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

OPTIMASI KOMPOSISI ETANOL-AIR SEBAGAI CAIRAN PENYARI DALAM PRODUKSI EKSTRAK KERING DAUN SAMBUNG NYAWA (*Gynura procumbens* (Lour.) Merr.)

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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 compositition 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 (R_{total}>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

ABSTRAK

Daun Sambung Nyawa (Gynura Procumbens (Lour.) Merr telah dipergunakan secara luas sebagai obat herbal sehingga membutuhkan peningkatan kualitas pembuatan ekstrak kering untuk industri. Optimasi pelarut ekstraksi adalah salah satu faktor kunci yang menentukan kualitas. Penelitian ini bertujuan untuk mengetahui komposisi etanol-air untuk pelarut ekstraksi yang optimal menggunakan metode Simplex Lattice Design (SLD) dengan parameter kualitas berupa flavonoid total dan fenol total serta aktivitas penangkalan radikal DPPH. Daun kering diserbukkan dan diayak pada Mesh 60, dimaserasi (1:5) dengan variasi komposisi etanol-air sebagai berikut 1:0; 0,7:0,3; and 0,5:0,5v/v, digojok selama 24jam kemudian disaring. Proses diulangi 2 kali. Filtrat dikumpulkan dan ditambah laktosa (1:2)b/b kemudian dilakukan pengeringan menggunakan spray dryer pada 100°C selama 30 menit. Ekstrak kering yang dihasilkan dievaluasi kualitasnya menggunakan metode SLD dengan fenol total, flavonoid total dan aktivitas penangkal radikal DPPH sebagai parameter. Respon optimal SLD response diketahui pada komposisi etanol-air 0,66:0,34-0,75:0,25 v/v (R_{total}>0,9). Tidak terdapat perbedaan signifikan antara data yang diperoleh dari eksperimen dan dari hasil perhitungan menggunakan formula SLD. Analisis korelasi antara fenol total dan flavonoid total, masing-masing terhadap aktivitas penangkal radikal bebas adalah berturut-turut sebesar 95,29% dan1,25%.
Kata kunci: Gynura procumbens, ekstrak kering, fenol total, flavonoid total, radikal penangkal DPPH

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

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used as herbal medicine for treatment of degenerative diseases i.e. hyperglikemia (Algariri *et al.,* 2013), hyperlipidemia (Zhang and Tan, 2000) and hypertension (Hoe *et al.,* 2011). Puangpronpritag *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-*O*-rutinoside, silica gel 60 F_{254} $FeCl_3$, $AlCl_3$, CH_3COONa, Folin Ciocalteau reagent, NaOH (Merck, Germany),.

Electric balance (Mettler teledo, 0.01-210g, eluent chamber, ultraviolet 254 and 366 lamps, spectrophotometer UV-VIS (Spectronic^R 20 Genesys^{TM}), 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 Supplemen 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 (R_1); total flavonoid (R_2); total phenolics (R_3) and DPPH radical scavenging activity (R_4) were determined as well as the degree of interest. Each resulted response was included in the SLD formula as follows:

$Y = a [A] + b [B] + ab [A] [B]$…….(**1**)

Y= measured response; A= portion of ethanol; B=portion of water; a= ethanol coefficient; b=water coefficient, ab= ethanol-water coefficient (A+B = 1). The resulted SLD formula is used to predict the optimized solvent mixture.

Table I. System used in *G. procumbens* dry extract production

Sample amount (g)	Ethanol (A)	Water (B)	Macerate (mL)	Lactose (g)	Inlet temperature	Outlet temperature	Pump speed
50.0	1	0	500	25	100	50	5
50.0	0.7	0.3	500	25	100	50	4
50.0	0.5	0.5	500	25	100	50	3

Table II. Extract ratios

Composition		Dry leaves (g)	Dry extract (g)			Extract ratio
Ethanol	Water		1	2	3	
1	0	50.00	8.22	9.04	10.75	5:1
0.7	0.3	50.10	5.38	6.31	7.34	7:1
0.5	0.5	50.00	3.12	3.56	3.52	14:1

Tabel III. Extract organoleptics

Composition		Form	Color	Smell	Taste
Etanol	Water				
1	0	Powder	Dark green	Aromatic	Bitter
0.7	0.3	Powder	Green brownish	Aromatic	Bitter
0.5	0.5	Powder	Light brown	Aromatic	Bitter

Tabel IV. Responses of dry extracts according to chosen parameters

Solvent Composition		LOD (%)	Total Flavonoid (% w/w QE)	Total Phenolics (% w/w GAE)	IC_{50} of DPPH radical scavenging activity
Ethanol	Water				
1	0	0.49±0.10	0.151±0.0049	0.142±0.005	35.826±0.810
0.7	0.3	2.63±0.26	0.186±0.0042	1.023±0.010	4.744±0.111
0.5	0.5	5.38±0.07	0.124±0.0021	0.781±0.014	6.490±0.525

Note: LOD: Loss of Drying (%) ± SD (n=3); QE: Quercetin Equivalent ±SD (n=3); GAE: Gallic Acid Equivalent ±SD (n=3); IC_{50} (mg/mL) ± SD (n=3).

Table V. Statistical analyses comparing the responses resulting from experimental and SLD formula calculation (Ethanol:water = 0.71:0.29 v/v)

Parameters	Calculation based results	Experimental based results	Difference based on statistical analyses
LOD (%)	2.63	1.99±0.493	Not significant
Total flavonoid (% QE)	0.19	0.18±0.004	Not significant
Total phenolics (% GAE)	1.02	1.07±0.021	Not significant
IC_{50} of DPPH radical scavenging activity (mg/mL)	4.74	4.91±0.164	Not significant

That having the optimum R_{total} (formula 2) is estimated to be the optimized mixture by calculation and should be verified by an experiment. R_{total} > 0.9 is considered as the optimized response area.

$R_{total} = R_1 + R_2 + R_3 + R_4$.....(**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):

$$N = \frac{X - Xmin}{Xmax - Xmin} \text{.....(3)}$$

Note :

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

R_{total} = (weight x N_{LOD}) + (weight x $N_{total\ phenolics}$) + (weight x $N_{total\ flavonoid}$) + (weight x $N_{DPPH\text{-}radical\ scavenging\ activity}$).....(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 exipient 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).

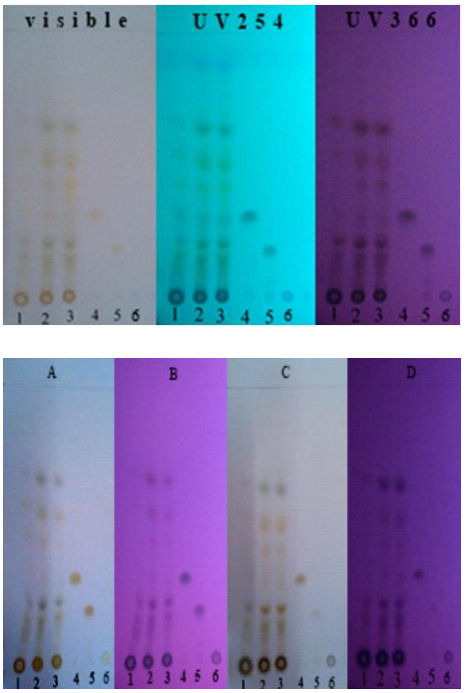

Figure 2. TLC chromatogram of the dry extracts

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 andUV 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-dicaffeoilquinate 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 (Kahkonen *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 Kaewseejan *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.

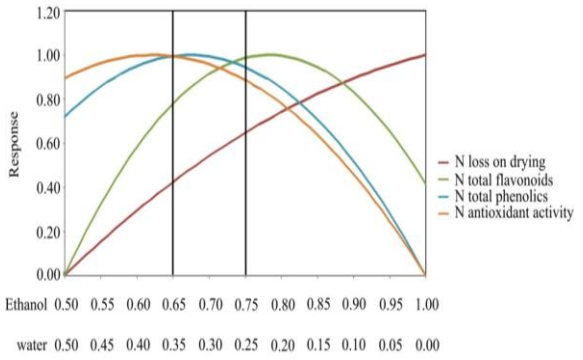

Figure 3. SLD diagram representing ethanol composition vs responses of choosen parameters

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 + 0.781B + 2.094AB; SLD formula of DPPH radical scavenging activity = 35.85A + 7.68B – 57.74B; A= ethanol composition and B = water composition

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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