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PROCEEDING

International Conference on Restorative Justice

4 January 2014, Grand Tjokro Hotel, Yogyakarta

"Consumer Protection Law and Pharmacy Perspectives"

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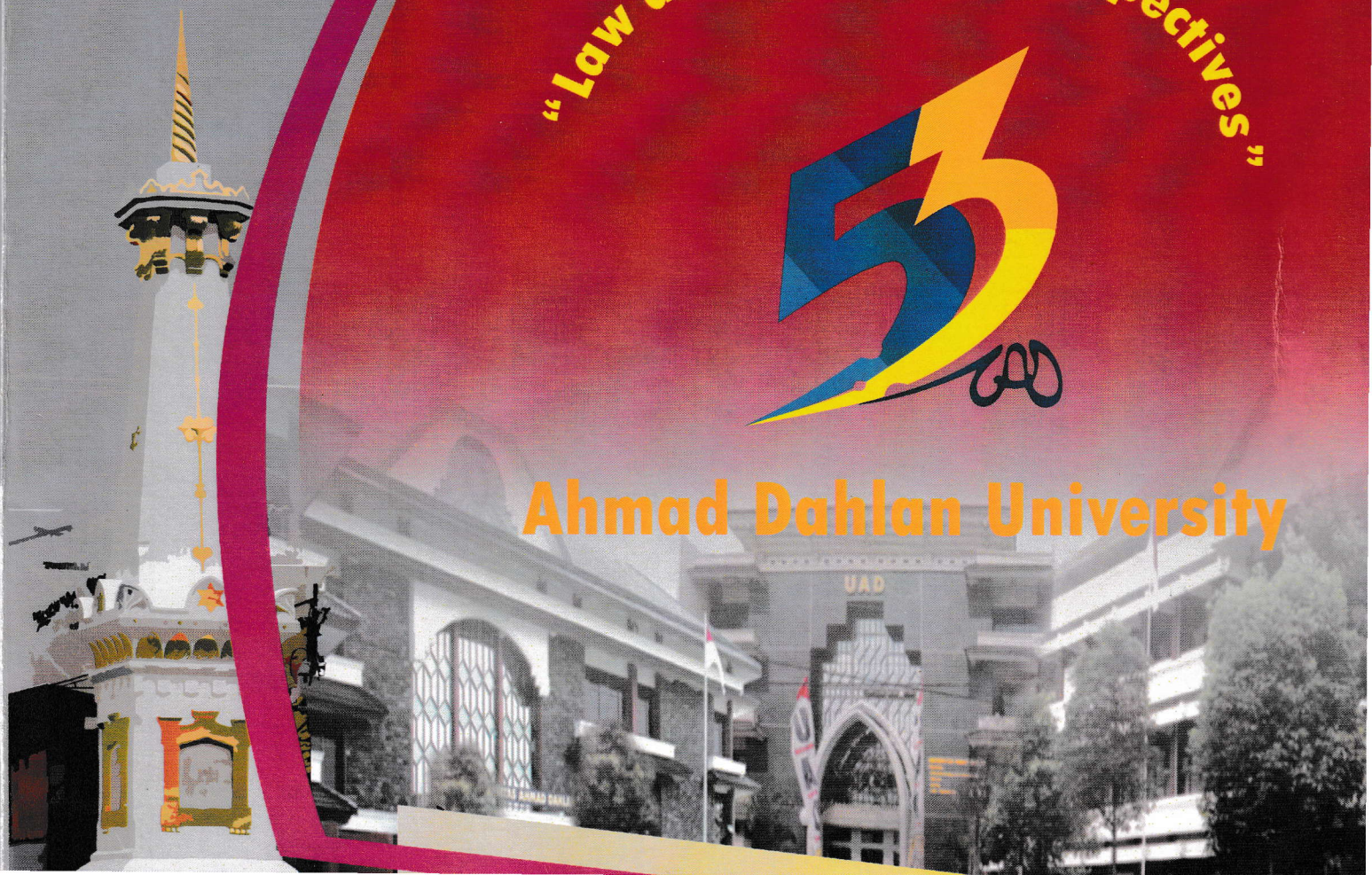
Ahmad Dahlan University



PROCEEDING

INTERNATIONAL CONFERENCE ON RESTORATIVE JUSTICE

Theme :
Consumer Protection: "Law and Pharmacy Prespective"

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Deputy Health Minister of Republic Indonesia
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Tabel Of Content

POSTER PRESENTATION

STUDY OF MACRO – MICROSCOPY AND THIN LAYER CHROMATOGRAM PROFILE OF HENNA LEAVES (*Lawsoniainermis* L.)

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ABSTRACT

Background: Safety and affectivity of an herbal medicine greatly influenced by the chemical constituents present incrude drugsasraw material. Botanicals standardization of crude drugs is very important because the plant materials have a wide variety of both biology and chemistry, especially chemical components contained in crude drugs.

Objective: The purpose of this research was to determine the characteristics of the macroscopic, microscopic and thin-layer chromatogram profiles of Henna leaves (*Lawsoniainermis* L.).

Methods: The research was descriptive experimental laboratory. Macroscopic analysis of the fresh and dried leaves is done by observing the shape, size, color, flavor and texture of the leaves. Microscopic analysis performed on fresh leaves and Henna leaves powder by microscope. Chromatographic profile analysis performed on methanol extracts of fresh leaves and Henna leaves powder. Stationary phase used silica gel plates F254 and eluted with chloroform: methanol (17:3) v/v as mobile phase.

Outcome measured: The characteristic of macro-microscopic of Henna leaves and thin layer chromatogram profile of methanol extract of Henna leaves.

Results: The result of macroscopic and microscopic analysis showed the characteristic of henna leaf. Henna leaf had anomocyticstomata, non-glandulartrichome,Ca oxalate in clusters form was present at leaf mesophyll. The TLC profile of methanol extract of Henna leaves confirms the presence of various phytochemicals such as naphthoquinones, flavonoids, polyphenols, alkaloids, saponins, and steroids.

Conclusion: In conclusion, the Henna leaves had specific characteristic of macro-microscopic and several of phytochemical that could be used to ensure the quality of crude drugs.

Keywords: Macroscopic, microscopic, thin layer chromatography, *Lawsoniainermis* L.

Introduction

One of the plants with highly potential to developas herbal medicine is henna (*Lawsoniainermis* L.). Besides being used as a dye, henna also has many biological activities such as antibacterial, antiviral, antimitotic and anti-parasitic (Babu&Subhasree, 2009). The water extract and methanol extract of henna leaves has antioxidant activity and can inhibit Cr (VI) induced oxidative toxicity to MDA - MB - 435S cells and pBR322 DNA (Guha*et.al.*, 2009). The antioxidant activity of the methanol extract is higher than the water extract, because the content of total phenolic compounds extracted in methanol is greater than water extract withrespectively 2.56 mg/g and 1.45 mg/g calculated as tannins with Folin - Ciocalteu method (Hosein&Zinab, 2007). Chloroform extract can inhibit the growth of Malassezia in concentrations of 3 and 4 (v/v %), methanol extract at 0.25 and 3 (v/v %), while the water extract at 0.25 and 0.5 (v/v %) (Berenji, *et.al.* 2010).

Efforts to ensure the quality and safety of herbal medicines should be done early in the process ranging from the selection and use of botanicals, the entire production process to such products circulating in the community. Manufacturers of herbal medicine have a major responsibility for the quality and safety of all the products that are marketed to the public. For that they should have an internal system that can monitor and control the quality of its products since the beginning of the process until the product is in the market (Sampurno, 2007).

Safety and effectiveness of a traditional medicine was mainly influenced by the components contained in it, especially botanicals as the raw materials. Standardization of crude drugs has become very important because the natural materials have a wide variety of both biology and chemistry, especially chemical components contained in crude drugs. The good quality of traditional medicine musthave repeatability in shape, safetyand benefits also needgood botanicals as raw material. Some guide books such as pharmacopoeia using macroscopic evaluation methods, microscopic and chemical profiles on botanicals as standardization parameters (Anonymous, 2011; Kushwaha *et.al.* 2010; Ahmed *et.al.* 2013). Then it is necessary to study the characteristics of crude drugs mainly to seek henna leaves macroscopically, microscopic and chemical content profiles.

Materials and Methods

Plant material

The Henna leaves (*Lawsoniainermis* L.) were collected from Yogyakarta province, Indonesia during April 2013. The whole plant was authenticated by determination in Biology Faculty of Ahmad Dahlan University, Yogyakarta, Indonesia.

Macro-microscopic characteristic assay

The macroscopic examination was conducted on fresh leaves, dried leaves and henna leaves powder. This examination was performed by observing the shape, size, color, surface characteristics, texture, fracture characteristics and surface texture pieces (Anonymous, 2011).

The microscopic examination was conducted on fresh leaves and henna leaves powder. Transverse section was prepared with a sliding microtome (MSE) and the powder observations were made using chloral hydrate solution (anonymous, 2008; Anonymous, 2011).

Profile of Thin Layer Chromatography

Profile of thin layer chromatography was performed on methanol extracts of bothfresh leaves and henna leaves powder.

Preparation of sample solution

Five hundred milligrams of henna leaves powder (*L. inermis* L.) was macerated using 5 ml of methanol while shaken for 1 hour. The filtrate obtained by filtration using filter paper then volume was made to 5.0 ml by adding methanol solvent.

Preparation of standard solution

The reference compounds were used Lawson (naphtoquinone), piperine (alkaloid), glycyrrhizin acid (saponin), quercetin (flavonoid and polyphenol), and tannic acid (polyphenol). All of the standard solution was made 0.1% w/v concentration in methanol.

Thin layer chromatography system

A set of five plates of silica gel 60 F254 were used for detection of naphthoquinones, alkaloids, saponins, flavonoids, and polyphenols groups.Five µl 20% w/v of fresh henna leaves methanol macerate, 5.0 µl 5%w/v of henna leaves powder methanol macerate, and 5.0 µl of standard solution were applied on each TLC plate. The plates were then developed with chloroform-Me OH (17:3, v/v) (Zainab, 2012) until 8 cm for distance. The dried TLC plates 1-5 were inspected under UV light (254 nm and 366 nm). TLC plate 1 was visualization with 5% KOH methanol for naphthoquinone identification. TLC plate 2 was visualization with Dragendorff spray reagent for alkaloids identification. TLC plate 3 was visualization with Liebermann Burchard for saponin identification.TLC plate 4 was visualization with 1% $AlCl_3$ for flavonoids identification. TLC plate 5 was visualization with 1% FeCl3 for polyphenol identification. All spots were recorded the Rf values, characteristic of the spots under UV254, UV 365 and colour that obtain after spray with certain reagent.

Results and Discussion

Macroscopic of henna leaves

The macroscopic analysis performed to determine the macroscopic characters include leaf shape, leaf blade, leaf base, leafsurface, the edge of the leaf, the veins, size, color, petiole, smell, and taste (Tjitrosoepomo, 1987). Macroscopic analysis performed on fresh leaves, dried leaves and henna leaves powder, the result can be seen in Fig1.

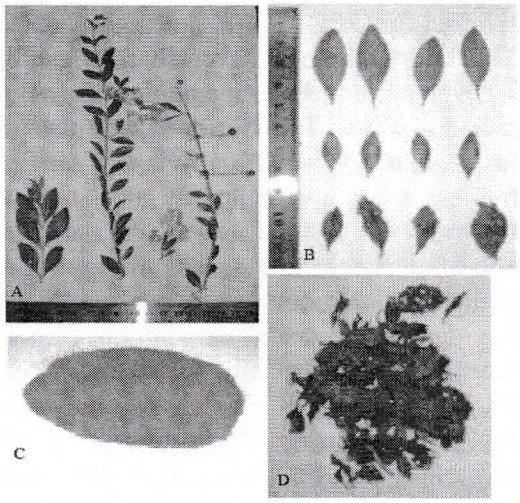

Figure 1. Morphology of fresh leaves (A, B), dried leaves (D) and henna leaves powder (C)

Microscopic of henna leaves

The microscopic analysis was conducted to obtain anatomical elements typical of henna leaves so that it can be obtained fragment marker that can be used for identification raw plant material. Microscopic analysis performed on fresh leaves and henna leaves powder. Fresh leaf examination was conducted by transversection sliding then placed on the object glass and gives several drop of distilled water and covered with a cover glass and observed under a microscope. Crude drug powder was observed in chloral hydrate solution (Anonymous, 2008; Anonymous 2011). The results of microscopic analysis of the leaves and henna leaves powder can be seen in Table II.

In Table II, shown in the lower epidermis layers of henna leaf content epidermal cells in wavy shaped, non-glandular trichomes and stomata with anisocytic types, each stomata surrounded by 3 to 5 neighbouring cells with similar cell size. In the leaf mesophile was found Ca oxalate crystals in clusters form.

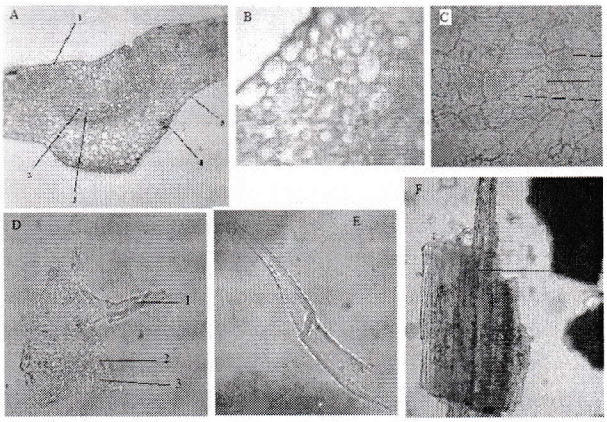

Figure 2. Microscopic of henna leaves. A. Transversection of henna leaf (1.upper epidermis, 2. Xylem, 3.Floem, 4.Trichome), B. Crystal of Ca oxalate in cluster form, C. Upper epidermis (1. Epidermis cell, 2. Cell guard, 3. Stomata), D. Microscopic of powder (1. Trichome, 2.Stomata, 3.Epidermis cell), E. trichome, F. Vascular bundle.

Fig E. showed multi-cellular covering trichome with two cell but Jain, et.al., 2010 found unicellular covering trichome and diacytic stomata are present on both the surface of *Lawsoniainermis* L. Agarwal, et.al., 2012 found that type of stomata are both anisocytic and anomocytic were present. Fragment of parenchyma cell were content of oil globule, crystals of calcium oxalate in rosette and prismatic form. This difference is due to the variation in the species of *Lawsoniainermis* L.

Thin layer chromatogram of henna leaves

Identification of chemical compound in the fresh leaves and Henna powder was done by TLC with silica gel F254 as stationary phase and mixture of chloroform: methanol (17:3) v/v as mobile phase. The results of TLC test can be seen in Table I and Fig.3.

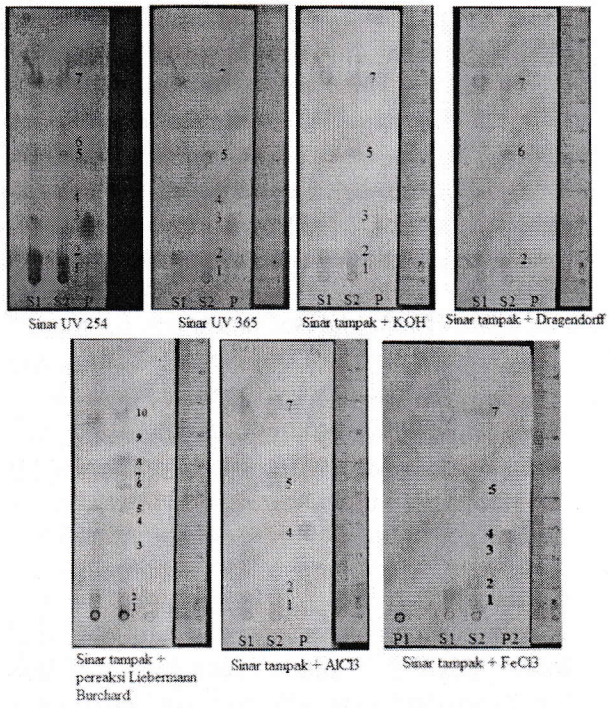

Figure 3. Thin layer chromatogram profile of henna leaves

Table I and Fig. 3 showed that fresh and powder leaves of Henna content of naphtoquinone, flavonoid, saponin, alkaloids, polyphenol and saponin. Raja, et.al. 2013 found glycosides positive with Botranger reaction, flavonoids, alkaloids, steroids and tannin.

Table I. Result of thin layer chromatography of fresh and powder leaves of Henna

No	Rf value		Detector		Chemical content
	Fresh leaves	Powder leaves	UV_{254}	UV_{366}	
1	0,04	0,04	Q	Blue	Naphtoquinones (polyphenols)
2	0,11	0,11	Q	Blue	alkaloids
3	0,26	0,26	Q	Blue	Naphtoquinones

4	0,30	0,30	Q	Blue	(polyphenols) Flavonoids (polyphenols)
5	0,36	-	-	-	Saponins (triterpenoid)
6	-	0,45	Q	Blue	-
7	-	0,54	Q	-	+
8	-	0,58	-	-	Saponins (triterpenoid)
9	0,65	0,65	-	-	Saponins (triterpenoid)
10	0,84	0,84	Q	Blue	
Rf standard of lawsone : 0,19			Q	orange	Naphtoquinones
Rf standard of piperin : 0,76			Q	Blue	alkaloids
Rf standard of tannic acid : 0,11			Q	Blue	polyphenols
Rf standard of quercetin : 0,38			Q	Blue	flavonoids
Rf standard of glycyrrhizid acid : 0,00			Q	-	saponins

Conclusion

1. Macroscopic characteristics of the nail henna leaves is a single green leaf, leaf lays the cross face, smooth leaf surface. Leave blade elliptic to lancet. Tapered tip and base of the leaf with the flat edge, pinnate's bone-leaves. Odourless fresh leaves with somewhat bitter taste.
2. Simplicia macroscopic characteristics intact and crude drug powder: brown, pungent aromatic odor with a bitter taste.
3. Microscopic characteristics of henna leave nails: slightly wavy shapes epidermis, stomata type anisositik, Ca oxalate crystals form clusters and are trichomes.
4. Methanol extract of fresh leaves and henna leaf powder nail crude drugs containing compounds of naphthoquinones, flavonoids, polyphenols, alkaloids and saponins group.

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