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Research Article

IDENTIFICATION OF STANDARD PARAMETERS OF KEPEL LEAVES [*STELECHOCARPUS BURAHOL* (BL.) HOOK. F. & TH.] AND THE EXTRACT AS RAW MATERIAL FOR ANTI-HYPERURICEMIC MEDICAMENTS

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ABSTRACT

Herb-drug product or product of natural medicine derived from plants is definitely influenced by the quality of raw material. The raw material quality is affected by some factors such as cultivation, post harvest and processing. Therefore, the standardization of the raw material is needed to ensure the quality of herb-drug product. The Kepel [*Stelechocarpus burahol* (Bl.) Hook. f. & Th.] is one of the Indonesian medicinal plant that possessed an activity as anti-hyperuricemic agent. The leaves was the main source of the raw material for the herb-drug product, so that, the some identified parameters were needed to ensure the safety, efficacy and the quality of the product. This study identified the some standard parameters that may be useful as guideline to choose the Kepel leaves for anti-hyperuricemic medicaments.

Keywords: Herb-drug product, Raw material, *Stelechocarpus burahol* (Bl.) Hook. f. & Th., Standard Parameter

INTRODUCTION

Kepel or *Stelechocarpus burahol* (Bl.) Hook. f. & Th. has been used as medicinal plant by society. The development of pharmaceutical science and the increase of using the natural medicine in the world encouraged the Indonesian government to conduct the increase of Indonesian natural medicine quality. One of the efforts that was taken is by creating the standardized product. It means the product has a good standard of chemically, biologically or pharmacy, including the guarantee of the stability as pharmacy product generally.

Product of natural medicine derived from plants (herb-drug) is strongly influenced by the quality of raw material. It is the main ingredient that contained the effective compounds. Many factors affect the raw material quality such as cultivation, post harvest and processing. Therefore, the standardization is needed to achieve the product that is safe, efficacious and has a good quality.

Kepel comes from Javanese and Malaya, it has synonym names as Burahol or Turalak. Kepel has been traditionally used by the Indonesian communities for gout, diuretic, cosmetic, renal inflammation and sometimes as abortifacient[1,2]. The chemical contents at the seed, kernel and root of Kepel are saponin, flavonoids and polyphenols. The seed of Kepel contains the alkaloid compounds, while the leaves contain flavonoids and polyphenols[3]. Some studies showed that Kepel has a potency as anti-hyperuricemia. Susilowati (2000)[4] evaluated that the water extract of Kepel leaves decreased plasma uric acid levels in rats.

The effect of the water extract of Kepel leaves in the decreasing of plasma uric acid levels in chicken reported by Hening (2002)[5]. The anti-hyperuricemic activity of the soluble and not soluble fractions of petroleum eter on chicken and the ethanol and hexane leave extracts of Kepel on rats have been evaluated by Sutomo (2003)[6] and Purwantiningsih (2010)[7], respectively. Based on the studies the Kepel leaves could be able to develop as a potential anti-hyperuricemic agent. Therefore, the standardization of the raw material from Kepel leaves was needed to ensure the quality of the product. The aim of this study was to determine some parameters that may be used as standard parameter for raw material of Kepel leaves and the n-hexane extract.

MATERIALS AND METHODS

Plant material

The Kepel leaves [*Stelechocarpus burahol* (Bl.) Hook. f. & Th.] were collected from Samigaluh area, Yogyakarta and from Ambal, Central Java, Indonesia, authenticated by Prof. Wahyono, Head of Department of Pharmaceutical Biology, Faculty of Pharmacy, Gadjah Mada University, Indonesia. A voucher specimen was deposited. The raw material was produced using a standard guideline[8,9]. The n-hexane extract was made by maceration of dried leaves of Kepel with ethanol 96% for 24 hours and re-macerate for two times. The mixture extract was concentrated by using the evaporator, then was extracted again by n-hexane and was evaporated until was got the concentrated extract.

Determination of standard parameter of raw material (dried leaves) and n-hexane extract of Kepel Leaves

Determination of standard parameters was done using the standard methodology in Indonesia and the extract common standard parameters of herb-drug[9]. It was the macroscopic and microscopic investigation, physicochemical parameters screening including water content, total ash, water soluble extractive, ethanol soluble extractive, loss on drying, contaminant, phytochemical analysis and the chemical content, in this case the flavonoid content because the previous studies suggest that the active compound that may has the anti-hyperuricemic activity was flavonoids, and the non-polar extract had a higher effect if compared with the polar-extract[6,7].

Determination of total flavonoid content in n-hexane extract of Kepel leaves

The total flavonoid content of n-hexane extract was determined using the procedure reported by Zhuang *et al.,* (2002)[10]. Rutin was used to make the calibration curve. It was made a stock solution, the concentration was 2.5 mg/mL. A known volume of extract or the stock solution (50, 75, 100, 125, 150, 175 and 200 µL) was taken out and was placed in a 10 mL volumetric flask. Then, it was added with 4 mL of distilled water and 0.3 mL of 5% sodium nitric solution. Aluminum chloride solution (0.3 mL; 10%) was added to this mixture after 6 min incubation. After 6 min, the sodium hydroxide solution (4 mL; 4%) was added. The mixture was made up 10 mL by adding distilled water. The solution was mixed well and the absorbance was measured against a blank by using a visible spectrophotometer (Perkin Elmer lambda EZ 150, United Kingdom) at 510 nm after incubation for 15 min at room temperature.

RESULTS AND DISCUSSION

The sample of Kepel leaves was taken from two different places. Subdistrict Samigaluh, Regency of Kulon Progo represented the hilly area becoming a part of the Menoreh mountain. The Location was at the height of 750 to 800 m on the surface of the sea. Location of Subdistrict Ambal, Kebumen represented the coastal area of Indies ocean. The region resided in the height of 5 to 50 m of the above of

the sea level. The different environment may affect the components in the Kepel leaves. The Kepel leaves were taken from the Kepel trees with 7 to 10 m height and in a diameter 30 to 50 cm. Fig. 1 shows the fruit, seed, and the leaves of Kepel.

Fig. 1: A part of Kepel tree (*Stelechocarpus burahol* (Bl.) Hook. F. & Th.): a) young leaves, b) old leaves, c) fruit, d) kernel and e) seed

Macroscopic study

Leaves of Kepel measuring about 12 to 27 cm long and 5 to 9 cm wide. The leaves were in elliptic, elongate-ovate or lanceolate shaped. Young leaves were in reddish color and dark green for old leaves, pointed or slightly pointed tip and not hairy (Fig. 1). Kepel Leaves from the two places were dried in the oven at 50ºC and were grinded. Fig. 2 shows the dried leaves from Ambal (2A) and Samigaluh (2B) and the powder after grinding process (2C and 2D). The powder from Ambal area shows a brownish color, and greenish for the powder from Samigaluh. The leaves have a dry weight 10% from the wet weight and the n-hexane extracts from Ambal and Samigaluh have a yield 0.95% and 1,28%, respectively. The extract was odorless and no taste.

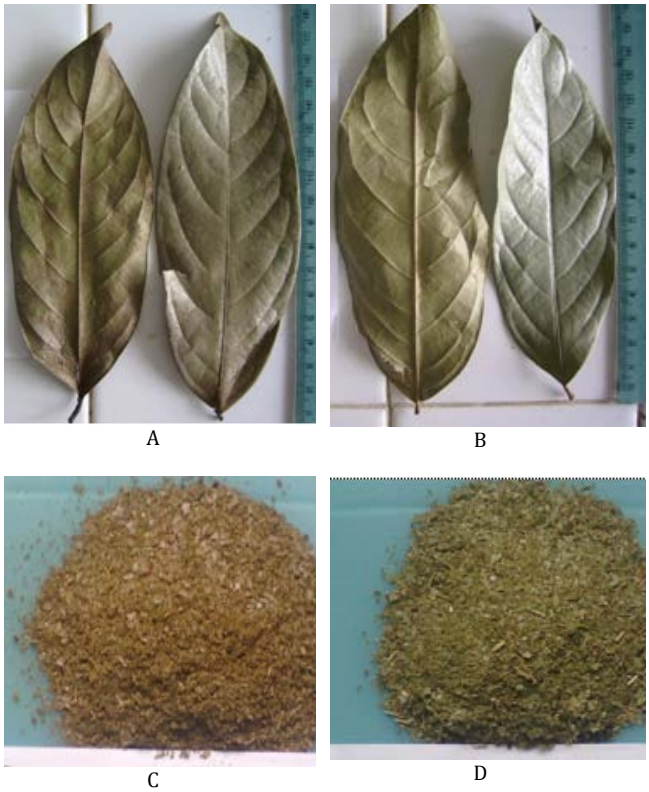

Fig. 2: Leaves of Kepel and the powder of the leaves from Ambal (A, C) and Samigaluh (B, D)

Microscopic study

Transverse section of Kepel leaves was showed in the following features (Fig. 3 to 5). Fig. 3 shows that the Kepel leaf cross section consisted a layer of epidermal tissues, a layer of palisade tissues and parenchyma cells at the middle and in irregular location. This tissues was called the spongy tissues.

The palisade and spongy tissues together were often called leaf mesophyll tissues. In the middle of the leaf bones seemed the carrier files. It was an open collateral and consisted the xylem and phloem (polygonal shaped) cells. The phloem cells were located on the inside of the xylem (Fig. 4A). Besides these four kinds of the tissues, there was one type, namely sclerenchyma tissues (Fig. 4B). This tissues consists sclerenchyma cells. The cells had a narrow lumen, because the cell wall has undergone secondary thickening. Therefore, this part appeared to harden, more obvious when the leaves were crushed, will look stiff and slightly sharp. In addition, from the fragment analysis was found stomata type, calcium-oxalate crystal, sclereids and sclerenchyma fiber (Fig. 5).

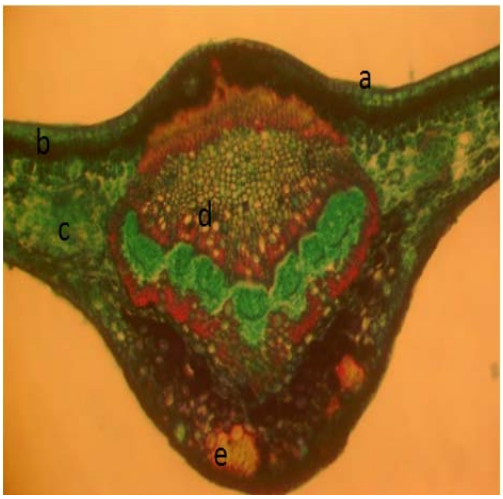

Fig. 3: Transverse section of Kepel leaves (magnification 10X10): a) epidermis, b) palisade tissues, c) spongy tissues, d) carrier files and e) sclerenchyma

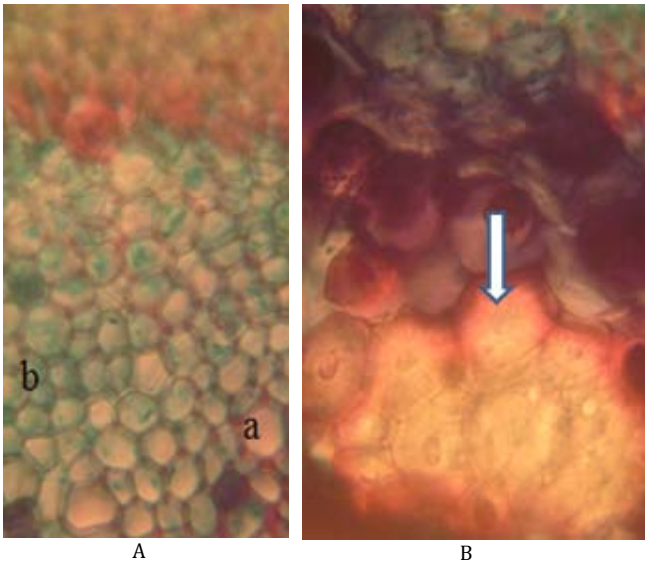

Fig. 4: Transverse section of Kepel leaves: A) the carrier files (magnification 10X10) with the xylem cells (a) and phloem cells (b), B) sclerenchyma (magnification 40X10)

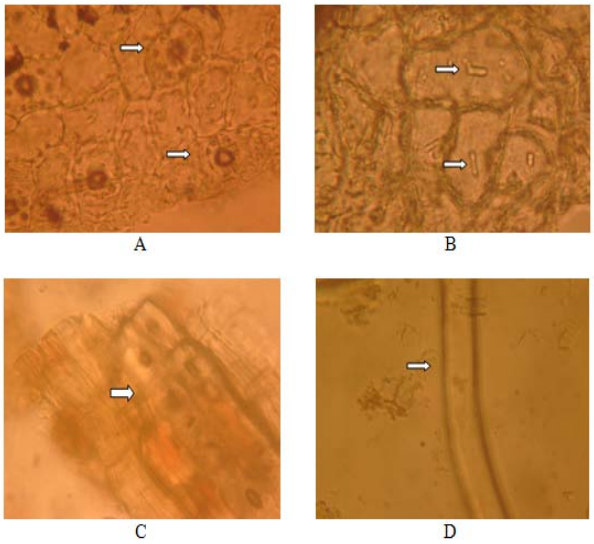

Fig. 5: Fragment analysis of Kepel leaves: A) upper epidermis with the stomata types, B) calcium-oxalate crystal in the upper epidermis, C) sclereids and D) sclerenchyma fiber

Physicochemical identification

The results of physicochemical identification is showed in Table 1. The water content in sample from Samigaluh was higher if compared with sample from Ambal area, as well the ethanol soluble extractive, water soluble extractive and the lost on draying for the n-hexane extract. But the total ash, the Ambal's sample has a high level for both dried leaves and the n-hexane extract. The sample of Samigaluh was taken from a mountain area that may has a rainfall higher than the coastal area, so that affected the water content. The water content should be no more than 10% because may affect the quality during storage and processing. The contamination of the bacteria and mold of the both samples were at the allowed level. Sample from Ambal has a high contamination of mercury, and at the not allowed level. Therefore, the raw material will not be taken from the area.

Phytochemical screening and determination of the flavonoid content

Chromatogram pattern (Fig. 6) was used to determine the approximate content of chemical compounds that was contained in the n-hexane extract. Table 2 shows that the chemical compounds in the n-hexane extract were terpenoid compounds, flavonoids and several compounds that cannot be identified using the thin layer chromatography (TLC) only. Anisaldehyde-sulfuric acid was a common reagent to detect the organic compounds. The terpenoid compounds and the derivates will be confirmed by the red spot or purple after the spot was sprayed by using the reagent (it shows by spots with hRf above of 80). The presence of flavonoids could be identified using the sytroborate reagent. The spots will be in a sharp violet color after spraying of reagent. The n-hexane extract contained at least the three types of flavonoids (especially these are showed by spots with hRf of 31, 43 and 64). The determination of flavonoids in the n-hexane extract was displayed in table 3.

The Dragendorff's reagent was used to detect the presence of alkaloid. The results showed that there was no alkaloids compound detected. Detection using UV_{254} and UV_{366} indicated a fluorescent compound. This was presented by redemption of the fluorescent of TLC system by the spot, or the spots will be in red or violet color under UV_{366} detection. The fluorescent compound was a compound that has a chromophore group. Unfortunately, the detection system was not be able to used to determine the group of the compound.

Fig. 6: Chromatogram pattern of n-hexane extract of Kepel leaves on the visual detection. The thin layer chromatography system was silica-gel as stationary phase and the mobile phase system of n-hexane:ethylacetate (1:1)

Flavonoids has been reported to be a potent xanthine oxidase inhibitors[11,12]. The xanthine oxidase inhibitor compounds reported has a good activity for the treatment of gout and rheumatism[13,14]. Purwantiningsih (2010)[7] evaluated the activity of the Kepel leaves extract as anti-hyperuricemic agent and as inhibitor of xanthine oxidase. The anti-hyperuricemic activity of the ethanol (60.86 to 78.33 %) and hexane extracts (78.23 to 88.52 %) are almost equivalent to that of the positive control of allopurinol (50.82 to 91.16%). While the ethanol extract showed the inhibitory activity lower than allopurinol. Sunarni and co-workers (2007)[15] found that Kepel leaves contained flavonoid compounds and one of them has been identified as 3, 7, 3', 4'-tetrahidroxy-5-methyl-flavone. The total flavonoid compounds in n-hexane extract form Samigaluh and Ambal were 11,543±0,889% and 9,535±0,331%, respectively. It may suggest that the anti-hyperuricemic activity of the n-hexane extract was contributed by flavonoid compounds. In conclusion, the standard parameters were identified from the two area of samples, especially from the physicochemical identification were at the allowed level unless the mercury contamination of sample from Ambal area. This study results may be useful for supplement information to choose the Kepel leaves if it will use as raw material for the anti-hyperuricemic medicaments.

Table 1: Result of physicochemical identification of raw material (dried leaves) and the n-hexane extract of Kepel leaves

Physicochemical parameters	Region	Sample form	Mean ± SD (n=3)		
1. Water content (%)	Samigaluh	dried leaves	7,87 ± 0,23		
		n-hexane extract	5,00 ± 0,00		
	Ambal	dried leaves	7,00 ± 0,00		
		n-hexane extract	2,00 ± 0,00		
2. Total ash (%)	Samigaluh	dried leaves	11,98 ± 1,05		
		n-hexane extract	0,65 ± 0,05		
	Ambal	dried leaves	14,40 ± 0,05		
		n-hexane extract	0,42 ± 0,03		
3. Lost on drying (%)	Samigaluh	dried leaves	NA		
		n-hexane extract	6,98 ± 0,26		
	Ambal	dried leaves	NA		
		n-hexane extract	5,41 ± 0,37		
4. Ethanol soluble extractive (%)	Samigaluh	dried leaves	7,27 ± 0,12		
		n-hexane extract	75,67 ± 2,52		
	Ambal	dried leaves	5,70 ± 0,26		
		n-hexane extract	62,00 ± 2,00		
5. Water soluble extractive (%)	Samigaluh	dried leaves	13,93 ± 0,21		
		n-hexane extract	NA		
	Ambal	dried leaves	10,16 ± 0,35		
		n-hexane extract	NA		
6. Contamination of bacteria (Total Plate Count score) (colony)	Samigaluh	dried leaves	$2.9x10^3$		
		n-hexane extract	NA		
	Ambal	dried leaves	$3.8x10^3$		
		n-hexane extract	NA		
7. Mold score (colony)	Samigaluh	dried leaves	$4x10^3$		
		n-hexane extract	NA		
	Ambal	dried leaves	$5x10^3$		
		n-hexane extract	NA		
			Hg	Cd	Pb
8. Heavy metal contamination	Samigaluh	dried leaves	< 0,62	< 0,01	< 0,03
Mercury/Hg (ppb), Cadmium/Cd (ppm) and Plumbum/Pb (ppm)		n-hexane extract	< 0,62	< 0,01	< 0,03
	Ambal	dried leaves	721,99	< 0,01	< 0,03
		n-hexane extract	< 0,62	< 0,01	< 0,03

Note: NA is not identified; ppb is part per billion; ppm is part per million

Tabel 2: Phytochemical screening result

hRf	Color spots were seen by the detector					
	UV_{254} light	UV_{366} light	Anisaldehyde-sulfuric acid reagent	Visible	Sytroborate reagent	Dragendorff's reagent
15	NA	NA	Violet	NA	NA	NA
20	NA	NA	Green	NA	NA	NA
31	NA	Violet	NA	NA	Violet	NA
29	NA	Violet	Violet	NA	NA	NA
43	NA	Violet	NA	NA	Violet	NA
49	NA	NA	Violet	NA	NA	NA
64	NA	Violet	NA	NA	Violet	NA
67	NA	Red	NA	Green	NA	NA
77	Damping	NA	Violet	NA	NA	NA
79	Damping	NA	NA	Yellow	NA	NA
81	Damping	NA	Green	NA	NA	NA
83	Damping	NA	Violet	NA	NA	NA
84	Damping	Red	Violet	Yellow	NA	NA
88	Damping	Red	NA	Green	NA	NA
92	Damping	NA	Violet	NA	NA	NA
94	Damping	Red	NA	Yellow	NA	NA

Note: hRf is the distance of the spot after elution from the initial spot was divided by the elution length and was multiplied by 100, NA is not identified, UV is ultraviolet

Table 3: Total flavonoids content of sample

Identification	Region	Sample form	Mean ± SD (%) (n=5)
Total flavonoid content	Samigaluh	dried leaves	NA
		n-hexane extract	11,543 ± 0,889
	Ambal	dried leaves	NA
		n-hexane extract	9,535 ± 0,331

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