvent System	No. of spots	Rf (UV 366 nm)	Spraying reagent (SO ₂)
Hexane	chloroform: ethyl-acetate (6:4)	9	0.15	yellow
			0.22	light yellow
			0.31	orange
			0.51	light brown
			0.61	yellow
			0.67	light violet
			0.73	orange
			0.83	light yellow
DCM	hexane: ethyl-acetate (2: 8)	7	0.55	blue
			0.62	blue
			0.67	yellow
			0.75	blue
			0.85	yellow
			0.90	violet
			0.98	blue
Ethanol (70%)	Ethyl-acetate -- formic acid-- acetic acid glacial--water = (5: 0.5: 0.5: 1)	3	0.25	blue
			0.37	brown
			0.50	yellow

Phytochemical evaluation:

Phytochemical screening of the extract gives a general idea regarding the nature of chemical constituents present in the crude drug. The outcome of the phytochemical screening of the extract indicated the presence of alkaloids, flavonoids, phenols, tannins, saponins, and terpenoids.

Total flavonoid content:

The total flavonoid content of the ethanolic extract leaves of *P. foetida* was 1.32 mg/g, expressed as quercetin equivalents. The total flavonoid concentration of the extract was determined using the aluminum chloride colorimetric method. The content of flavonoids was expressed in terms of quercetin equivalent with the standard curve equation. The concentration of flavonoid in this extracts depends on the

polarity of solvents used in the extract preparation.

DISCUSSION:

According to the World Health Organization¹⁸, the macroscopic and microscopic 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. In recent years, identification and evaluation of plant drugs by pharmacognostical study is still more reliable, accurate and inexpensive. Macroscopical and microscopical evaluations are the step towards authentication of an internal structure of the plant to establish proper identification by revealing tissue arrangement. This is done by identifying internal structures of the transversal section of the leaf, stem, and root; also the structures of the epidermis, vascular bundles, collenchyma, types and arrangement of vascular bundles, sclerenchyma, and any other specific features that lie therein. In this microscopical character of *P. foetida* leaves powder showed the presence of raphides needle of calcium oxalate crystal and stoma (paracytic type) in the drug. Physicochemical parameters are important for qualitative standards and useful in determining the authenticity and purity of crude drugs. Physical constants like ash and extractive values help in establishing the pharmacopeia standards of a drug. The ash determination is helpful in determining the authenticity and purity of the drug. Water soluble ash in the measure of physiological inorganic components of the crude drug. Acid-insoluble ash gives an idea about the physiological ash produced due to the adherence of inorganic dirt, dust to the crude drug. Increased acid insoluble ash means adulteration due to dirt, soil or sand. Extractive values are useful for the determination of exhausted or adulterated drugs. It gives an idea about the nature of the chemical constituents present in the crude drug. 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. Fluorescence study is an important parameter for the standardization of crude drugs. Many drugs fluoresce when their powder is exposed to ultraviolet radiation. It is important to observe all materials on reaction with different chemical reagents under UV light. The phytochemical screening showed the presence of many important groups of phytoconstituents such as alkaloids, flavonoids, phenols, tannins, saponins, and terpenoids which influence the pharmacology activities of the plants. Thin layer chromatographic profile of hexane, DCM and ethanolic extracts were carried out with the different mobile phase system, have shown 9, 7 and 3 phytoconstituents respectively. Each compound give different Rf values and colored in different solvent systems.

CONCLUSION:

These botanical characters, physical constants will serve as a reference for quality control of the preparations from this plants. Ash values, extractive values can be used as reliable aid for detecting adulteration. This preliminary phytochemical screening of the plant extract confirmed the presence of several bioactive compounds like alkaloids, flavones, phenols, terpenes and tannins which could be responsible for the versatile medicinal properties of these plants. Pharmacognostical studies help in the identification and authentication of the plant compounds isolated from leaves of plants. This preliminary information is necessary for standardization of the plant material used in the herb medicines. This variation of Rf values and color of the phytoconstituents in the TLC profile show a very important clue in understanding of their polarity. The pharmacognostic study and phytochemical characters of *P. foetida* will be useful to identify the plant in the powder form and in the elimination of adulterants. Moreover, the data of this study could be useful in the preparation of the pharmacopoeial monograph of the plant drug.

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CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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