ISSN 0975-2331 (Print)
0975-4385 (Online)
DOI: 10.5958/0975-4385.2019.00015.3

Vol. 11| Issue-02|
April- June, 2019

Available online at
www.anvpublication.org

Research Journal of
Pharmacognosy and Phytochemistry
Home page *www.rjpponline.org*

RESEARCH ARTICLE

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

Rindita*, Hanani E, Saidah N.
Department of Pharmacognosy—Phytochemistry, Faculty of Pharmacy and Sciences, Universitas Muhammadiyah Prof. Dr. HAMKA Jl. Delima II/IV Klender, Jakarta 13460, Indonesia.
*Corresponding Author E-mail: **rindita@uhamka.ac.id**

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 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.

Received on 09.04.2019 Modified on 28.04.2019
Accepted on 03.05.2019
Res. J. Pharmacognosy and Phytochem. 2019; 11(2):81-86.
DOI: 10.5958/0975-4385.2019.00015.3

This aromatic climbing plant is a leafy vegetable that can be eaten raw or steamed. It has also been reported for antinociceptive, antiviral[2], antidiarrheal[3], the antiinflammatory[4], antitussive[5], hepatoprotective[6], and antioxidant avtivity[7,8]. The leaf extract of *P. foetida* also showed remarkable antihyperglycemic[8] 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 plant[9]. 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 at 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 and microscopic description of a medicinal plant is the simplest and cheapest methods to start with, to establish the correct identification and determination of the purity of the source materials[13]. 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 Pharmacopoeia[14] and Evans WC[15]**.** 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 guidelines[18] 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 Pharmacopoeia[14]. 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 light[19].

Moisture content:

As many as 2 g of the air dried powdered material was placed in a watch glass, kept in oven at 105^0C 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 to 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 was 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 400ºC), 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

aside for 18 hours. The solution was filtered rapidly through a dry filter. As much as 25.0 ml of the filtrate was evaporated 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.

Water-soluble extractive value:
As many as 10 g of air-dried coarse powdered material was weighed accurately, in a glass-stopper conical flask, in 100 ml of chloroform water macerated for 24 hr shaking frequently during the first 6 hours, then allowed to stand for 18 hours. Filtered rapidly taking precautions 25 ml of the filtrate and evaporated to dryness in a tarred flat bottomed dish, dried at 105ºC and weighed. The percentage of the value of water-soluble extractive was calculated with reference to the air-dried drug.

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

Fluorescence character:
Fluorescence study of leaves powder was performed as per reported procedure by Kokhasi[19]. A small quantity of the powder was observed with or without chemical, under visible and ultraviolet (366 nm) light. The chemicals were methanol, 2N hydrochloric acid, 50% sulphuric acid, 50% nitric acid, 50% sodium hydroxide and ammonium hydroxide.

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 minutes[20]. The extraction was filtered and concentrated using a rotary evaporator. The ethanolic extract was used to analyze qualitatively various phytoconstituents such as alkaloids, glycosides, steroids, phenolic compounds, tannins, flavonoids and carotenoids using standard procedures, described by Indonesian Herb Pharmacopoeia[14] and Harborne[21].

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%, respectively[22]. The extracts were concentrated using a rotary evaporator. Thin layer chromatography profile of *n*-hexane, DCM, ethanolic extracts was performed using standard method[23] 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 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 observed under ultraviolet and visible light after spraying with 10% sulfuric acid followed by heating at 105º C for 5-10 minutes. Next, the Rf values were determined[24].

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 the calibration curve. The diluted extract or quercetin (2.0 mL) was mixed with 0.1 mL of 10% (w/v) aluminum chloride solution and 0.1 mL of 0.1 mM sodium acetate solution. The mixture was incubated at room temperature for 30 minutes. Then the maximum absorbance of the mixture was measured at 415 nm using a uv–vis spectrophotometer. The total flavonoid content was expressed as mg of quercetin/g of extract and counted using the equation of the standard curve.

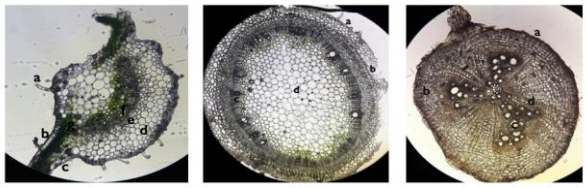
RESULTS:

Macroscopic and microscopic analysis:
Paederia foetida belonging to family Rubiaceae is a perennial twining vine from woody rootstock; stems to 7 m or more, climbing, or prostrate and rooting at the nodes; leaves are green 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.

A B C D E

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

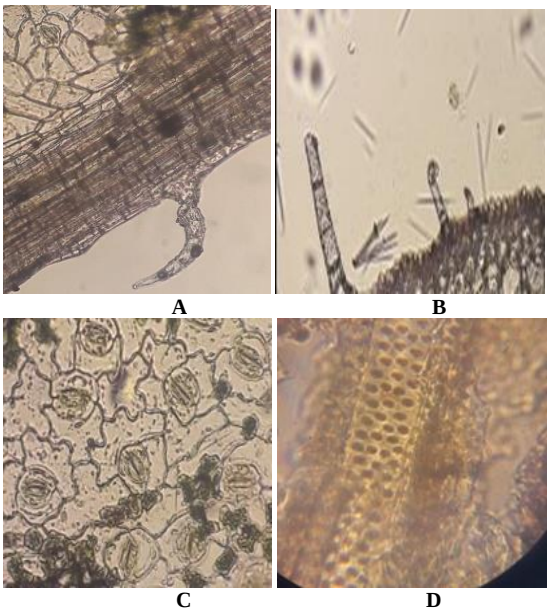
Leaves and stems have a disagreeable odor, especially when crushed (Fig. 1A, B, C). The taproot has the same color as the stem, brown (Fig. 1D). Flowers are small, grayish pink or lilac, in broad or long, "leafy," curving clusters, terminal or at leaf axils; corolla densely hairy, tubular with 5 (usually) spreading lobes (Fig. 1E). Fruits are a shiny brown, nearly globose capsule, to 0.7 cm wide, with 2 black roundish seeds, often dotted with white raphides[6]. 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.



A B C

Fig. 2: A. T S 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 parenchyma 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* steam 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.



A B C D

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**	
	Loss on drying	4.42
2	**Ash Value**	
	a. total ash	6.93
	b. water soluble ash	2.16
	c. 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 materials[20].

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_2SO_4	Greenish yellow	Light yellow
+ 50% HNO_3	Light Brown	Yellow
+ 50% NaOH	Brownish yellow	Brownish green
+ NH_40H	Brownish yellow	Yellow
DCM		
+ 2N HCl	Green	Light yellow
+ 50% H_2SO_4	Green	Yellow
+ 50% HNO_3	Orange	Brownish yellow
+ 50% NaOH	Brownish yellow	Brownish yellow
+ NH_40H	Yellowish brown	Brownish yellow
Ethanolic (70%)		
+ 2N HCl	Light yellow	Yellow
+ 50% H_2SO_4	Light yellow	Yellow
+ 50% HNO_3	Yellow	Light yellow
+ 50% NaOH	Yellowish brown	Brownish yellow
+ NH_40H	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	6.93
	b. water soluble ash	2.16
	c. acid insoluble ash	1.81
3	**Extractive values**	
	a) Alcohol soluble Extractive	11.58
	b) Water soluble extractive	24.34
	c) Ether	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]. Finally, Rf values and colored were calculated and shown in Table 4.

Table 4: Chomatographic evaluation of different extraxt of *P. foetida*.

Extract	Solvent System	No. of spots	Rf (UV 366 nm)	Spraying reagent (H_2SO_4)
Hexane	**chloroform: ethyl-acetate (6:4)**	9	0.15 0.22 0.31 0.51 0.61 0.67 0.73 0.83 0.91	yellow light yellow orange light brown yellow light violet orange light yellow yellow
DCM	**hexane: ethyl-acetate (2: 8)**	7	0.55 0.62 0.67 0.75 0.85 0.90 0.98	blue blue yellow blue yellow violet blue
Ethanol (70%)	**Ethyl-acetate -- formic acid-- acetic acid glacial--water = (5: 0,5: 0,5: 1)**	3	0.25 0.37 0.50	blue brown 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* ethanolic 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 Organization[18], 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, crystals 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 non-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 fluorescence 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 may 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 the 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.

ACKNOWLEDGEMENT:

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

CONFLICT OF INTEREST:

The authors declare no conflict of interest.

REFERENCES:

1. Khushbu C, Patel A, Patel M, Macwan C, Roshni S, Subodh A. *Paederia foetida* Linn. as a potential medicinal plant: A Review. J of Pharmacy Research. 2010; 3(12): 3135-3137.
2. Wadud MA, Akhter R, Chowdhury SF, Narjish SN, Mannan MM, Nasiruddin MM, *et al*. In vivo pharmacological investigations of leaf extracts of *Paederia foetida* (L.) Int J Pharm Sci Rev Res. 2015; 31(2):166-172.
3. Afroz S, Alamgir M, Khan MTH, Jabbar S, Nahar, N, Choudhuri MSK. Antidiarrheal activity of the ethanol extracts of *Paederia foetida* Linn. (Rubiaceae). J Ethnopharm. 2006; 105:125-130.
4. De S, Ravishankar B, Bhavsar GC. Investigation of the anti-inflammatory effects of *Paederia foetida*. J Ethnopharm.1994; 43:31-38.
5. Nosalova G, Mokry J. Antitussive activity of the ethanolic extract of *Paederia foetida* (Linn.) in non Anaesthetized cats. J of the University of Veterinary and Pharmaceutical Sciences. 2007; 76:27-33.
6. De S, Shukla VJ, Ravishankar B, Bhavsar GC. A preliminary study on the hepatoprotective activity of methanol extract of *Paederia foetida* leaf. J of Fitoterapia.1996; 67:106-109.
7. Osman H, Rahim AA, Isa NM and Bakhir NM. Antioxidant activity and phenolic content of *Paederia foetida* and *Syzygium aqueum*. Molecules. 2009; 14:970-978.
8. Kumar V, Anwar F, Ahmed D, Verma A, Ahmed A, Damanhouri ZA, Mishra V, Ramteke PW, Bhatt PC and Mujeeb M. *Paederia foetida* Linn. leaf extract: an antihyperlipidemic, antihyperglycaemic and antioxidant activity. BMC Complementary and Alternative Medicine. 2014; 14:76.
9. Shukla YN, Lloyd HA, Morton JF, Kapadia GJ. Iridoid glycosides and other constituent of *Paederia foetida*. Phytochemistry. 1976; 15:1989-1990.
10. Wong KC, Tan GL. Steam volatile constituents of the aerial parts of *Paederia foetida* L. Flavour Fragr. J.1994; 9:25-28.
11. Raj RS, Radhamany PM. Pharmacognostic and physicochemical analysis on the leaves of *Brunfelsia americana* L. Asia Pac J Trop Biomed. 2012; 2:305-307.
12. Phillipson JD. Phytochemistry and pharmacognosy. Phytochemistry. 2007; 68:2960-72.
13. Thomas S, Patil DA, Patil AG, Naresh C. Pharmacognostic evaluation and physicochemical analysis of *Averrhoa carambola* L. fruit. J Herb Med Toxicol. 2008; 2:46-51.
14. Departemen Kesehatan Republik Indonesia, Farmakope Herbal Indonesia. Ed I. (Ministry of Health of RI, Indonesian Herb Pharmacopoeia 1st ed.), 2008; Jakarta, Indonesia.
15. Evans WC. Trease and Evans pharmacognosy 15th edition, Edinburgh, London, New York, Philadelphia, St Louis, Sydney, Toronto: WB. Saunders, 2000.
16. Wallis TE. Practical pharmacognosy, 5th. London: J and A Churchill Ltd. 1984.
17. Muhammad N, Saeed M, Barkatullah, Ibrar M, Khan H. Pharmacognostic studies of *Viola betonicifolia*. Afr J Pharm Pharmacol. 2012; 6:43-47.
18. Anonymous. Quality control methods for medicinal plant materials. World Health Organisation, Geneva. 1998; 25-30.
19. Kokashi CJ, Kokashi RJ, Sharma M. Fluorescence of powdered vegetable drugs in ultra-violet radiation. J American Pharm Assoc. 1958; 47:715–717.
20. Hanani E, Prastiwi R, Karlina L., Indonesian *Mirabilis jalapa* Linn.: A pharmacognostical and preliminary phytochemical investigation. Pharmacog J. 2017; 9(5): 683-688.
21. Hanani E, Ladeska V, Astuti AC. Pharmacognostical and phytochemical evaluation of Indonesian *Peperomia pellucida* (Piperaceae). International J of Biological and Pharmaceutical Research. 2017; 8(1):10-17.
22. Harborne JB. Phytochemical investigation of certain medicinal plants 2nd edition. London: Chapman and Hall, 1984; 37-40.
23. Stahl E. Thin layer chromatography a laboratory handbook. Berlin, Heidelberg, New York: Springer-Verlag, 1969; 241-247, 427-431.
24. Wagner H, Bladt S, Zgainski EM. Plant drug analysis, a thin layer chromatography atlas. Berlin, Germany: Springer-Verlag,1984;1-17.
25. Chang CC, Yang MH, Wen HM, Chern J. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal. 2002; 10(3):178-182.
26. Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S, *et al*. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. J of Foods and Drud Analysis. 2014; 22: 296-302.