THE STANDARDIZATION OF ETHANOL-WATER EXTRACTS OF *KAYU ANGIN* PLANTS (*Usnea flexuosa*, Tayl) FROM THREE GROWING PLACES

Mumpuni E, Sudjaswadi W, Sureni E. Melania, Layla G.
Faculty of Pharmacy, Pancasila University of Jakarta
esti_mumpuni@yahoo.com

Abstract

Usnea flexuosa, Tayl is a plant that has long been used by Indonesian people to cure the various types of diseases. One of the major obstacles for the development of fitho-pharmaceutical industry in Indonesia is the insufficient raw materials of standardized herbal medicines. In this study, the standardization of ethanol-water extracts of Usnea flexuosa Tayl from three growing places: Mount Slamet, Mount Gede and Mount Lawu, was done. Results of the standardization of extracts show that the hRf of usnic acid was ± 65, drying shrinkage was 6.1-16.8%, ash content was 0.3 to 1.4%, water content was 2.7-9.8%, there was no pesticide residues and heavy metal contamination; the extract contain microorganisms (bacteria) in the allowable amount of <1100 each gram. There was difference in usnic acid content of each sample tested (0.22%-20.9%).

Key words : *Standardization, extract, Usnea flexuosa Tayl*

ISBN : 978-979-18458-5-4

INTRODUCTION

One of the major constraints in the development of fitho-pharmaceutical industry in Indonesia is the insufficient raw materials of herbal medicines, so that the availability of effective and efficient procedures for the standardization of the medicinal plant extracts becomes important, particularly specific standardization directly related to the content of the active substances of medicinal plants. The standardization of medicinal plant extracts is very important, particularly those for pharmaceutical industries using and producing the extracts because preparations that are made and consumed by people must assure its content diversity, safety and efficacy.

Usnea flexuosa Tayl is one of the plants contained in herbal preparations commercially available in Indonesia. The preparations are given in the form of powder in capsule that is greatly recommended to drink for people with sinusitis, bronchitis, pneumonia, influenza and infections due to fungi. *Usnea flexuosa* Tayl also acts as antibiotic. The wood in herbal preparations is made a herbal infusion, by brewing it with boiling water. Also, it is frequently used for the outside drugs such as in fungal infections, vaginal infections, and sores or scabs, in a form of herbal deodorant.

Usnea flexuosa Tayl contains the active compounds of usnic acid, namely, the compounds that are semi-polar in nature, soluble in chloroform, ethyl acetate, acetone, and efficacious as antibacterial, antifungal and antibiotic agents, to treat skin cancer, analgesic, anti-inflammatory, and against thyroid cancer. To obtain a safe, efficacious, and standardized extract, the standardization of three kinds of extracts are carried out using ethanol and water solvents. In this study, to obtain the optimum standardization, *Usnea flexuosa*, Tayl is selected from three growing places because the different conditions of climate and soil to grow will affect the content of usnic acid and other organic compounds in plants studied. Moreover, standardization is also done by a more sensitive method, i.e. the high-performance liquid chromatography (HPLC) to analyse usnic acid content in each extract.

MATERIALS AND METHODS

Materials

Materials used in this study were *Usnea flexuosa* Tayl obtained from three growing different places, i.e. Tawangmangu (Mount Lawu), Baturaden (Mount Slamet) and Cibodas (Mount Gede).

The chemicals used were reference standards of usnic acid, ethanol 96%, aquadest, methanol pro HPLC, distilled water, reference compounds of pesticide, reference standard of heavy metal (Pb), filter paper, Millipore filter paper, silica gel GF 254 plates, microbiological media for the contamination of microbes, volumetric tools, analytical balance glass tools (Sartorius 1872), micro balance (Mettler MT 5), Rotavapor vacuum (Buchi 205), oven (Memmert 854), chromatographic laver, ultrasonic bath (LC 30 H), Absorption Spectrophotometer Atom, Gas Chromatography, High Performance Liquid Chromatography (HPLC) (Shimadzu SPD-20A I-20AD LC), UV-VIS spectrophotometry (Shimadzu UV-1601).

Method

The materials used in this study were collected by plant determination, simplicia preparation, the making of ethanol 50%, 96% extracts and water extract, TLC identification, drying shrinkage determination, dry extract determination, water content determination, ash content determination, pesticide contamination test, microbial contamination test, heavy metal contamination (AAS) test, usnic acid content determination by HPLC method.

RESULTS AND DISCUSSION

Results of the Plant Determination and Extraction

Plant determination was done in Herbarium Bogoriense, Botany Research, Centre for Biology Research, LIPI Bogor. Based the information letter obtained, it can be known that

the plants taken from Tawangmangu (Mount Lawu), Baturaden (Mount Slamet) and Cibodas (Mount Gede) were *Usnea sp* with a type of *Usnea flexuosa* Tayl of *Usneaceae*.

Results of Usnic Acid Identification in TLC

Plate: Silica gel GF 254, eluent: chloroform: methanol (7:3), creepage distance: 20 cm, Detection: 254 nm UV lamp, Reference standards: Usnic Acid.

This TLC identification is an initial investigation to find out the presence of the usnic acid compounds in the extract of *Usnea flexuosa* Tayl. The examination was done qualitatively by comparing the value of hRf from patches of the extract of *Usnea flexuosa* Tayl and that of usnic acid standard compound. After spotting was observed on 254 nm UV lamp, it can be known that in the extracts of ethanol 96%, ethanol-water 50%, and water collected from three growing places with the usnic acid content, hRf was 65.3-66.6.C. Result of the Determination of Extract Drying Shrinkage

Result of the Determination of Extract Drying Shrinkage

Table I. Result of the determination of extract drying shrinkage

Usnea flexuosa, Tayl	Solvent	% Drying Shrinkage
Mount Lawu	Ethanol 96%	8,06 %
	Ethanol-water 50%	15,44 %
	Water	16,54 %
Mount Slamet	Ethanol 96%	6,10 %
	Ethanol-water 50%	14,36 %
	Water	15,73 %
Mount Gede	Ethanol 96%	9,03 %
	Ethanol-water 50%	15,94 %
	Water	16,78 %

$$\% \text{ Drying Shrinkage} = \frac{[(Bo + Be) - Bo] - [Bi - Bo]}{[(Bo + Be) - (Bo)]} x100$$

Drug Extract Ratio (DER)

Table II. Results of the determination of Drug Extract Ratio (DER

Extract of Kayu Angin		DER_{ratio}
Cibodas (Mount Gede)	Ethanol 96%	29 : 1
	Ethanol 50%	36 : 1
	Water	45 : 1
Tawangmangu (Mount Lawu)	Ethanol 96%	25 : 1
	Ethanol 50%	38 : 1
	Water	52 : 1
Baturaden (Mount Slamet)	Ethanol 96%	31 : 1
	Ethanol 50%	24 : 1
	Water	54 : 1

Based on results of the extractions of *Usnea flexuosa* Tayl from Cibodas (Mount Gede) and Tawangmangu (Mount Lawu), the best solvent for use was ethanol 96%, while those from Baturaden (Mount Samet) the best solvent for use was ethanol 50% with values of DER ratio of 29:1, 25:1, and 24:1, respectively. It means that every 29 g, 25 g and 24 g, simplicia could produces 1 g of extract, respectively, so that the extraction by the solvent produces more extracts

Water Content

Results of the determination of water content from each extract could be seen in the following table :

Table III. Results of the determination of water content

Extract of Kayu Angin		Mean ±SD
Cibodas (Mount Gede)	Ethanol 96%	3,874 ± 0,712
	Ethanol 50%	7,545 ± 0,744
	Water	9,600 ± 0,220
Tawangmangu (Mount Lawu)	Ethanol 96%	3,098 ± 0,421
	Ethanol 50%	7,917 ± 0,336
	Water	8,791 ± 0,344
Baturaden (Mount Slamet)	Ethanol 96%	2,992 ± 0,299
	Ethanol 50%	6,301 ± 0,817
	Water	8,571 ± 0,211

From the table, it can be seen that the extract with smallest water content was the extract of ethanol 96%. Therefore, it can be concluded that all the extracts met the standard requirements of extract with the value of water content was less than 10%.

Results of the ash content determination

Table IV. Result of the ash content determination

Extract of Kayu Angin		Ash Content (%)
Cibodas (Mount Gede)	Ethanol 96 %	1,1695 ± 0,0782
	Ethanol 50 %	1,3234 ± 0,0259
	Water	1,3915 ± 0,0225
Tawangmangu (Mount Lawu)	Ethanol 96 %	0,6549 ± 0,0234
	Ethanol 50 %	0,9711 ± 0,0195
	Water	1,0494 ± 0,0477
Baturaden (Mount Slamet)	Ethanol 96 %	0,4402 ± 0,0969
	Ethanol 50 %	0,9285 ± 0,0183
	Water	1,1065 ± 0,0101

From the above data, the largest ash content was produced by the extract of *Usnea flexuosa* Tayl from Cibodas (Mount Gede) with the three solvents used, i.e. ethanol 96%, ethanol 50%, and water.

Pesticide residues

a. Result of the identification of pesticide in organoclorus class DDT (hRf of 60.6-65) by TLC shows that no spot was available. It means that all the extracts made were free of pesticide.

b. Result of the identification of organophosphorus pesticide/Malathion (hRf of 53.8-63.12) by TLC show that no spot was available. It means that all the extracts were free of pesticide.

The extract of *Usnea flexuosa* Tayl could be stated as free of organoclorus and organophosphorus pesticides that can be seen from result of the identification of extract compared with the reference standards of pesticides. It is also related to the growing places at altitudes of more than 800 meters above sea level, causing the low probability of plants to contact with pesticides usually used in agriculture.

Heavy metal contamination

From data on the Atomic Absorption spectrophotometry, it can be known that each of the extracts had absorption with very little Pb light, i.e. under LOD (0.0874 ppm) and LOQ (0.2915 ppm). Therefore, it can be concluded that the compounds did not contain the heavy metal contamination of Pb.

Microbial contamination

Based on tesult of the Most Probable Number (MPN) values of coliform, it can be seen in the following table:

Table V. Result of the Most Probable Number (MPN) values of coliform

Extract of Kayu Angin		MPN Values of Coliform
Cibodas (Mount Gede)	Ethanol 96 %	3
	Ethanol 50 %	6,2
	Water	15
Tawangmangu (Mount Lawu)	Ethanol 96 %	3
	Ethanol 50 %	6,1
	Water	9,2
Baturaden (Mount Slamet)	Ethanol 96 %	3
	Ethanol 50 %	6,1
	Water	16

From the data above, it can be seen that the extract of water from each site contained more micro-organisms (bacteria). It was because water is a good media for the growth of micro-organisms (bacteria), but each of these extracts contained the micro-organisms (bacteria) in the allowable amount of <1100 in each gram.

Result of the mold/yeast test can be seen in the following table:

ISBN : 978-979-18458-5-4

Table VI. Result of the mold/yeast test.

Extract of Kayu Angin		Total Mold/Yeast
Cibodas (Mount Gede)	Ethanol 96 %	< 10
	Ethanol 50 %	1×10^{-4}
	Water	$1{,}55 \times 10^{-3}$
Tawangmangu (Mount Lawu)	Ethanol 96 %	< 10
	Ethanol 50 %	1×10^{-6}
	Water	$5{,}5 \times 10^{-4}$
Baturaden (Mount Slamet)	Ethanol 96 %	< 10
	Ethanol 50 %	1×10^{-5}
	Water	$22{,}75 \times 10^{-1}$

From the data above, the extract with the solvent of water contained more the colony of mold/yeast.

The determination of Usnic Acid Content (HPLC)

From the table VII, it can be seen that the highest content of usnic acid are contained in the extract of ethanol 96% from Cibodas (Mount Gede), namely, 20.9973%.

Table VII. Result of the determination of usnic acid content

Usnea flexuosa, Tayl	Solvent	Mean (%)	SB	KV (%)
Mount Lawu	Ethanol 96%	18,5870	0,0867	0,5716
	Ethanol-water 50%	0,5455	0,0027	0,6102
	Water	0,3071	0,0032	1,2931
Mount Slamet	Ethanol 96%	17,3386	0,1110	0,7846
	Ethanol-water 50%	0,4000	0,0061	1,8867
	Water	0,2208	0,0010	0,6030
Mount Gede	Ethanol 96%	20,9973	0,1728	1,0084
	Ethanol-water 50%	0,7807	0,0103	1,6193
	Water	0,3704	0,0031	1,0400

In view of the growing places, it was because Cibodas has rainfall with a higher intensity, lower temperature, and high humidity if compared with two other places. It strongly supports the growth of *Usnea flexuosa* Tayl. However, after the ANOVA statistical analysis was done, the result indicates there was no significant difference between usnic acid content and the growing places. Meanwhile, in view of the three solvents used, the highest content of usnic acid was available in the solvent of ethanol 96% and lowest one was in the solvent of water. It was affected by the solubility of usnic acid, i.e. soluble in ethanol 96% and poorly soluble in water. Therefore, it can be concluded that the use of *Usnea flexuosa* Tayl as the traditional ingredients in herbal preparation consumed by many people in the form of herbal decoction is safe for consumption because the content of usnic acid was very small. However, the high content of usnic acid is toxic in nature.

CONCLUSION

Result of the analysis indicates that the hRf of usnic acid was ±65, drying shrinkage was 6.1-16.8%, ash content was 0.3-1.4%, water content was 2.7-9.8%, there was no pesticide residue, and there was no heavy metal contamination, the extracts contained micro-organisms (bacteria) in the allowable amount of <1100 of each gram. The extract with water solvent contained more mold/yeast colonies and there was difference in acid usnat content from each sample tested (0.22%-20,9%). In the ethanol 96% extract, usnic acid content was 17.34% from Mount Slamet (Baturaden), 18.59% from Mount Lawu (Tawangmangu) and 20.99% from Mount Gede (Cibodas). In the ethanol-water 50% extract, it was 0.40% from Mount Slamet (Baturaden), 0.55% from Mount Lawu (Tawangmangu) and 0.78% from Mount Gede (Cibodas). In the water extract, it was 0.22% from Mount Slamet (Baturaden), 0.31% from Mount Lawu (Tawangmangu) and 0.37% from Mount Gede (Cibodas).

ACKNOWLEDGMENTS

The study was funded by the Directorate General of Higher Education, the Department of National Education, the Agreement Letter of Research Grant No. 279/SP2H/PP/DP2M/IV/2010 dated on April 12, 2010.

REFERENCES

Departemen Kesehatan Republik Indonesia, *Materia Medika Indonesia,* Jilid II. 1978. Hal. 96-100

E. Hale, M. *How to Know The Lichen,* 2nd edition. USA: Wm. C. Brown Company Publisher; 1989.

Gembong Tjitrosoepomo. *Taksonomi Tumbuhan Schizophyta, Thallophyta, Bryophyta, Pteridophyta.* Yogyakarta: Gajah Mada University Press; 1994. hal. 173-7.

Heyne K. *Tumbuhan Berguna Indonesia Jilid I.* Diterjemahkan oleh Badan Litbang Kehutanan Jakarta, Jakarta: Yayasan Sarana Wana Jaya; 1987. Hal. 76-7.

Johnny R.P, Soerahso, Yuli Widiyastuti. *Ragam Penggunaan Kayu Angin Sebagai Obat Luar dan Dalam Obat Tradisional.* Warta Tumbuhan Obat Indonesia. 1992 ; 1(4) : Hal. 24-6.

Priyono Suwarso. *Lichen Tanaman Suku Rendah Berpotensi Sebagai Sumber Senyawa Kimia Bahan Alam Baru*. Yogyakarta: Gajah Mada University Press; 1991. hal. 103-4.

Subagus Wahyuono, Sudarsono. *Kayu angin (Usnea sp.). Tanaman Obat Indonesia Sebagai Sumber Produksi Asam Usnat dan Potensi Asam tersebut Sebagai Antibakteri*. Laporan Penelitian Yogyakarta : Fakultas Farmasi Universitas Gajah Mada. 1994. Hal. 1-10.

Underwood A.L. JR Day R.A. *Analisis Kimia Kuantitatif Edisi VI.* Jakarta: Erlangga; 2001. Hal 499-500, 553-66.

ISBN : 978-979-18458-5-4

United Stated Pharmacopoeia Convention. *The United Stated Pharmacopoeia 28. The National Formulary 23.* Rockville: United Stated Pharmacopoeia Convention Inc; 2005. Hal. 178, 2748-5