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Research Article

PHARMACOGNOSTICAL AND PHYTOCHEMICAL EVALUATION OF INDONESIAN *Peperomia pellucida* (PIPERACEAE)

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ABSTRACT

Peperomia pellucida (L.) Kunth. belongs to family Piperaceae, is used in traditional system of medicine for treatment of several disorders. The leaves was the main source of the raw material for the herb-drug product, so the material and some parameters identified were needed to ensure the safety, quality and efficacy of the product.The present study was to evaluation revealed interesting macros- and microscopic characteristic of the Indonesian plant and establishment of its quality parameters including fluorescence characters, physicochemical, and phytochemical screening.Fluorescence characters of powdered material were analysed under ultraviolet and ordinary light, which signifies their characteristics. Moisture content, alcohol and water soluble extractive were also determined, and were found to be 9.68 , 18.12 and 6.23%. Physicochemical parameters such as total ash value, water soluble and acid insoluble ash value were determined which were 7.03, 5.27, 0.55% respectively. The TLC profile of different extracts (hexane, DCM and methanol) of *P. pellucida* showed 10, 5 and 4 spot respectively. Phytochemical screening of aqueous ethanolic extract of leaf of the *P. pellucida* showed the presence of alkaloid, flavanoids, glycosides, phenols, saponin, steroids, terpenoids, tannins and carbohydrates. These present study help in identification and authentication of the plant material, such as information for correct identification of the plant and also will be useful in making monograph of the plant.

Key Words: Chromatography profile, Pharmacognostical, Physicochemical, Phytochemical, *Piperomia pellucida*.

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INTRODUCTION

Peperomia pellucida belongs to the Piperaceae family, is an annular herb, roots are fibrous; stems translucent pale green, erect or ascending (until 45 cm). Basically the native range of this plant is Tropical America, but now widely cultivated throughout the tropic countries. It is found in the major part in India, and also used in Ayurvedic medicine (Majumder and Kumar., 2011). In different parts of Indonesia, people commonly known as "suruhan, saladaan, tumpangan air" and also used in Indonesia traditional medicine ("Jamu"). The plant has been applied for treating abdominal pain, gout, headache, renal disorders, gout and arthritis (Tilaar *et al.*, 2010).This plant is occurring at elevations of sea

level to about 400 m as a weed along road sides, in plantations, on damp ground in shady places near houses, and occasionally along forest trails (Depkes, 1997).Plant growth and development, and often the nature and quantity of secondary metabolites, are affected by environment conditions, such the altitude area, soil and nutritive requirements, temperature, rainfall, day-length and radiation characteristics, and cultivated or wild plants (Evans WC, 2002). This study will help in authentication of this Indonesian plant and ensures reproducible quality of herbal product which to lead safety and efficacy of natural product. The pharmacognostical study of the raw material medicine plant is very essential for the identification of medicinal plants and prevention of adulteration (Ismail *et al.,* 2011).This plant indicated also possess various pharmacological activities such as hypoglycemic, anti-inflammatory, analgesic (Sheikh *et al.,* 2013), antipyretic (Khan A, 2008) and antibacterial activities (Igwe *et al*., 2014).Since ancient time plants and plant products have been used for treating various illnesses. To ensure reproducible quality of herbal products, authentication of the starting material is essential (Chandra S, 2014). 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 (WHO, 1998).The present research work is concerned to characterization of different pharmacognostical parameters, has included here, botanical identification, microscopic study, powder characteristics, analytical standardization, florescence study etc. A preliminary phytochemical screening has also been done. 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. Thus, all the experimentations had done in this regard have been described below.

MATERIALS AND METHODS

Collection and Authentication

The plant of *Peperomia pellucida* (L.) Kunth. (Piperaceae) were collected from Botanical Garden, Bogor, West-Java, Indonesia, in the month of September 2015. The taxonomical identification and authentication of the plant was done by the Herbarium Bogoriense, Bogor, Indonesia. The voucher specimen was deposited in our laboratory for further reference. The physicochemical parameter studied were moisture content**,** total ash, water soluble and acid insoluble ash, alcohol and water soluble extractives, were determined using methods described in the Farmakope Herbal Indonesia (FHI = Indonesian Herb Pharmacopoeia**,** 2008.

PHARMACOGNOSTICAL EVALUATION

Macroscopic study

Morphological studies of leaves such as color, size, odor, taste, surface characteristic and fracture were examined using the terms and outlined given in Evans WC**.,** 2002. In these tests taste, colour, odour, size of leaf and powder were observed and noted and photographs were taken in the original environment.

Microscopic study

Microscopic of transverse sections of fresh leaves was performed. For this purpose a transverse section by free hand was prepared. The midrib of the leaf was cut using a sharp razor including a small portion of lamina. The portion of midrib 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. Small amount of different powder material macerated with chloral hydrate suspension. One drop of solution was taken on a slide and then it was heated on spirit lamp and then examined under microscope. Different tissues were observed under the microscope and were photographed (Wallis TE, 1984; Muhammad *et al.,* 2012).

Moisture content

Weigh about 2 g of the air dried powdered material 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 weight before and after the material dried in oven.

Total ash value

Weigh accurately 2 g of the air dried powdered material in a silica crucible which was previously ignited and weighed. Spread the powdered material in a fine even layer at the bottom of the crucible, and ignite it by gradually increasing the heat to 400°C until it is white, that indicating it is free from carbon. The crucible was cooled in a desiccator 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 above was boiled with 25 ml of distilled 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 min at a temperature (not exceeding 400^oC, cooled and weighed. The procedure was repeated to get the constant weight. The percentage

of water soluble ash was calculated with reference to the air dried drug.

Acid insoluble ash value

The total ash obtained above 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.The same procedure was repeated 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

Weigh accurately 10 g of air dried coarse powdered material, in a glass-stopper conical flask. Macerated with 100 ml of 90% ethanol for 24 hours, shaking frequently during 6 hours, then to allowed for 18 hours. The solution filtered rapidly through a dry filter, taking precautions 25 ml of the filtrate and evaporated to dryness in a tarred flat bottomed dish, dried at 105°C in oven and weighed. Calculate the value of extractable matter with reference to the air-dried material.

Water soluble extractive value

Weigh accurately 10 g of air dried coarse powdered material, in a glass-stopper conical flask, in 100 ml of water macerated for 24 hrs 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 105^{o}C and weighed .The percentage of the value of water soluble extractive was calculated with reference to the air-dried drug.

FLUORESCENCE OBSERVATION

Fluorescence character

Fluorescence characters of powdered, plant material were sieved through 60 mesh and observations with different chemical reagents, observed under visible and ultraviolet (254 and 366 nm) light. The reagents were methanol, 2N hydrochloric acid, 50% sulphuric acid, 50% nitric acid and5% sodium hydroxide (Kokashi *et al.*,1958).

PHYTOCHEMICAL SCREENING

Preliminary phytochemical screening was carried out using 20 g powdered material and subjecting it to successive extraction in a reflux apparatus with 200 ml methanol for 30 minutes. The extraction was filtered and concentrated using a rotary evaporator and the obtained extracts were concentrate. Presence of various phytoconstituients *viz.*, carbohydrates (Fehling and Molish test),protein (Biurete and Ninhydrin test), alkaloids (Dragendorff, Mayer and Bouchardat reagent), anthraquinones (Borntrager test), flavonoids (Shinoda and ammonia test), glycosides (Molisch and Fehling solution test), phenols (ferric chloride test), saponins (foam test), steroids and terpenoids (Liebermann Burchard test, Salkowski test) and tannins (gelatin and lead acetate test) were tested, as per the standard procedures described by Harborne JB.,1984 and Dep Kes RI., 2008. The intensity of the coloration determines the abundance of the compound present.

CHROMATOGRAPHIC PROFILE

Chromatographic profile was carried out using 10 g powdered material and subjecting it to successive extraction in a Soxhlet apparatus with 150 ml solvents *viz*., hexane, dichloromethane (DCM) and methanol.The extraction was continued until the solvent became colourless. The 3 extracts were concentrated using a rotary evaporator then analyzed by TLC. Prepared silica gel 60F254 TLC plates were used 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 plates were developed in a chromatographic tank using the different solvent systems as Hexane –DCM (5:5), chloroform- methanol (9:1), ethyl acetate – methanol (6:4). 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^{o}C for 5-10minutes in an oven (Wagner S, 1984).The retention factor (Rf) value was calculated by using the following formula.

$$Rf = \frac{\text{Distance moved by the solute/compound}}{\text{Distanced moved by the solvent front}}$$

RESULT

Pharmacognostical evaluation

Macroscopic study

Macroscopic characters were: the leaves are green on upper surface, lower surface light green, thinly fleshy, shiny, heart-shaped, alternate, broadly ovate, 1.5-4 cm long, 1-3 cm wide, glabrous, apex acuminate, with characteristic odour, acid taste and the texture are soft and waxy. *P. pellucida* usually growing to a height about 15-40 cm, petioles 0.5-3 cm long, glabrous.The flowers well-spaced and fruits were subglobose, ca. 0.5 mm long, blackish colour. Roots (were 4-7 cm, light brown colour and multiple branches (Fig 1).

Microscopic Studies

Microscopical examination of the different parts of plant showed the following: the transversal

section (TS) of leaf showed single layered epidermis and multi layered palisade parenchyma (Fig 2.a.), TS of stem:epidermis covered with cuticle, parenchyma, vascular bundle, xylem, and phloem (Fig 2.b.), the cross section of well-developed roots showed an outer layer of cork tissue, xylem, phloem composed of thin walled cells, and vascular bundle in spiral form (Fig 2.c.), the cross section of the seed: outer layer of the seed, phloem, xylem starch grains and vascular bundle (Fig 2.d).

Powder Microscopy

Physicochemical Parameters

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

Moisture is an inevitable component of crude drugs, which must be eliminated as far as practicable. The moisture content was 9.68% which were not so high as to facilitate to the proliferation of microorganisms. The total ash, water soluble ash and acid insoluble ash value which were determined to be not more than 7.03, 5.27, 0.55 % w/w respectively which indicated the presence of the total foreign inorganic matter. The ash value represents the inorganic salts present in the drug. Extracts obtained by exhausting crude drugs are indicative of approximate measures of certain chemical compounds they contain, the diversity in chemical nature and properties of contents of drug.

Fluorescence Observation

The results of the fluorescence of the powder and extract of *P. pellucida* 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 help full for preliminary chemical study as well as for standardization of specific plant materials. The colour was seen day light and ultra-violet rays at 254 nm and 366 nm for observing any specific fluorescence.

Phytochemical Screening

Phytochemical screening of the extract gives general idea regarding the nature of chemical constituents present in the crude drug. The outcome of the phytochemical screening of the extract showed the presence of carbohydrates, alkaloids, flavonoids, glycosides, phenols, saponins, steroids, terpenoids and tannins (Table 3). The pharmacological action of the crude drug is largely depends on the metabolites present in it. In the present investigation, the qualitative screening by using prepared extracts revealed the presence of a wide range of phytoconstituents.

Chromatographic Profile

The Rf values were calculated for the optimum solvent system revealed the presence of promising spots as shown in Table 4.

It was observed that the thin layer chromatographic profile of *P. pellucida* hexane extract showed the presence of 10 spots with Rf value of 0.05, 0.11, 0.23, 0.30, 0.51, 0.63, 0.68, 0.85, 0.91 and 0.98 respectively in hexane – DCM (5:5) solvent system. The thin layer chromatographic analysis of DCM extract of *P. pellucid* showed the presence of 5 spots with Rf value of 0.21, 0.43, 0.73, 0.80, 0.98 respectively in chloroform – methanol (9:1) solvent system. On another hand in the methanol extract of *P. pellucida*, there were showed 4 spots in the TLC, with Rf value of 0.74, 0.80, 0.91, 0.96 respectively in ethyl acetate – methanol (6:4) solvent system.

Table 1. Physicochemical parameter of powder of *P. pellucida*

No.	Parameters	Average (%w/w)
1	Moisture content Loss on drying	 9.68
2	Ash Value a. total ash b. water soluble ash c. acid insoluble ash	 7.03 5.27 0.55
3.	Extractive values a) Alcohol soluble Extractive b) Water soluble extractive	 18.12 6.23

Table 2. Fluorescent analysis of various powder and extract of *P.pellucida*

Material	Day light	Short UV (254 nm)	Long UV (366 nm)
Powder			
+ methanol	Dark green	Black	Black

+ 2N HCl	Dark brawn	Black	Greenish black
+ 50% H_2SO_4	Black green	Brawn	Greenish black
+ 50% HNO_3	Yellowish brawn	Black	Greenish black
+ 5% NaOH	Dark brawn	Black	Black
Ethanol Extract			
+ methanol	Dark green	Black	Greenish black
+ 2 N HCl	Dark green	Light green	Green
+ 50% H_2SO_4	Black	Dark Green	Greenish black
+ 50% HNO_3	Yellowish green	Yellowish	Greenish black
+ 5% NaOH	Greenish brawn	Black	Black

Table 3. Phytochemical screening of material plant extracts of *P. pellucida*

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	+
Saponins	Foam	+
Steroids and terpenoids	Liebermann BurchardSalkowski	+ +
Tannins	Gelatin Lead acetate	+ +

+ = presence; **-** =Absence;

Table 4. Chromatographic evaluation of different extract of *P. pellucida*

Extract	Solvent system (Ratio)	No. of spots	Visual	Spraying reagent (10% H_2SO_4)	Rf (UV 366 nm)
Hexane	Hexane –DCM (5:5)	10	1. Green 2. Gray 3. Gray 4. Yellow 5. Yellow 6. -- 7. -- 8. -- 9. -- 10. --	Brown Brown Light brown Light brown Yellowish Pink Pink Yellowish Yellowish Yellow	0.05 0.11 0.23 0.30 0.51 0.63 0.68 0.85 0.91 0.98
DCM	Chloroform-methanol (9:1)	5	1. Brown 2.Brown 3. Grey 4. Grey 5. --	Grey Grey Light blue Light blue Yellowish	0.21 0.43 0.73 0.80 0.98
Methanol	Ethyl acetate – methanol	4	1. – 2. –	Light blue Light blue	0.74 0.80

	(6:4)		3. -- 4. Brown	Yellowish Yellowish	0.91 0.96

Fig 1. *Peperomia pellucida*: a, b: plants, c: leaf, d: roots, e: flower and f: seeds

Fig 2. Microscopy of TS of *P. pellucida*: a. leaf, b. stem, c. root, and d. seed

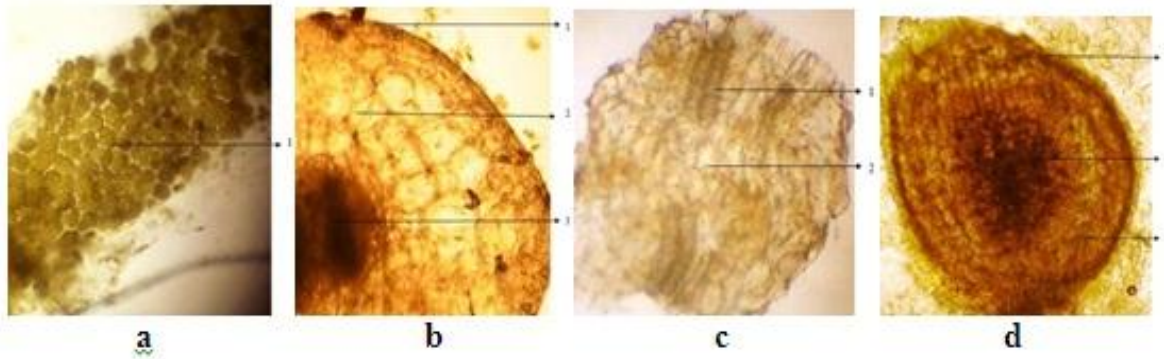
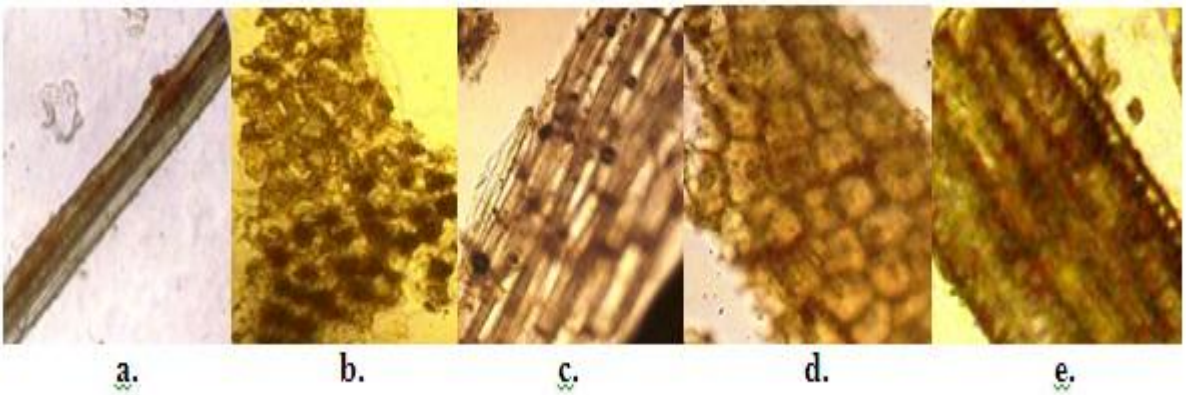


Fig 3. Powder Microscopic Characteristic of *Peperomia pellucida*



(a. vascular bundle, b. calcium oxalate crystals, c. parenchyma cells, d. stomata, e. vascular bundle (spiral type)

DISCUSSION

Microscopical evaluation is a step towards authentication of internal structure of the plant to establish proper identification by revealing tissue arrangement. This is done by identifying internal structures such as epidermis, collenchyma, vascular bundles, types and arrangement of vascular bundles, sclerenchyma, crystals and any other specific features that lie there in**.** The physicochemical parameters which as certain the quality, purity and also help in evaluating the medicinal plant material.

The extractive values can serve as a valuable source of information and provide suitable standards to determine the quality of plant material in future investigations or application. Extraction values are useful for determination of plant material and it gives

an idea about the nature of the chemical constituents present. The solvent used for the extraction should be in position to dissolve appropriate quantities of desired substances.The phytochemical screening showed the presence of many important groups of phytoconstituents such alkaloids, flavonoids, glycosides, phenols, saponins, steroids, terpenoids, tannins and carbohydrates, which may influence the pharmacology activities of the plants. The chromatographic profile of all three extracts gives a remarkable result that directing towards the presence of number of phytoconstituents, that give different Rf values in different solvent system. This variation in Rf values of the phytoconstituents, show a very important clue in understanding of their polarity. Combination of solvents with changeable polarity in dissimilar ratio can be used for separation of unpolluted compound from plant extract.

CONCLUSION

The macros- and microscopic characteristics of this plant were the important first step to identify the origin crude drug. The physicochemical parameters and phytoconstituents were established which can be important in detecting adulteration and mishandling of the crude drug. The present finding of phytochemical screening of the plant extract confirmed the presence of several bioactive compounds like carbohydrates, alkaloids, flavonoids, glycosides, phenols, saponins, steroids, terpenoids, tannins. Which could be responsible for the versatile medicinal properties of these plants. This investigation is in the line with the earlier reports of the Indian *P. pellucida*, and found to be significant and encouraging towards the goal for standardization. The generated information of the present study will provide data which is help full in the correct identification and authentication of this medicinal plant and may help in preventing its adulteration.

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CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

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