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QUALITY STANDARDIZATION OF BROTOWALI *(Tinospora crispa)* STEM EXTRACT

STANDARDISASI KUALITAS EXSTRAK BROTOWALI

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ABSTRACT

Brotowali (Tinospora crispa) has been traditionally used for the treatment of gout and scientifically reported as analgesic, anti-inflammatory, and antihyperuricemic agents. Tinospora crispa stem is one of herbal medicine material that its quality should be standardized. This study aims to determine the quality parameters of the T. crispa ethanolic extract included specific and non-specific parameters. Brotowali stem were macerated using ethanol 70%, then the non-specific parameters such as the water content, total ash, total contaminant number of bacteria and fungus were determined. The specific parameters including organoleptic properties, water soluble extract, ethanol soluble extract, and the thin layer chromatography (TLC) profile have also been determined. The parameter values were compared to the qualification of traditional medicine from Department of Health (Depkes R.I.). The result showed that T.crispa stem ethanolic extract has the water content was 8.12±0.06% and the total ash was 5.20 ± 0.12%. The microbiology results showed that the total contaminant of bacteria as much as 5 x 10^2 CFU/g and fungus as much as 5 x 10^3 CFU/g. This extract was brown viscous extract, bitter taste and characteristic odor with water soluble fraction was 45.09 ± 0.67% and ethanol soluble fraction was 14.19 ± 0.14%. The TLC profile of ethanolic extract indicates the existence of flavonoids and alkaloids. Total flavonoids of brotowali extract (32.65 ± 0.20%) rutin equivalent.

***Key words**: Tinospora crispa, brotowali, quality standardization, standardized extract*

ABSTRAK

Brotowali (Tinospora crispa) secara tradisional telah digunakan untuk pengobatan asam urat dan secara ilmiah telah dilaporkan sebagai analgesik, antiinflamasi, dan antihiperurisemi. Batang brotowali termasuk salah satu bahan jamu yang perlu dilakukan standardisasi mutu. Penelitian ini bertujuan untuk menetapkan parameter mutu ekstrak etanolik batang brotowali yang meliputi parameter umum dan spesifik. Ekstrak batang brotowali dibuat dengan metode maserasi menggunakan etanol 70% selama 3 x 24 jam. Parameter umum yang ditetapkan meliputi kadar air, kadar abu total, angka lempeng total, dan angka kapang, sedangkan parameter spesifik seperti organoleptik, kadar sari larut air dan etanol serta profil kromatografi lapis tipis juga ditentukan. Nilai parameter yang diperoleh dibandingkan dengan pedoman standardisasi mutu ekstrak tumbuhan obat. Hasil penelitian menunjukkan bahwa ekstrak memiliki kadar air sebesar 8,12±0,06% dan kadar abu total 5,20±0,12%, sedangkan angka lempeng total 5x10^2 CFU/g dan angka kapang 5x10^3 CFU/g. Ekstrak etanolik batang brotowali memiliki karakteristik berupa ekstrak kental berwarna coklat tua, berasa pahit dan berbau khas dengan kadar sari larut air sebesar 45,09±0,67% dan kadar sari larut dalam etanol sebesar 14,19±0,14%. Selain itu, profil kromatografi lapis tipis ekstrak etanolik menunjukkan adanya senyawa alkaloid dan flavonoid. Ekstrak ini memiliki kandungan total flavonoid sebesar 3,71±0,05% setara dengan rutin.

INTRODUCTION

Indonesia is a second largest biodiversity country that provides many traditional medicines for various diseases. Over 30,000 species of plants and more than 1,000 species of medicinal plants grow in Indonesia have been used in traditional medicine industries. The number of these industries especially home scale (IKOT) increased significantly from 907 in 2002 to 1,413 in 2010 (Wahyuningsih, 2006; Dewoto, 2007). Most of traditional medicine products were prepared in the form of extract. The kinds of extract were viscous extract, dry extract, and liquid extract that

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produced according to the active constituent and the dosage forms, such as capsule, tablet, liquid, pill, and etc. The extract should be standardized to ensure the quality and safety (Hariyati, 2005).

Brotowali (*T. crispa*) is well known as a bitter medicinal plant but it has various efficacy and has been empirically used to treat rheumatism, gout, bruise, and fever, also to stimulate appetite (Dalimartha, 2008). Chemical compounds of brotowali were reported as columbine, tinocrisposide, quaternary alkaloids, saponins, tannins, polyphenols, glycosides, and flavonoids (Sudarsono *et al.*, 2006; Handayani, 2010). The antioxidant activity of brotowali stem according to the method used by Irianti *et al.* (2011). The others studies also showed that *T. crispa* stem extract have analgetic (Sulaiman *et al.*, 2008) and anti-inflammatory effect (Hipol *et al.*, 2012). Coss *et al.* (1998) reported that flavonoids and alkaloids could be correlated to xanthine oxidase inhibitor activity. It is can inhibit production of uric acid, an endogenous substance involved gout disease.

Brotowali has the potential compounds to be developed as a raw material of standardized herbal medicine or phytopharmaca, especially for antihyperuricemia (anti gout). Raw material of extract which will be developed as a standardized herbal medicine needed standardization process. Accordingly, this study about standardization of brotowali ethanolic extract was aimed to determine the quality parameters of raw materials included specific and non-specific parameters.

METHODOLOGY

Materials

Stem of brotowali (*T. crispa*) used in this research was collected from two different areas that are Sumbang, Banyumas and Buayan, Kebumen, Central Java, Indonesia. The plant was authenticated at Laboratory of Plant Taxonomy, Faculty of Biology, Universitas Jenderal Soedirman. The voucher specimen was stored in a herbarium of the Laboratory of Pharmaceutical Biology, Universitas Jenderal Soedirman. The chemicals included ethanol 70%, TLC plate silica gel 60 F_{254} (Merck, Germany), Dragendorff; citroboric reagent, rutin, Nutrient Agar/NA (Merck, Germany) and Potato Dextrose Agar/PDA (Merck, Germany).

Preparation and extraction of sample

Stem of brotowali was selected from Sumbang district, Banyumas regency, Central Java, Indonesia. It was throughly washed, wet sortation, dried, and grinded into powder. One kilogram sample were extracted by maceration using ethanol 70% (in a 1: 5 ratio) for 24 hours, subsequently filtered. Residue was re-extracted twice with the same method and solvent. Ethanol extract were concentrated using rotary vacuum evaporator at 80°C and followed by using waterbath.

Determination of non-specific parameters of extract

Physical evaluation of extract was conducted on water content and total ash value by gravimetric method based on the Indonesian Herbal Pharmacopeae (Ministry of Health, 2012). While the contaminants of total bacteria and total fungus were determined by total plate count method with three times of replication (Department of Health, 2000).

Determination of specific parameters of extract

The specific parameters included organoleptic, water soluble extract, ethanol soluble extract, the phytochemical properties, and total flavonoid content. The organoleptic of brotowali extract include colour, odor, flavour and the consistency. Determination of water soluble extract and ethanol soluble extract was conducted based on the Indonesian Herbal Pharmacopeae (Ministry of Health, 2012). The phytochemical of brotowali extract was identified by TLC method. Total flavonoid content was determined based on modified colorimetric method of Chang *et al.* (2002) using rutin as a reference standard.

Data analysis

The data were descriptively analysed according to the guidebook about quality standardization of extract from Department of Health Republic of Indonesia (Depkes R.I, 2000).

RESULTS AND DISCUSSION

In the present study, extraction of one kg *T. crispa* dry stem with 70% ethanol yielded 193.4 g of a viscous ethanolic extract (19.34%) which is more than the rendement of 96% ethanolic extract reported by Irianti *et al.* (2011) only 12.02%. However, this result is less than the rendement of 96% ethanolic extract was 20.25% (Mutiatikum *et al*, 2004). Accordingly, the higher polarity of the solvent, the more yield of extract.

Quality standardization of brotowali extract was determined by non-specific and specific parameters. The water content was measured by gravimetric method, while the microbial contaminations such as total bacteria and fungus number were determined by microbiological testing.

Table I. Non-specific parameters of brotowali ethanolic extract

Parameters	Measured values	Quality standard for extract
Water content	7.8 ± 1.9%	≤ 10%
Total ash	4.75 ± 0.25%	≤ 5%
Total contaminant of bacteria	1 x 10^4 CFU/g	< 10^6 CFU/g
Total contaminant of fungus	0.33 x 10^4 CFU/g	< 10^4 CFU/g

Table II. Percentage of total flavonoid content in ethanolic extract of *T. crispa* stem

Sample concentration[a] (ppm)	Absorbance (n) (λ 415 nm)	Total flavonoid in each sample[b] (ppm)	Total flavonoid content[c] (RE % b/b)
400	0.314	129.0	32.25
	0.321	131.8	32.95
	0.319	131.0	32.75
		Mean ± SEM	32.65 ± 0.20

Explanation : RE = Rutin Equivalent; **Error! Reference source not found.**

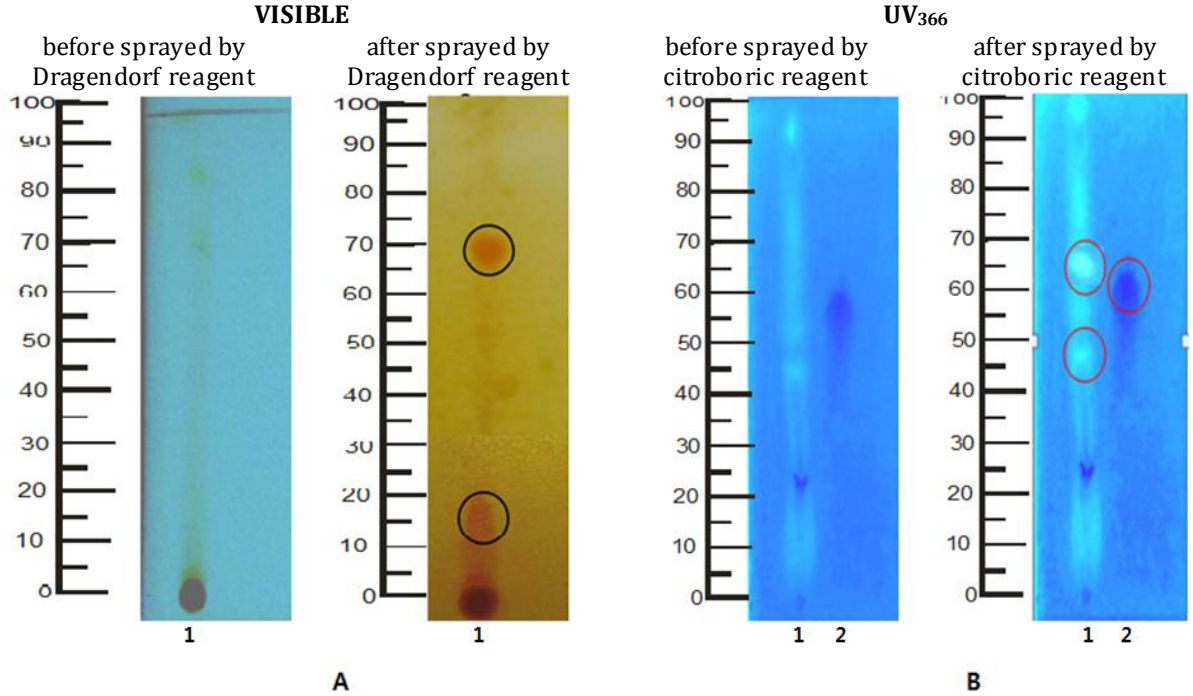

Figure 1. TLC profile of brotowali ethanolic extract (1) and rutin (2) on silica gel 60 F_{254} plate as stationary phase and chloroform: methanol (9:1) (A); BAW (4:1:5) (B) as mobile phase

Several factors determine the microbiological quality of medicinal plants included plant composition (antimicrobial compounds) as intrinsic factors, and also extrinsic factors such as location, post-harvesting, and exogenous microbial contaminations (Kneifel *et al.*, 2002).

Non-specific parameters of brotowali ethanolic extract were shown in table I. The values of each parameter does not exceed the maximum limits or ranges were allowed by the requirements of a guidebook. However, non-specific parameters was determined for brotowali ethanolic extract had been asserted complying with the quality standard. These parameters showed correlated to purity and contamination in that extract (Department of Health, 2000).

Organoleptic examination showed that brotowali ethanolic extract has brown viscous extract, bitter taste, and specific odor. Determination of water soluble fraction (45.087±0.636%) had highest solubility than ethanol soluble fraction (14.194 ± 0.143%).

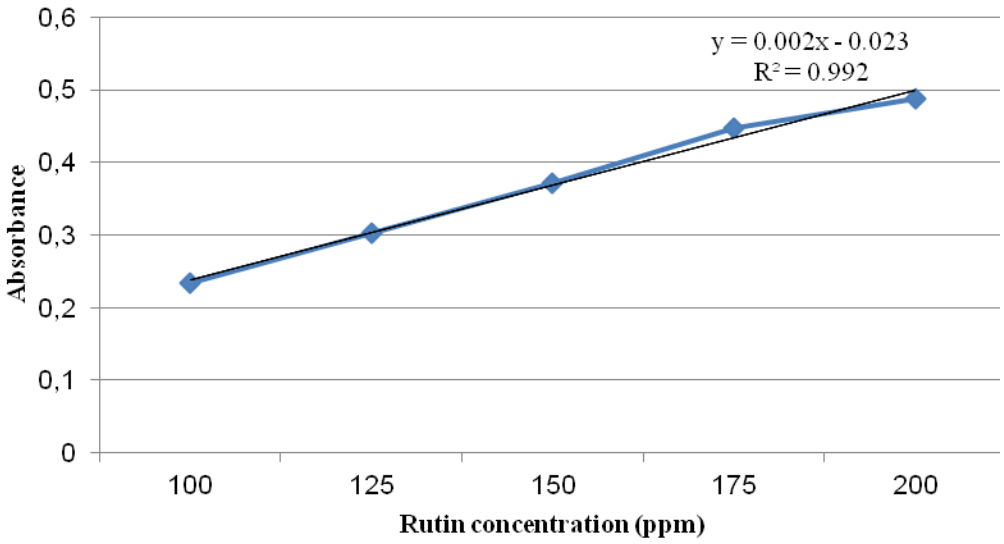

Figure 2. Linear curve of rutin concentration (ppm) versus absorbance for determination of total flavonoid content in *T. crispa* stem ethanolic extract

The result indicated that brotowali ethanolic extract contain mostly polar compounds. Brotowali ethanolic extract exhibited the presence of alkaloids and flavonoids based on the TLC profile (Fig. 1). The positive result of alkaloids was characterized by the appearance of orange color after sprayed by Dragendorff reagent (Fig. 1A) (Harborne, 1996).

Based on the chromatogram on Figure 1B, brotowali ethanolic extract exhibited a clear separation when developed using a mobile phase *n*-buthanol-glacial acetic acid-water/BAW (4:1:5 v/v, upper phase). The TLC profile showed some spots that have hRf values of 10; 44; 52; 62; 70 with brownish yellow fluorescens under UV_{366} after sprayed by citroboric reagent. Moreover, rutin spot as a reference also showed a yellowish brown fluorescens at hRf 60 under UV_{366} after sprayed by this reagent. TLC profile of extract showed the hRf values 5-20 and 70-80 whose yellow fluorescence showed higher intensity of flavonoid detected by UV_{366} (Fig. 1B). The flavonoid type which expected are flavonols without free 5-OH group or flavonols with unsubstituted 5-OH group (Wagner and Bladt, 1996). Flavonoids types contained in brotowali which were previously reported such as O-glycoside flavonoids (apigenin) and flavone glycosides, namely luteolin 4'- methyl ether 7-glycoside, genkwanin 7-glycoside, luteolin 4'-methyl ether 3'-glycosides, diosmetin and genkwanin (Cotelle, 2001), catechin, luteolin, morin, and rutin (Amom *et al.,* 2009).

Total flavonoid content was determined by colorimetric method according to Chang *et al.* (2002) using rutin as a reference standard. Principally, the procedure is related to the formation of complex between flavonoid and $AlCl_3$ that produces a yellow coloured solution. The absorbance was measured by spectrophotometer UV-Vis at maximum wavelength of 415 nm. The absorbances of concentration series of quercetin were plotted to their concentration to yield a linear calibration curve of rutin (y = 0.0026x - 0.023) with coefficient of correlation (r^2) value of 0.992 (Figure 2). In this study, total flavonoid content of brotowali extract was 32.65±0.20%. It means that each 100 g dry weight of ethanolic extract contained total flavonoid equivalent to 33g of rutin.

In the present study, ethanolic extract of brotowali stem showed high content of total flavonoids. Rutin is one kind of flavonoid compounds in brotowali, but there are another flavonoids like apigenin and luteolin. Reportedly, these flavonoids exhibited antihyperuricemic activity due to their potential effect on lowering uric acid level (Chen *et al.*, 2011; de Souza *et al.*, 2012). Brotowali extract whose flavonoids content also responsible for antioxidant (Irianti *et al.*, 2011) and antihyperuricemic activity (Harwoko *et al.*, 2015). Antioxidant activity naturally occurring in plants was expected to limit microbial contaminant (Mansour and Khalil, 2000). However, medicinal plants as material of herbal medicine originally not contaminant-free. Thus several hygiene parameters have to be considered in routine control, especially when the plant would be applied for medical purposes.

CONCLUSION

Ethanolic extract of *T. crispa* stem showed the general standardization parameters i.e the water content 7.8±1.9%; total ash content 4.75±0.25%; total contaminant of bacteria and fungus less than 10^4 CFU/g, respectively. In addition, the specific parameters included water soluble fraction 45.09±0.67% and ethanol soluble fraction 14.19±0.14%, as well as the total flavonoids content was 32.65±0.20% equivalent to rutin.

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