OPTIMIZATION OF ETHANOL-WATER COMPOSITION AS EXTRACTION SOLVENT IN PRODUCING SAMBUNG NYAWA (Gynura procumbens (Lour.) Merr.) LEAVES DRY EXTRACT

ABSTRACT

Sambung Nyawa leaves (Gynura procumbens (Lour.) Merr has been widely used as herbal medicine which requires a quality improvement of the dry extract for industrial production. Extraction solvent optimization is one key factor which determines the quality. This research aims was to find out the optimal ethanol-water composition as extraction solvent by using Simplex Lattice Design (SLD) method of which the total phenolics, total flavonoids and DPPH radical scavenging activity were used as quality parameters. Dried leaves as raw materials were pulverized and screened at Mesh 60, macerated (1:5) with ethanol-water composition as 1:0; 0.7:0.3; and 0.5:0.5v/v, shaked for 24h, filtered. The procedure was repeated twice. Filtrates were collected of which lactose were added (1:2)w/w and spray dried at 100°C for 30min. Dried extracts yielded were evaluated the quality by using SLD method of which the total phenolics, total flavonoids as well as DPPH radical scavenging activity were used as parameters. Optimal SLD response was revealed at the ethanol:water composition of 0.66:0.34-0.75:0.25v/v (Rtotal>0.9). No significant difference of the above mentioned parameters between the values resulted from the experiment and SLD formula. Correlation analyses of total phenolics and total flavonoids towards DPPH radical scavenging activity were found as 95.29% and 1.25%, respectively.

Keywords: Gynura procumbens, dry extract, total phenolics, total flavonoids, DPPH radical scavenging

INTRODUCTION

Modern production of herbal medicine requires qualified raw material which is usually in form of dry extract. Choosing the right extraction solvent can optimize the extracted target constituents. Simplex Lattice Design (SLD) is one of the optimization method which can be applied in determining the most optimum solvent composition.

Sambung Nyawa (Gynura Procumbens (Lour.) Merr., Asteraceae) leaves has been widely...
used as herbal medicine for treatment of degenerative diseases i.e. hyperglykemia (Algariri et al., 2013), hyperlipidemia (Zhang and Tan, 2000) and hypertension (Hoe et al., 2011). Puangprongpritag et al. (2010) and Akowuah and collaborators, (2002) reported that compounds responsible for the activities were flavonoid. The ability of flavonoid and phenolics as antioxidant have been widely accepted, mostly due to the compounds have the ability to scavenge the free radicals (Giorgio, 2000). Therefore, those parameters were used as parameters in determining the quality of the dry extract.

The total phenolics and total flavonoid contents of the raw materials have been determined in our preliminary experiments to be 0.1201-0.003% GAE w/w and 0.3675-0.003 %QE w/w, respectively, of which the kaempferol content was detected as 0.12% w/w.(Hertiani and Effendi, 2015, unpublished data). Spray drying was used to reduce the exipient content, while lactose was used as exipient based on our preliminary experiment. Spray drying principle based on suspending exipient in filtrate of which the suspension can act as droplet spray drying process. The suspension can convert the suspension into dry extract in one step drying process (Patel et al., 2009). Spray drying method produces material particle size below 100μM which may water solubility. The disadvantage of the method is heat used for drying process may destroy heat sensitive constituents (Barbosa-Canovas, 2005).

**METHODOLOGY**

**Material and Equipments**

Raw materials used was fresh leaves of *G. procumbens* which were collected from 7th row from the bud of around 1 year old plants, having 10-12cm length. The sampling location was at Gligir plot, Mangunan village, Imogiri District, Yogyakarta, Indonesia and collected in February 2015.

Ethyl alcohol 96% and distilled water (technical grades) were used as solvent extraction; lactose (PT. Brataco, Indonesia); DPPH (2,2-diphenyl-1-picrylhydrazyl), quercetin, kaempferol, kaempferol-7-O-rutinoside, silica gel 60 F254, FeCl3, AlCl3, CH3COONa, Folin Ciocalteau reagent, NaOH (Merck, Germany).

Electric balance (Mettler teledo, 0.01-210g, eluent chamber, ultraviolet 254 and 366 lamps, spectrophotometer UV-VIS (Spectronic® 20 Genesys™), spray dryer (Labplant UK Ltd., Hunmanby, UK), delivery pipettes (Gilson pipetmen) volume 20-200μL ans 100-1000μL.

**Methods**

Fresh leaves were washed with flowing water, decanted and left air dried for 6h, followed by oven drying at 50°C for 6h. Dried leaves were pulverized and screened with Mesh 60.

**Extract production**

Dried pulverized samples (50.0g) were put into three different 500mL Erlenmeyer flasks, macerated with 250mL extraction solvent (table I). Our preliminary research used 5 different solvent composition i.e., the ethanol:water (1:0); (0.7:0.3); (0.5:0.5); (0.3:0.7) v/v and 100% distilled water, however, the higher concentration of water (up to 30%) used caused gelling form of which the separation with the residue was not possible. Therefore, in the SLD calculation, the ethanol:water (1:0) v/v was considered as A = 1 and the ethanol:water (0.5:0.5) v/v was considered as B = 1.

After 24h shaking, the macerates were filtered. Residues were remacerated twice and the filtrates were combined later on. Filtrates, each in amount of 500mL was added with 25g lactose and dried by using spray dryer for 30min, of which the drying process specifications were as described in table I.

**Determination of Dry extracts specification**

The dried extracts were assayed for the parameters as follow

Physical parameters: Lost of Drying (%), physical appearance. Chemical parameters: TLC profiling; total flavonoid and total phenolics were measured according to Farmakope Herbal Indonesia Suplemen I (Ministry of Health RI, 2010), while the DPPH-radical scavenging activity was measured by method as described by Kikuzaki et al. (2002). All measurements were done in triplicate.

**Simplex Lattice Design Calculation**

Target responses for each parameters, i.e.,

Lost on drying LOD (R1); total flavonoid (R2); total phenolics (R3) and DPPH radical scavenging activity (R4) were determined as well as the degree of interest. Each resulted response was included in the SLD formula as follows:

\[ Y = a [A] + b [B] + ab [A] [B] \ldots \ldots (1) \]

\[ Y = \text{measured response}; A = \text{portion of ethanol}; B = \text{portion of water}; a = \text{ethanol coefficient}; b = \text{water coefficient}; ab = \text{ethanol-water coefficient} \]

The resulted SLD formula is used to predict the optimized solvent mixture.
That having the optimum $R_{\text{total}}$ (formula 2) is estimated to be the optimized mixture by calculation and should be verified by an experiment. $R_{\text{total}} > 0.9$ is considered as the optimized response area.

$$R_{\text{total}} = R_1 + R_2 + R_3 + R_4, \ldots \ldots \ldots (2)$$

$R_{1,2,3,4}$ are responses from each parameter of which each has been given certain weight according to the degree of interest/importance. DPPH radical scavenging activity ($IC_{50}$) was given weight 0.4 due to main parameter; total phenolic contents was given 0.3 considering its higher content in the leaves, while total flavonoid content was given 0.2 due to flavonoid being part of phenolics and has less content in the leaves. LOD was given the smallest weight (0.1). The total amount is 1. Considering that the each response has different metric system, a normalization should be applied as derived by formula (3):
OPTIMIZATION OF ETHANOL-WATER COMPOSITION

\[ N = \frac{X - X_{\text{min}}}{X_{\text{max}} - X_{\text{min}}} \]..............(3)

Note:
X: experimental response; X_{\text{min}}: minimum value of desired response; X_{\text{max}}: maximum value of desired response; Furthermore, R_{\text{total}} was calculated as follows:

\[ R_{\text{total}} = (\text{weight} \times N_{\text{LOD}}) + (\text{weight} \times N_{\text{total phenolics}}) + (\text{weight} \times N_{\text{total flavonoid}}) + (\text{weight} \times N_{\text{DPPH-radical scavenging activity}}) \]..............(4)

Verification of SLD formula

The dry extract was produced by using the mixture of ethanol-water in the area resulting optimized response. All parameters were tested accordingly, and the difference of the response with the expected response resulted from calculation of the SLD formula was statistically analyzed by one sample t-test (significance level at 0.05).

RESULTS AND DISCUSSION

Raw materials were collected from the University Farming at Mangunan, Yogyakarta, Indonesia based on our previous study of high quality of dried materials after comparison to other locations in Yogyakarta and surrounding (Figure 1). Collection was taken at once to limit variation due to different sampling condition.

Figure 1. Sambung Nyawa leaves

Extract ratio describes how much extract yield from a certain method of extraction. It is not necessarily define the extract quality directly, especially in the case of different methods of extraction were applied. In this research, the same total amount of extraction solvent as well as lactose as expipient were used. Table II describes that the higher composition of water caused higher extract ratio, means that more raw materials were needed to gain the same weight of dry extract.

Only slight difference in organoleptic observation found as seen in table III. TLC profile of the resulted dry extracts as seen in figure 2 showed similar patterns. However table V showed significant difference in all parameters. Nevertheless, the LOD was all meet the requirement as dry extract (<10%). LOD less than 10% results more preserved product as microbes and enzymatic reaction which are responsible for deterioration or chemical changes will be limited (Buckle et al., 1992).

Figure 2. TLC chromatogram of the dry extracts

Note: TLC system: stationary phase silica gel 60 F\(_{254}\) and mobile phase hexane:ethyl acetate:formic acid (6:4:0.1 v/v) Above: Chromatogram before spraying with reagents 1. Ethanol : water (0.5:0.5); 2. Ethanol : water (0.7:0.3); 3. Ethanol : water (1:0); 4. kaempferol 1 mg/mL; 5. quercetin 1 mg/mL; 6. kaempferol-O-rutinoside 1 mg/mL; from left to right: detected in visible, UV 254 nm and UV 366 nm. Below: Chromatogram after spraying A. AlCl\(_3\), detection: visible; B. AlCl\(_3\), UV 366 nm; C. FeCl\(_3\), visible; D. FeCl\(_3\), UV 366 nm

The highest contents of total flavonoid and total phenolics were observed in the dry extract of ethanol-water (0.7:0.3) v/v, which also showed the highest activity as DPPH radical scavenger (Table IV). However, the correlation analyses of total phenolics and total flavonoids vs DPPH-radical scavenging activity exhibited that the total phenolics correlates to the activity but not to the total flavonoid. The total phenolics contributed 95.29% to the DPPH radical scavenging activity, while the total flavonoid contribution was only 1.25%. It is interesting to note that only 10.55% of flavonoid contributed to the total phenolics...
content, suggests the higher proportion of phenolics other than flavonoid contained in the extracts. Tan et al. (2013) reported that flavonol and phenolic acid as the active constituents of Sambung Nyawa, which has been identified as kaempferol, quercetin, kaempferol-3-O-β-D-glucopyranoside, kaempferol-3-O-rutinoside, rutin, chlorogenic acid and 3,5-dicaffeoylquininate methyl ester. Antioxidant activity of phenolics mostly depends on oxidation-reduction reaction of which the compounds play role as reductor, hydrogen donor and radical scavenger (Kakhonen et al., 1999). The fact that the extract resulted from the ethanol-water 1:0 v/v had moderate flavonoid level (in comparison to the other two extracts) but the least of phenolics content and the lowest DPPH radical scavenging activity, suggested that the phenolics had more contribution to the activity. It is expected that more aglycons were extracted by increasing portion of ethanol. Those aglycons such as myricetin and kaempferol are actually strong antioxidant flavonoids. As reported by Kaewsuejan et al. (2015) myricetin and kaempferol showed higher antioxidant activity in comparison to the phenolic acids of G. procumbens. However, TLC profile showed similar pattern suggested similar type of compound extracted, but in different proportion (Figure 2). Higher deviation of the phenolics contents amongst samples underlined the bigger contribution of this particular group of compounds to the DPPH radical scavenging activity of the resulted extract.

Optimal SLD response ($R_{total}>0.9$) was found at the ethanol:water composition at 0.66:0.34 – 0.75:0.25 v/v (Figure 3). No significant difference of the above mentioned parameters between the values resulted from the experiment and SLD formula, tested at a mixture of ethanol:water 0.71:0.29 v/v.

Optimal response ($R_{total}>0.9$) was observed at the ethanol-water composition as follows (0.66:0.34) - (0.75:0.25) (v/v) of which the most optimum was observed at composition ethanol-water 0.70:0.30 v/v (R$_{total}$=0.9182). SLD formula was verified to be applicable. Correlation analyses showed total phenol, showed that 95.29% of DPPH radical scavenging activity was caused by total phenolics content, however, total flavonoid contribution was only 1.25%.

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Figure 3. SLD diagram representing ethanol composition vs responses of chosen parameters
Note: SLD formula of LOD = 0.4900A + 5.3912B + 2.8843AB; SLD formula of total flavonoid content = 0.151A + 0.124B + 0.2051AB; SLD formula of total phenolic content = 0.142A + 0.701B + 2.094AB; SLD formula of DPPH radical scavenging activity = 35.85A + 7.68B – 57.74B; A= ethanol composition and B = water composition