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DETERMINATION OF STANDARD PARAMETER OF ETHANOL EXTRACT OF ROSEMARY HERB

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ABSTRACT

Rosemary (Rosmarinus officinalis L.) is A kind of potpourri who have aromatic odour and very prospectif to be developed because showing an high antioxidant activities. In order to get the information about standardization parameter on ethanol extract of rosemary herb,a research was done about specific and non specific parameters, also chemical content of rosemary herb from Lembang and Yogyakarta. The result of this research indicated that rosemary herb has 25.81-30.60% extractive matter; 36.00-48.33 % v/b water content; 1.17-4.83% ash content; 3.67-4.33% soluble ash content; 2.00-2.67% insoluble acid ash content; 1.67-2.30% water soluble extractive matter; 6.83-13.83% ethanol soluble extractive matter; 42-44.38% loss on drying; 0.936-0.977 specific grafity. Phytochemical screening showed the presence of flavonoids, monoterpenoids, sesquiterpenoids, steroids and quinone.

Key words : Standardization, extract, Rosmarinus officinalis L.

INTRODUCTION

Rosemary (*Rosmarinus officinalis* L.) is one of aromatic plant that begin to be developed in Indonesia. The plant has purple flowers, and live on dry air area and cool conditions. If the land was flooded, rosemary would be easily killed and attacked by pests (Christman, 1999).

Chemical contents of rosemary in bark was flavonoid (diosmetol, methoxylated flavon C-6 and or C-7), fenolamic acid (2-3%), kafeic derivate (kafeic acid, chlorogenic acid, and rosmarinic acid), tricyclic diterpene (carnosic,acid carnosol), rosmanol (more than 4%), triterpene (ursolic acid and oleanolic acid). In the leaves was Volatile oil 1- 25%, therein 1,8%, cineole 30%, champore 15-255, borneol 16-20%, bornyl asetat 7% and essential oil (Armengol, 1995). In recently, rosemary uses as repellent, potpourri, aromatherapy, expectorance, antibacterial agents, hair tonic, anti wringkle, analgetic, nerve relaxans, rheumatoid, influenza, and spices plant (Ratih, 2007).

Extract that used as raw materials and medicinal products was obtained from crude drug. The chemical compound of crude drug can not always be guarantee stable or constant. This is because of the diversity of seedlings, growing place, climate, conditions at harvest, and post-harvest processing, including drying and storage stages. Fulfillment of quality standards can not be separated from quality control, meaning that a standardized process may ensure a standardized product (Hafid, 2001). So this research was done to provide standardized value or database of rosemary herb.

METHODS AND RESULTS

Material:

Rosemary herb *(Rosmarinus officinalis* L.*)* obtained from Lembang and Yogyakarta. Alcohol 70% (BRATACO-Indonesia), chloroform (BRATACO-Indonesia), toluene (BRATACO-Indonesia), Mayer, Dragendorff, Lieberman-Burchard, vanillin-sulfuric acid

10% , chloric acid 2N, potasium hydroxide , ammonia, ferric(III)chloride, gelatin 1%, amil alcohol, and Mg.

Tools:
Distillation (azeotrop distillation), cruz porcelain, blender, digital camera (SONY, T100), UV lamp (CAMAG), maserator, microscope (OLYMPUS), oven (MEMMERT), *Rotavapor* (BIBBY,RE200B), analytical balance (NAGATA), and common equipment in laboratory.

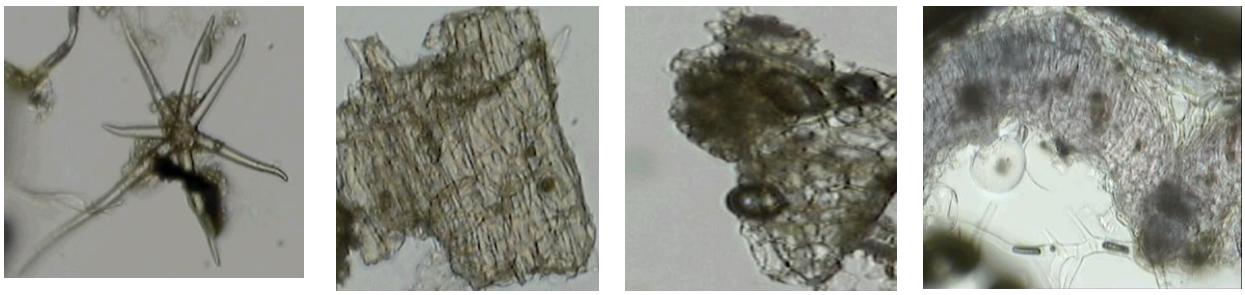
Sample preparation:
Rosemary herbs was collected from Lembang and Yogyakarta, Indonesia. The Material was sorted, cleaned, air-dried then finally grinded. The plant was identified in Herbarium Jatinangor, Plant Taxonomy Laboratory of Biology Department, Faculty of Mathematic and Natural Sciences Universitas Padjadjaran. The result showed that materials was belong to Lamiaceae, species *Rosmarinus officinalis* L.

Extractions:
Powdered crude drug was extracted by maceration using 70% ethanol (3x24 hour). Filtrate was condensed first using vacuum rotavapor then water bath. Extractive matter was 30,6% of rosemary extract from Lembang (EL) and 25,81% of extract rosemary from Yogyakarta (EY).

Determination of paramater quality
Extract were determined by strandard procedure [Depkes RI, 1987; 1995], parameters was covered water-soluble extractive matters, ethanol-soluble extractive matters, loss on drying, total ash content, water-soluble ash content, acid-insoluble ash content, and water content. Specifically to crude drug followed by macroscopical and microscopical analysis.

Macroscopical analysis showed that rosemary herb was green,the leaves are shaped like a needle 2,5 cm of lenght, acuted tip, and has aromatic odour. By microscopical analysis fragments identified were oil glands, schlerenchime, upper epidermis, and filament hair. The result of determinations are available in Figure 1, Figure 2 and Table 1.

Figure 1 Macroscopic of rosemary herb



a b c d

Figure 2 Microscopic of rosemary herb

Note: a. filament hair; b. schlerenchime; c. parenchime; d. upper epidermis

Table 1 Characteristic of Quality

Parameter	Content of Extract (% w/v)	
	From Lembang	From Yogyakarta
Specific grafity	0,977	0,936
Water-soluble extractive matters	1-3	1-3
Ethanol-soluble extractive matters	13,5-14,5	6,5-7,0
Loss on drying	43,75-45,00	40-44
Total ash content	4,52-4,53	1,00-1,10
Acid-insoluble ash content	2,30-2,50	1,90-2,30
Water content (% v/w)	30-40	45-50

Chemical Content Investigation

Chemical content investigation was done by phytochemical screening and thin layer chromatography (TLC). Phytochemical screening were determined from extract from Lembang and Yogyakarta that covered alkaloid, flavonoid, tannin, polyphenol, saponin, Monoterpen & Seskuiterpen, triterpenoid, steroid, and quinone, using standard procedures (Depkes RI, 1995). The results are available in Table 2.

Ethanol extract of rosemary herb from Lembang and Yogyakarta were investigated by TLC using precoated silica gel GF254 and (7:3) mixture solvent of n-hexane:ethyl acetate. Chromatogram was observed visually, under UV 254 nm, 366 nm and after sprayed by 10% of sulfuric acid (V-S), revealed the profile or numerous chemical contents of rosemary herb marked by every spots. The results showed in Table 3 and Table 4.

Table 2 Result of Phytochemical Screening

Secondary metabolite	Rosemary Extract
Alkaloid	-
Flavonoid	+
Tannin	-
Polyphenol	-
Saponin	-
Monoterpen & Seskuiterpen	+
Triterpenoid	-
Steroid	+
Quinone	+

Note: (+) = Present (-) = Absent

Table 3 TLC Result of Extract from Lembang

Spot	Rf	Visual	UV		V-S
			254nm	366 nm	
1	0,786	-	-	-	-
2	0,688	Green	Purple	-	-
3	0,686	Brown	Purple	Red	Dark blue
4	0,678	-	-	-	-
5	0,675	Brown	Purple	Red	Green
6	0,600	Brown	Purple	Red	-
7	0,550	-	-	-	Green
8	0,500	-	-	-	-
9	0,440	Brown	Purple	-	Dark blue
10	0,400	-	-	-	-
11	0,313	Brown	Purple	-	-
12	0,300	-	-	Red	Dark blue
13	0,275	Grey	Purple	Red	-
14	0,250	-	-	-	Dark blue
15	0,125	-	-	-	Yellow

Table 4 TLC Result of Extract from Yogyakarta

Spot	Rf	Visual	UV		V-S
			254nm	366 nm	
1	0,786	Green	Purple	Red	-
2	0,688	-	-	-	-
3	0,686	Brown	-	Red	Dark Blue
4	0,678	-	Purple	-	-
5	0,675	Brown	Purple	-	-
6	0,600	-	Purple	Red	Green
7	0,550	Brown	-	-	Green
8	0,500	Brown	Purple	-	-
9	0,440	-	-	Red	Dark Blue
10	0,400	Brown	Purple	-	-
11	0,313	-	-	Red	-
12	0,300	Grey	-	-	Dark Blue
13	0,275	-	Purple	Red	-
14	0,250	-	-	-	Dark Blue
15	0,125	-	-	-	yellow

DISCUSSIONS

Maceration is extraction process at room temperature, this used to avoid the destruction of thermolabile compounds contained in *R.officinalis* L. A 70% ethanol used as solvent for maceration due to a very good solubility, polarity, and volatility.

The result of phytochemical screening indicated that extract of rosemary herb from Lembang and Yogyakarta has flavonoid, monoterpen & seskuiterpen, steroid and quinone. In chromatogram of TLC, showed that selected mobile phase was a good solvent for separation process of chemical contents of extracts. Every spots with their Rf values represent that both extract has similar of chemical contents.

Data showed that value of parameter standardized of extract from Lembang and Yogyakarta was not exactly the same. This may appear because of different growth area. Until now, there is no data showed the standardized extract of rosemary. So this value was provided to basic guide line for further reseach.

CONCLUSIONS

Determination of standard parameter of ethanol extract of rosemary herb was done by determination of paramater quality and chemical content investigation. This research obtained range value to each parameters and basic profile of chemical contents from rosemary herb.The data may resulted in standardized extract of Rosemary herb. The result of this research indicate that rosemary herb has 25.81-30.60% extractive matter; 36.00-48.33 % v/b water content; 1.17-4.83% ash content; 3.67-4.33% soluble ash content; 2.00-2.67% insoluble acid ash content; 1.67-2.30% water soluble extractive matter; 6.83-13.83% ethanol soluble extractive matter; 42-44.38% loss on drying; 0.936-0.977 specific grafity. Phytochemical screening showed the presence of flavonoids, monoterpenoids, sesquiterpenoids, steroids and quinone.

Further research should be done on determining the parameters specified from extract of different regions or climate conditions, so that the data obtained will be more accurate.

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