Research Article

Determination of quality standards parameters of kemangi (*Ocimum basilicum* L.) leaves extract from three locations

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ABSTRACT

Objective: The objective of this study was to determine the parameters of the quality standard of kemangi (basil, *Ocimum basilicum* L.) leaves originating from three regions of Indonesia. **Materials and Methods:** The research method included collecting basil from three regions, namely Bandung, Cianjur, and Solo. The basil sample was then extracted using the maceration method followed by the phytochemical screening test. Determination of extract quality standardization parameters in accordance with the reference book parameters of Indonesian extract quality standards which included determination of moisture content, determination of ash content, determination of water-soluble ash content, determination of acid insoluble ash content, specific gravity, determination of water-soluble extractive content, and determination of ethanol soluble extractive content. Determination of essential oil contents using the thin-layer chromatography and gas chromatography/mass spectrometry (GC-MS) methods. **Results:** The standardization parameter value of Bandung basil leaf extract was 9.5%, 4.68%, 82.86%, 1.47%, 0.82, 4.33%, and 64.33%, Cianjur basil leaf extract was 9.83%, 7.78%, 93.37%, 2.08%, 0.85, 22.33%, and 53.00%, and Solo basil leaf extract was 13.67%, 9.98%, 92.59%, 5.95%, 0.87, 41.67%, and 49.67%. Phytochemical screening of basil leaf extract showed that the metabolites contained were flavonoids, tannins, steroids, and saponins. The essential oil content found in basil leaves was 0.132%. GC-MS results in the three samples showed the linalool content in Bandung basil leaf extract was 2.62%, Cianjur 19.85%, and Solo 27.80%. **Conclusion:** It was found that there were differences in the quality standard parameters of each basil including the content of secondary metabolites and essential oils. Each region has a different standard of quality which cannot be generalized which was thought to be caused by differences in soil nutrient origin of plants.

KEY WORDS: Essential oil, Gas chromatography-mass spectroscopy, Kemangi, Maceration, *Ocimum basilicum*

INTRODUCTION

A tradition of using medicinal plants and traditional medicine is part of Indonesia's biodiversity. As a very potential wealth, the continuity of the tradition of drug use and traditional treatment of the ancestral heritage must be maintained, preserved, and developed for national health development.[1] The Kemangi (Indonesian), basil plant (*Ocimum basilicum* L.) belongs to the family Lamiaceae, a plant that is green to brownish green has a distinctive aroma and tastes rather spicy. Heyne[2] describes the plant morphology and ecology as follows: Upright shrubs, often branching out, 0.3–1.5 m high, spread throughout Java from the lowlands to approximately 600 m above sea level. These plants have different designations in each region in Indonesia: Klampes, Surawung (Sunda), Kemangen (Java), Kemanghi (Madura), Uku-uku (Nusa Tenggara), Balakana (Manado), Basil basil, Ruku-ruku, and Lufe-lufe (Maluku).[2]

The chemical content of basil leaves is essential oils, tannins, flavonoids, and steroids. In the world market, basil oil (basil oil) is known as sweet basil oil. Essential oils contained in basil herbs around 0.04–0.7% with the main components, namely methyl cavikol, citral, geraniol, ocimene, 1.8 sincole, eucalyptol, limonene, eugenol, furfural, methyl cinnamate, and farnesol.[3] Basil plants have many uses. All parts of this plant that can be used both in fresh and dried conditions are used as rheumatoid medicine, cough medicine, respiratory problems, cancer drugs, etc. In addition, essential oils contained in basil leaves can be used as

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antiseptics, antispasmodics, carminatives, expectorants, antipyretics, and antidepressants.[4,5]

On the basis of the many uses of basil plants, the idea arises to develop basil plants into standardized herbs. Therefore, it needs to be examined standardization of extracts and chemical content; this needs to be known to obtain complete information that is very useful in the development and use of basil plants so that they can be used for the utilization of quality, efficacious, and safe Original Indonesian Medicine. In this study, standardization of basil leaf extract which consisted of non-specific parameters, specific parameters, essential oil content, and the content of chemical compounds of basil leaf extract was reported guided by parameters of Indonesian extract quality standards and Materia Medica Indonesia.[6,7]

MATERIALS AND METHODS

Material

The research material used was basil (*O. basilicum* L.) leaves obtained from Bandung, Cianjur, and Solo. Mayer reagent, Dragendorff reagent, chloroform, 2N HCl, ammonia, FeCl3, 1% gelatin, amyl alcohol, 10% vanillin in concentrated sulfuric acid, magnesium powder, sulfuric acid in 10% ethanol, Lieberman–Burchard reagent, KOH, and 95% ethanol were used. Unless otherwise stated, all chemicals were analytical grades.

Tools

The tools used in this study are water content determinants, ash content detectors, stirring rods, blenders, cameras, vaporizer plates, Petri dishes, filter funnels, thin-layer chromatography (TLC) equipment (Camag), UV lamps, macerator, oven (Memmert), water bath, Rotary evaporator (IKA), and gas chromatography-mass spectrometry (GC-MS) (Shimadzu GC-17 A; GC/MS. QP5050A).

Research Methods

The method used in this study was carried out through several stages, namely: Collection and processing of materials, determination of plants, maceration extraction, extract phytochemical screening, and determination of the standardized quality of extracts in accordance with the reference book parameter of quality standards for extracts medicinal plants and Materia Medica Indonesia, including: Determination of water content, determination of ash content, determination of water-soluble ash levels, determination of acid insoluble ash content, specific gravity, determination of water-soluble extractives, determination of ethanol soluble extractives, and analysis of extracts of chemical compounds using TLC and GC-MS methods.

RESULTS AND DISCUSSION

Collection and Processing of Materials

Kemangi leaves were collected and then cleaned from the soil, washed using running water and then dried and then sliced or chopped with ± 3 cm long and dried with indirect sunlight for about 1 week. After drying, the sample was ground until smooth, then stored in a clean and closed container.[8,9] The sample was determined at the Laboratory Taxonomy, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, and been confirmed as Kemangi (*O. basilicum* L.).

Extraction

The extraction method used in this study was maceration. The selection of this method was done to prevent the occurrence of damage to the thermolabile chemical compounds contained in the ganda leaves leaf. The maceration was carried out by soaking the sample in the macerator then leaving it for 24 h at room temperature with stirring occasionally.[10,11] In making the extract, it was used 95% ethanol solvent, because 95% ethanol had a small water content, thus minimizing the possibility of an enzymatic reaction in simplicia. The extract was rotary evaporated followed by drying on the water bath. It was found that vicious extract yields from Bandung, Cianjur, and Solo were, respectively, 8.50, 11.25, and 11.82 w/w.

Three regions of the origin of basil were chosen based on different heights above the sea surface. The city of Bandung is at an altitude of ± 768 m above sea level,[12] whereas Cianjur[13] and Solo[14] were at ± 1450 m and ± 98 m.

Phytochemical Screening

Tests were carried out on ganda leaf extract which includes: Alkaloid, flavonoid, saponin, triterpenoid, steroid, quinone, and monoterpenoids/sesquiterpenes. The test was guided by the Farnsworth method.[15,16] Table 1 showed the results of phytochemical screening of kemangi leaves.

Table 1: Phytochemical screening of basil leaves extract

Secondary metabolites	Basil leaves extract of		
	Bandung	Cianjur	Solo
Alkaloids	+	+	+
Flavonoids	+	+	+
Polyphenols	−	−	−
Tannin	+	+	+
Quinone	−	−	−
Saponins	+	+	+
Steroids	+	+	+
Triterpenoids	+	+	+
Monoterpenoids/ sesquiterpenoids	−	−	−

+: Detected, −: Not detected

This results from basil cultivated in Iran as reported by Khair-ul-Bariyah *et al*.[17] contained terpenoids, alkaloids, flavonoids, tannins, saponin glycosides, and ascorbic acid in their plant. Fathiazad *et al*.[18] mentioned that their phytochemical screening indicated the presence of phenolic compounds (5.36%) and flavonoids (1.86%), rosmarinic acid was the principal phenolic compound with a 15.74% existence. Phytochemical analysis of the aqueous leaf extract revealed that the concentration of saponin and alkaloids was high, flavonoids, terpenes, and steroids were present in medium quantity, while traces of tannins and carbohydrates were also present in the basil aqueous extract.[19]

Determination of Non-specific Parameters

The results of non-specific parameter determination of basil leaf extract can be seen as follows.

Determination of water content

The results of determining the water content of basil leaf extract can be seen in Table 2.

Determination of ash content

The results of determining the ash content of basil leaf extract can be seen in Table 3.

Determination of water-soluble ash content

The results of determining the water-soluble ash content of basil leaf extract can be seen in Table 4.

Determination of acid insoluble ash content

The results of determining the acid insoluble ash content of basil leaf extract can be seen in Table 5.

Determination of density

It was found that the specific gravity of basil leaf extract of Bandung, Cianjur, and Solo was 0.82, 0.85, and 0.87, respectively.

Specific Parameter Determination of Basil Leaves Extract

Organoleptic

The three types of basil leaves have similar characteristics, which have a thick green color, a slightly bitter taste and have a distinctive odor.

Determination of specific parameter of basil leaves extractives

Table 6 shows the specific parameter of basil leaves extractives.

Table 2: The water content of basil leaves extracts

Area of origin	Extract amount (g)	Water volume (mL)	Water content (%)	Average water content (%)
Bandung	20	2.2	11.0	9.5
	20	1.9	9.5	
	20	1.6	8.0	
Cianjur	20	1.5	7.5	9.83
	20	2.0	10.0	
	20	2.4	12.0	
Solo	20	2.8	14.0	13.67
	20	2.4	12.0	
	20	3.0	15.0	

Table 3: Ash content of basil leaves extracts

Area of origin	Empty crucible weight (g)	Sample ash+Crucible weight (g)	Ash content (%)	Average ash content (%)
Bandung	21.37	21.46	4.43	4.68
	15.52	15.63	5.07	
	19.36	19.46	4.54	
Cianjur	17.36	17.52	7.44	7.78
	21.82	21.99	7.73	
	20.52	20.69	8.17	
Solo	20.25	20.47	10.89	9.98
	19.75	19.96	9.72	
	21.34	21.54	9.34	

Table 4: Water-soluble ash content of basil leaves extracts

Area of origin	Sample weight (g)	Water-soluble ash weight (g)	Water-soluble ash content (%)	Average water-soluble ash content (%)
Bandung	2.2	1.8	81.81	82.86
	2.4	2.06	85.83	
	2.1	1.70	80.95	
Cianjur	2.4	2.20	91.67	93.37
	2.3	2.16	93.91	
	2.2	2.08	94.54	
Solo	2.5	2.29	91.60	92.59
	2.3	2.15	93.47	
	2.2	2.04	92.72	

Table 5: Acid-insoluble ash content of basil leaves extracts

Area of origin	Sample weight (g)	Acid-insoluble ash weight (g)	Acid-insoluble ash content (%)	Average acid insoluble ash content (%)
Bandung	2.1	0.03	1.42	1.47
	2.2	0.04	1.81	
	2.0	0.02	1.20	
Cianjur	2.3	0.05	2.17	2.08
	2.3	0.05	2.26	
	2.2	0.04	1.81	
Solo	2.4	0.14	5.83	5.95
	2.3	0.13	5.65	
	2.2	0.14	6.36	

Table 6: Results of determination of a specific parameter of basil leaves extractives

Determination	Results (% w/w)		
	Bandung	Cianjur	Solo
Water-soluble extractives	4.33	22.33	41.67
Acid-soluble extractives	64.33	53.00	49.67
Essential oil content	0.16	0.16	0.15

Table 7: Thin-layer chromatography results of basil leaves extract using vanillin-H_2SO_4 as spot viewer

Spots	Rf	Color
1	0.187	Light green
2	0.212	Light green
3	0.237	Light green
4	0.262	Light green
5	0.275	Light green
6	0.300	Light green
7	0.462	Dark green
8	0.475	Dark green
9	0.487	Dark green
10	0.600	Light green
11	0.612	Light green
12	0.625	Light green
13	0.755	Dark green
14	0.775	Dark green
15	0.787	Dark green

TLC Results

TLC uses the n-hexane:ethyl acetate (7:3) developer was carried out on basil leaf extract. The results of TLC using n-hexane:ethyl acetate (7:3) developers have presented in Table 7. This table represented spots of the three basil leaves.

GC-MS Results

GS-MS used ethanol solvent, injected in micro size at an initial temperature of 600°C for 2–10 min until the final temperature reached 3200°C for 7 min. Table 8 shows compounds content from volatile oil of basil leaves extract whereas Figure 1 shows an example of GC-MS chromatogram of basil leaves extract.

Equality index was applied [Figure 1 and Table 8]. From Table 8, the Bandung basil extract, the compound with the largest amount of content was Linolenic (11.95%), whereas in the extracts of the Cianjur and Solo regions the compound with the largest amount of content were linalool (19.85% and 27.80%). The most influential factor in getting good results in GC-MS was the temperature regulation of the instrument. The GC-MS results from the three extract samples showed that not all of the compounds contained were the same in each region. The levels of the compounds contained also vary in each region. This was thought influenced by several factors, one of which was climate, soil, and degree of acidity. Joshi[20] in the study of chemical composition and antimicrobial activity of the essential oil of *O. basilicum* L. (sweet basil) from Western Ghats of Northwest Karnataka, India reported 19 compounds of their basil essential oil. Murárikova *et al.*[21] found in their Czech basil species study that the particular compounds were eucalyptol, fenchone, fenchyl acetate, β-linalool, α-bergamotene, caryophylene, isocaryophylene, 4-carvomenthol, β-farnesene, estragole, β-cubebene, α-bulnesene, γ-cadinene, eugenol, and γ-cadinene. Turkey's basil was identified having major compounds in the volatile oil of *O. minimum* were geranyl acetate (69.48%), terpinen-4-ol (2.35%), and octan-3-yl-acetate (0.72%). The essential oil of *O. basilicum* was characterized by its high content of methyl eugenol (78.02%), whereas the most important essential oil constituent of *O. minimum* was geranyl acetate (69.48%). Zeković[22] from Serbia reported that the dominant compounds detected in all investigated samples (EO obtained by hydrodistillation and different SFE extracts) were linalool, as the major compound of basil EO (content from 10.14% to 49.79%, w/w), eugenol (from 3.74% to 9.78%), and δ-cardinene (from 3.94% to 8.07%).

CONCLUSION

From the results of the quality standardization study of basil leaf extract that had been done, it could be concluded that the phytochemical screening results showed that the metabolites contained were flavonoids, tannins, steroids, and saponins. The results of parameter determination for water content, total ash content, water-soluble ash content, acid-insoluble ash content, specific gravity, water-soluble extract content, and soluble ethanol extract content for basil leaf extract were different for each region originating from basil. The GC-MS results in the three samples showed that the linalool content in Bandung basil leaf extract was 2.62%, Cianjur 19.85%, and Solo 27.80%.

Table 8: GC-MS results of basil leaf extracts

Compounds	Concentration (%)		
	Bandung	Cianjur	Solo
Ocimene	1.75		
Neophytadiene	2.39		
n-Butylphthalate	0.88		
2-Hexadecanoic acid	8.76	10.83	1.91
6-Octadecenoic acid	1.12	1.55	
Urs-12- en- 28- ol	1.46	1.76	1.11
2,3-Beta quinoline	0.37		
Oxirane	4.56		
Linolenin	11.95	3.40	
Linalool	2.62	19.85	27.80
Tetradecanal	1.03		
1,2-Benzenedicarboxylic acid	2.17	1.25	0.89
Farnesol	2.99	2.75	1.89
Trans caryophyllene		1.31	0.54
Trans-alpha-bergamotene		1.14	
Alpha-humulene		3.57	1.87
Caryophyllene		0.98	
Neophytadiene		2.37	4.39
12,15- Octadecanoic acid		0.89	2.35
Pentadecanoic acid		0.92	0.75
Phytol		11.22	7.71
Octadecanal		2.07	
Hexadecanal		0.75	
4h-pyran-4-one			2.08
5-Hydroxymethyl furfural			9.61
Geraniol			1.03
Thujyl alcohol primer			0.85
Pentadecanal			1.14
2-Hexadecanoic-1-ol			1.65
Total	13 compounds	17 compounds	17 compounds

GC-MS: Gas-chromatography/mass spectrometry

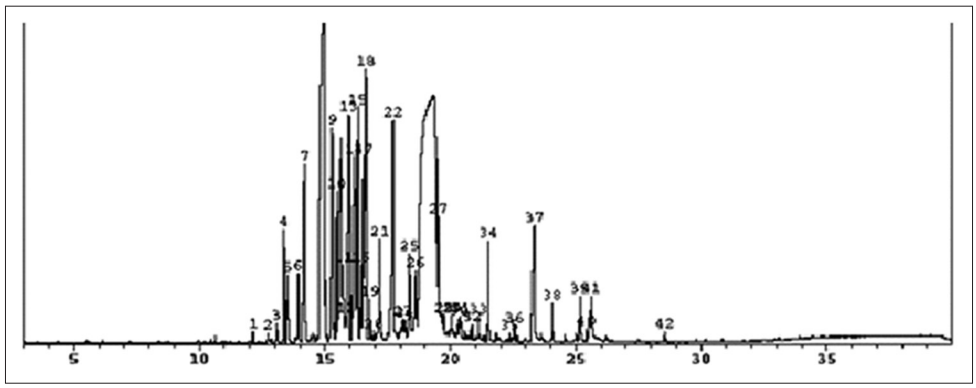

Figure 1: Gas-chromatography/mass spectrometry chromatogram of basil extract

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REFERENCES

1. Sukasediati N, dan Loupaty AM. The Main Thought Towards the Integration of Indonesian Native Medicines/Natural Medicines into Health Services (Indonesian: Pokok Pemikiran Menuju Integrasi Obat Asli/Obat Bahan Alam Indonesia ke Dalam Pelayanan Kesehatan). Jakarta: Badan Pengawas Obat dan Makanan Deputi Bidang Pengawasan Obat Tradisional, Kosmetik dan Produk Komplemen; 2006. p. 1-2.
2. Heyne K. Useful Plants of Indonesia III, Print I (Indonesian: Tumbuhan Berguna Indonesia III, Cetakan I). Badan Penelitian dan Pengembangan Kehutanan, Yayasan Sarana Wahana Jaya. Jakarta: Departemen Kehutanan; 1987. p. 1704-5.
3. Budi M, Hadipoentyanti E, Sukamto S. Plasma nutfah nabati. J Balai Penelitian Tanaman Rempah Obat 2004;16:52-6.
4. Agusta I. Minyak Atsiri Tumbuhan Tropika Indonesia. Bandung: Penerbit ITB; 2000.
5. Gill K. Why Everyone Should Eat Basil; 2018. Available from: https://www.medicalnewstoday.com/articles/266425.php. [Last accessed on 2018 Jul 12].
6. Depkes RI. Materia Medika Indonesia. Vol. 6. Jakarta: Departemen Kesehatan Republik Indonesia; 1989. p. 182.
7. Depkes RI. General Standard Parameters of Medicinal Plant Extracts (Indonesian: Parameter Standar umum Ekstrak Tumbuhan Obat). Jakarta: Direktorat Jenderal Pengawasan Obat dan Makanan; 2000. p. 9-37.
8. Sirait M. How to make Simplicia (Indonesian: Cara Pembuatan Simplisia). Jakarta: Direktorat Jenderal Pengawasan Obat dan Makana; 1985. p. 1-3, 105-7.
9. Mustarichie R, Ramdhani D, Iskandar Y. Characteristics and alopecia activity of pakis gajah (*Angiopteris evecta* (G.Forst)

Hoffm.) growing in galunggung mountainside, West Java. Asian J Pharm Clin Res 2017;10:337-40.
10. Handa SS. An Overview of Extraction Techniques for Medicinal and Aromatic Plants. Trieste, Italy: International Centre for Science and High Technology; 2008. p. 21-54.
11. Seindel V. Natural Product Isolation; 2005. Available from: http://www.springer.com/978-1-58829-447-0. [Last accessed on 2018 Jul 12].
12. Badan Pusat Statistik. Area Height Above Sea Level (Indonesian: Tinggi Wilayah di Atas Permukaan Laut); 2017. Available from: https://www.bandungkota.bps.go.id/statictable/2018/07/11/157/tinggi-wilayah-di-atas-permukaan-laut-dpl-menurut-kecamatan-di-kota-bandung-2017.html. [Last accessed on 2018 Jul 15].
13. Badan Pusat Statistik. Area Height Above Sea Level (Indonesian: Tinggi Wilayah di Atas Permukaan Laut); 2017. Available from: https://www.cianjurkab.bps.go.id/./ketinggian-wilayah-kabupaten-cianjur. [Last accessed on 2018 Jul 15].
14. Badan Pusat Statistik. Area Height Above Sea Level (Indonesian: Tinggi Wilayah di Atas Permukaan Laut): 2015-2017. Available from: https://www.jateng.bps.go.id/statictable/2017/10/26/1513/tinggi-wilayah-di-atas-permukaan-laut-dpl-menurut-kabupaten-kota-di-provinsi-jawa-tengah-2015-2017.html. [Last accessed on 2018 Jul 15].
15. Farnsworth NR. Biological and phytochemical screening of plants. J Pharm Sci 1996;55:225-76.
16. Rahmawati RP, Mustarichie R. Determination of anti-alopecia compounds from water fraction of the *Angiopteris evecta* (G. Forst.) Hoffm. L roots. Drug Invent Today 2018;10:1869-81.
17. Khair-ul-Bariyah S, Ahmed ID, Ikram M. *Ocimum basilicum*: A review on phytochemical and pharmacological studies. Pak J Chem 2012;2:78-85.
18. Fathiazad F, Matlobi A, Khorrami A, Hamedeyazdan S, Soraya H, Hammami M, *et al.* Phytochemical screening and evaluation of cardioprotective activity of ethanolic extract of *Ocimum basilicum* L. (basil) against isoproterenol induced myocardial infarction in rats. Daru 2012;20:87.
19. Sanni S, Onyeyili PA, Sanni FS. Phytochemical analysis, elemental determination and some *in vitro* antibacterial activity of *Ocimum basilicum* L. Leaf extracts. Res J Phytochem 2008;2:77-83.
20. Joshi RK. Chemical composition and antimicrobial activity of the essential oil of *Ocimum basilicum* L. (sweet basil) from western ghats of North West Karnataka, India. Anc Sci Life 2014;33:151-6.
21. Murárikova A, Ťažký A, Neugebauerová J, Planková A, Jampílek J, Mučaji P, *et al.* Characterization of essential oil composition in different basil species and pot cultures by a GC-MS method. Molecules 2017;22:E1221.
22. Zeković ZP, Filip SD, Vidović SS, Adamović DS, Elgndi AM. Basil (*Ocimum basilicum* L.) essential oil and extracts obtained by supercritical fluid extraction. Acta Period Technol 2015;46:259-69.

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