



Improving Simplicia of Kerangas Forest by Minimizing Microbial Content Under Ultraviolet Radiation Treatment

Kissinger¹ · Huldani Huldani² · Amar V. Nasrulloh³

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Abstract Kerangas forest is known as a source of traditional medicinal plants and thus potentially the source of simplicia. Medicinal plant preparations of simplicia are usually dried to meet water content standards (<10%). However, in some cases the drying process carried out still does not eliminate the microbial content in the simplicia. This study aims to analyze the phytochemical compounds contained in three types of medicinal plant simplicia and the total microbial content by treatment with ultraviolet (UV) irradiation. The types of simplicia used were *Baeckea frutescens* leaves, *Shorea balangeran* bark and *Vitex pubescens* leaves (obtained from the kerangas forest). Before testing the total

microbial content, simplicia first received UV irradiation. The first simplicia sample was not irradiated, while the next simplicia sample was given UV irradiation with a power of 60 watts and an irradiation time of 1, 2 and 4 h. The microbial content test used the total plate count method with the pour plate analysis technique. *S. balangeran* is unexposed species to microbes, even without UV irradiation. Radiation with a UV lamp for 1 h with a lamp power of 60 watts and a distance of the sample with a lamp of 30 cm effectively eliminates microbes in *B. frutescens* simplicia. Radiation for 4 h with a UV lamp of 60 watts and a sample distance of 30 cm was only able to eliminate the microbial content in *V. pubescens* simplicia. The results of this study indicate that *S. balangeran* has high potential to be developed as an antibacterial.

Significance's statement The use of medicinal plant simplicia must meet various requirements regarding the quality of the simplicia. The often-used traditional drying methods are by air drying or drying under the sunlight. Modern drying methods usually use tools such as ovens, microwaves and freeze dryers. Different drying methods produce different quality simplicia. The three medicinal plants simplicia from kerangas forest which are *Baeckea frutescens* leaves, *Shorea balangeran* bark and *Vitex pubescens* leaves contain phytochemicals including flavonoids, phenolhydroquinone, triterpenoids, and tannins. UV irradiation has various effects on the total microbial content of each medicinal plant simplicia. The present study provides data that *S. balangeran* simplicia did not contain microbial colonies either without irradiation or with UV irradiation. As such, *S. balangeran* simplicia is potentially high to be developing as an antimicrobial or antibacterial.

Keywords Kerangas forest · Microbes · Medicinal plants · Simplicia · Ultraviolet

Introduction

Kerangas forest is known as a source of natural ingredients of medicinal properties. Previous study found that out of 40 plant types, there are 35 plants can be used as medicinal ingredients [1]. Utilization of medicinal plants is conducted

✉ Kissinger
kissinger@ulm.ac.id

Huldani Huldani
huldani@ulm.ac.id

Amar V. Nasrulloh
amarvijai01@ulm.ac.id

¹ Faculty of Forestry, University of Lambung Mangkurat, Banjarbaru, Indonesia

² Department of Physiology, Faculty of Medicine, University of Lambung Mangkurat, Banjarbaru, Indonesia

³ Physics Study Program, University of Lambung Mangkurat, Banjarbaru, Indonesia

by boiling or brewing hot water [2]. With regard to utilization for a long time, it is necessary to prepare plant materials in the form of *simplicia*. *Simplicia* is a dried natural material that is used for treatment and has not processed unless otherwise stated and the drying temperature is not more than 60 °C [3]. Plant bioactivity does not only depend on the phytochemical content, but also depends on the quality of the obtained *simplicia*. The *simplicia* quality parameters are including water content and microbial contamination.

The use of medicinal plant *simplicia* must meet various requirements regarding the quality of the *simplicia*. Based on Regulation of the Head of the Food and Drug Supervisory Agency (BPOM) of Republic of Indonesia Number 12 year 2014 concerning Quality Requirements for Traditional Medicine states that one of the requirement as *simplicia* is the number of microbial contamination. *Simplicia* in the form of chopped and powder one of the conditions must have microbial contamination with a Total Plate Count (TPC) value 106. The required water content of *simplicia* is < 10% [3, 4]. Fulfillment of moisture content reaches 10% by a drying process. The process of microbial spoilage and metabolic activity of degrading enzymes is minimized by drying, so that plant material can be stored for a long period of time [5]. The drying process can reduce microbial activity and minimize physical and chemical changes during storage of *simplicia* [6].

The often-used traditional drying methods are by air drying or drying under the sunlight. Modern drying methods usually use tools such as ovens, microwaves and freeze dryers [7]. Different drying methods produce different quality *simplicia*. Drying by an oven at a temperature range of 50–60 °C and a relative humidity of 20–30% produces standardized *Zingiberaceae sp* *simplicia*, but drying with the wind-dry method under the sunlight does not produce lean *simplicia* [8]. The use of sunlight during the drying of *Angelica keiskei* is not recommended since it has a lower total phenolic content and antibacterial activity than in the oven. The problem that occurs is the *simplicia* of medicinal plants produced by local communities generally through the drying process using sunlight. It takes between 7 and 9 days in the drying process by drying in the sun [9]. Meanwhile, *simplicia* with a water content of 10% may still contaminated with microbes. Further treatment needed so that microbial contamination in *simplicia* can be minimized.

The ultraviolet (UV) radiation is one of the methods to control the growth of pathogenic bacteria. Ultraviolet (UV) light is part of the electromagnetic spectrum with wavelengths between 100 and 400 nm [10]. The advantage of UV light is highly effective in killing most pathogenic bacteria such as *E. coli*, *Giardia Lamblia* and *Cristosporidium* [11]. Research conducted with *Bacillus sp* showed that irradiating 38 watts of UV light for 10 and 15 min with a distance of 45 cm on media containing *Bacillus sp* bacteria showed

no colonies growing, while on control media that were not irradiated with UV, colony growth was very high and uncountable [12]. UV irradiation with a lamp power of 60 watts and irradiation for 50 min effectively reduce the total microbe [13].

There are nine native species of medicinal plants that is found in various locations all in kerangas forest, namely *Vitex pubescens*, *Adina minutiflora*, *Shorea balangeran*, *Callophylum lowii*, *Melaleuca cajuputi*, *Cratoxylon arborescens*, *Combretocarpus rotundatus*, *Tristaniopsis obovata* and *Baekkea frutescens* [14]. Three of the nine species of kerangas forest plants are most often-used by local communities in medicine are *B. frutescens*, *S. balangeran*. and *V. pubescens*. Local people generally understand that medicinal plant *simplicia* uses sunlight, so it has the potential to experience microbial contamination. This study aimed to analyze the effect of UV exposure on the microbial content of three species of *simplicia* from drying under direct sunlight kerangas forests.

Material and Methods

The material used in this study was *simplicia* in powder form from the leaves of *B. frutescens* and the bark of *S. balangeran*. The *simplicia* material dried using direct sunlight for 9 days. After the water content < 10% is met, the medicinal plant material *simplicia* is grinded into powder. Meanwhile, *Simplicia* leaves of *V. pubescens* were obtained from *V. pubescens* trees and washed flowing fresh water until dirt or foreign objects are not found on the leaves. After cleaning, the leaves cut into small pieces to speed up the drying process. The leaves are then dried in the sun for 7 days and then grinded into powder [15].

The content of secondary metabolites was identified qualitatively using standardized methods. The test of qualitative phytochemicals was conducted by using color visualization [16]. Alkaloid test: 0.5 g of sample was put into a microplate, then 0.5 mL of 1% HCl was added, and 2–3 drops of Mayor's reagent, Wagner's reagent or Dragendroff's reagent was added. A creamish or brownish red or orange precipitate indicated the presence of alkaloids. Flavonoid test was performed by adding 5 ml of dilute ammonia solution to a portion of the aqueous filtrate of each plant extract, followed by addition of concentrated H₂SO₄. A yellow color in each extract indicated the presence of flavonoids. The yellow color disappeared on standing. Few drops of 1% aluminum solution was added to portion of each filtrate. A portion of the powdered plant sample was in each case heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered, and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution.

A yellow color indicates opposite test for flavonoids. Test for phenolhydroquinone was 500 mg of powdered sample and added with 50 mL of water and boiled for 5 min. Three drops of concentrated sample was added to form layer. Few drops of NaOH 1 N was added. The red color indicated the presence of phenol hydroquinone. Test for steroids was performed by mixing 500 mg sample powder with 20 mL ether and maceration for 2 h, and then those 3 drops of concentrated sample were added to form a layer. Next step is to add few drops of glacial acetic acid- H_2SO_4 (Lieberman–Bourchard reagent) to the concentrated sample. The red color indicated the presence of steroids. Triterpenoids test was performed by mixing 5 ml of each extract with 2 ml of chloroform and 3 ml concentrated H_2SO_4 carefully added to form a layer. A reddish brown color of the interface was formed to show positive results for the presence of triterpenoids. Tannin test was performed with 0.5 g of sample added to water and then boiled for several minutes.

After filtering, the filtrate was added with 3 drops of FeCl_3 . The dark blue or greenish black color formed indicates the presence of tannins. The saponin test was carried out by boiling about 2 g of sample powder in 20 ml of distilled water using a water bath. Then boiled water sample (filtrate) was filtered. A total of 10 ml of filtrate was mixed with 5 ml of distilled water and then shaken vigorously until stable foaming. Foam formation is indicating the presence of saponins. Before testing the total microbial content, simplicia first received UV irradiation treatment. The first simplicia sample was not irradiated, while the next simplicia sample was irradiated with a UV lamp with a power of 60 watts and an irradiation time of 1, 2 and 4 h. During the irradiation process, the simplicia was wrapped in black cloth. The irradiation holder is made of aluminum box with the position of the lamp being 30 cm away from the sample.

The tools used in testing the total microbial content are Erlenmeyer, petri dish, oven, incubator, autoclave, hotplate, electric balance, aluminum foil, and pipette. The materials used are distilled water, Plate Count Agar (PCA), Violet Red Bile Agar (VRBA), and 95% alcohol. The testing process consists of two stages, which are the manufacture of agar media and testing for microbial content using the Total Plate Count (TPC) method with pour plate analysis technique.

The sequence of activities is as follows [17]:

(1) Media creation

- Prepare and sterilize Erlenmeyer for samples and agar media.

- Enter distilled water into the Erlenmeyer followed by using an autoclave for 15 min at a temperature of 121 °C and a pressure of 2 atm.
- Weigh the agar media and dissolve it with distilled water that has been sterilized.
- Heat the agar medium solution in an Erlenmeyer glass and stir until evenly mixed.
- Autoclave at 121 °C and 2 atm pressure for 15 min.
- Maintain the temperature of the solution in the water bath at 45 °C.

(2) Preparation of test materials and TPC testing.

- Prepare two test samples, each weighing 10 g, which diluted with 100 ml of sterilized distilled water.
- Pipette 1 ml of sample into a petri dish.
- Pour 12–15 ml of agar medium into a petri dish containing the sample.
- Invert the petri dish and incubate for 48 h at 37 °C.
- Count all colonies after those in the dish with a colony counter.
- Record the total colonies on the Microbiological Analysis Report.

The microbial content test used the Total Plate Count (TPC) method with the pour plate analysis technique. TPC analysis was carried out in the Biopharmaceutical Laboratory of IPB Bogor. The ingredients for making agar media consist of casein enzyme hydrolysate, yeast extract, dextrose, and agar. The agar medium was dissolved in aqua distillate by forming a suspension of 22.5 g/L and then sterilized in an autoclave for 15 min at 121 °C. The equipment used is analytical balance, autoclave, oven, micropipette, hot plate, spatula, Erlenmeyer, 100-ml measuring cup, petri dish, stirring rod, and test tube.

A total of 5 g of powder sample for each simplicia was put into a 100-ml Erlenmeyer containing 45 ml of sterile physiological saline, then homogenized with a vortex and allowed to stand for about 10 min and followed by dilutions of 10–2, 10–3, and 10–4. A total of 1 ml of the 10–4 dilution sample was put into a sterilized petri dish and then 12–15 ml of PCA media was poured. The petri dish was shaken slowly so that the sample and media were well mixed and solidified. Then the samples were incubated at a temperature of 37 °C for 2 × 24 h. The microbes that grow were counted with the colony counter [17].

Results and Discussion

Content of Phytochemical Compounds of 3 Simplicia Medicinal Plants from Kerangas Forest

The phytochemical compounds contained in the 3 simplicia are listed in Table 1. All types of simplicia tested contained flavonoid compounds, phenol hydroquinone, triterpenoids, and tannins. The results shown in Table 1 indicate that the medicinal plant simplicia from the kerangas forest has the potential to have bioactivity as antimicrobial or antibacterial. Flavonoid compounds, steroids, tannins, saponins, and phenol hydroquinone contained in *Nypa fruticans* leaf extract have antibacterial capacity [18]. Flavonoid compounds [19], terpenoids [20], and tannins [21] have antibacterial capacity. The flavonoid content in the ethyl acetate extract of *Drimys beccariana* has moderate to strong inhibition against *Escherichia coli* and *Bacillus subtilis* [22]. Phenol hydroquinone is one of the phenol group compounds, and an antimicrobial compound is widely used in the pharmaceutical industry, such as disinfectants and oral anesthetics [23]. Steroid compounds were also act as antibacterial and antifungal. These compounds damage bacterial cell membranes and inhibit bacterial growth [24]. Only *Vitex pubescens* does not contain saponins. Saponins also have antibacterial activity [25]. In this research, it is found that *S. balangeran* as a simplicia contains alkaloids. Pfoze et al. in [26] stated that alkaloids have potential as antibacterials.

Effect of Long UV Radiation on Total Bacteria in 3 Medicinal Plant Simplicia

The results of the measurement of total bacteria are presented in Table 2. The value of the number of microbes of all types of simplicia still meets the standard allowed value (≤ 106). The drying process in the sun for 9 days can meet the standard standards for microbial content that are allowed in simplicia, especially for simplicia the processing method brewed with hot water or boiled [3]. UV irradiation has varied effects on the total microbial content of each medicinal plant simplicia. Ultraviolet irradiation against pathogenic bacterial contamination in liquid food was not effective in killing pathogenic bacteria during the waiting time of 60 and 120 min. Penetration variations of material characteristics and irradiation time affect the effectiveness of bacterial inhibition [27].

Radiation with a UV lamp for 1 h with a lamp power of 60 watts and the distance of the sample with a lamp with a simplicia of 30 cm is quite effective in eliminating the microbes contained in the simplicia *B. frutescens*. Methanol extract of *B. frutescens* did not show antibacterial capacity against *Escherichia coli*, *Morganella morgani*, *Serratia marcescens*, *Salmonella typhi*, and *Listeria monocytogens*, but the p-cymene content in *B. frutescens* is known to be antibacterial [28]. Radiation for 4 h with a UV lamp of 60 watts and a sample distance of 30 cm was only able to eliminate the microbial content in *V. pubescens* simplicia. These results indicate that *V. pubescens* leaf simplicia has less potential as an antibacterial. The n-hexane, ethyl acetate and dichloromethane fractions from *V. pubescens* leaves were

Table 1 Phytochemical compounds of three medicinal plant simplicias from the kerangas forest

List of plant species	1	2	3	4	5	6	7	Part of plant
<i>Baeckea frutescens</i>	–	+	+	+	+	+	+	Leaves
<i>Shorea balangeran</i>	+	+	+	–	+	+	+	Bark
<i>Vitex pubescens</i>	–	+	+	+	+	+	–	Leaves

1. Alkaloid, 2. Flavonoid, 3. Phenolhydroquinone, 4. Steroid, 5. Triterpenoid, 6. Tannin, 7. Saponin

Table 2 Total bacterial content in simplicia with various UV irradiation times

Simplicia	Total colony (without UV) Colony/gram		Total colony (1 h) Colony/gram		Total colony (2 h) Colony/gram		Total colony (4 h) Colony/ gram	
	1	2	1	2	1	2	1	2
Bark of <i>S. balangeran</i>	0	0	0	0	0	0	0	0
Leaf of <i>B. frutescens</i>	0	1.1×10^3	0	0	0	0	0	0
Leaf of <i>V. pubescens</i>	3.3×10^4	3.6×10^4	1.2×10^5	3.1×10^5	2.5×10^5	2.8×10^5	0	0

Sub-column '1' is first repetition and sub-column '2' is second repetition

not able to inhibit the activity of *Staphylococcus aureus* and *Escherichia coli* bacteria [29].

No microbial colonies were found on the simplicia skin of *S. balangeran*, although it did not receive UV irradiation. The content of alkaloids, flavonoids, phenol hydroquinone, triterpenoids, tannins, and saponins thought to be factors that influence the absence of bacterial colonies on the simplicia bark of *S. balangeran*. These results indicate that *S. balangeran* has potential as an antibacterial. Local people around the kerangas forest use the skin of *S. balangeran* as a diarrhea medicine. [13].

Conclusions

The three medicinal plants simplicia from kerangas forest contain relatively complete phytochemicals including flavonoids, phenolhydroquinone, triterpenoids, and tannins. The drying process in the sun for 9 days is sufficient to meet the quality standards for microbial content in simplicia (≤ 106). UV irradiation has various effects on the total microbial content of each medicinal plant simplicia. *S. balangeran* simplicia did not contain microbial colonies either without irradiation or with UV irradiation. Results of the experiment indicate that *S. balangeran* simplicia is potentially high to be developing as an antimicrobial or antibacterial.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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References

- Kissinger K, Zuhud EA, Darusman LK, Siregar IZ (2013) Diversity of medicinal plants species from kerangas forest. *J Hutan Tropis* 1(1):2337–2799
- Kinho J, Arini DID, Tabba S, Kama H, Kafiar Y, Shabri S, Dan Karundeng MC (2011) Traditional medicinal plants in North Sulawesi Province 1. Manado Forestry Research Institute, Forestry Research and Development Agency. Ministry of Forestry, Republic of Indonesia
- Badan Pengawas Obat dan Makanan (BPOM) (2014) Regulation of the head of the food and drug supervisory agency (BPOM) of the Republic of Indonesia Number 12 of 2014 concerning Quality Requirements for Traditional Medicines
- Ministry of Health (2008) *Farmakope Herbal Indonesia*, 1st edn. Indonesian Ministry of Health, Jakarta
- Rahimmalek M, Goli SAH (2013) Evaluation of six drying treatments with respect to essential oil yield, composition and color characteristics of thymys daenensis subsp. daenensis Celak leaves. *Ind Crops Prod* 42:613–619
- Fatouh M, Metwally MN, Helali AB, Shedid MH (2006) Herbs drying using a heat pump dryer. *Energy Convers Manage* 47:2629–2643
- Hamrouni-Sellami I, Rahali FZ, Rebey IB, Bourgou S, Limam F, Marzouk B (2013) Total phenolics, flavonoids, and antioxidant activity of sage (*Salvia officinalis* L.) Plants as affected by different drying methods. *Food Bioprocess Technol* 6(3):806–817
- Manalu L, Adinegoro H (2018) Drying process conditions for producing Simplicia standard of zedoary. *J Standardisasi* 18(1):62–68
- Zamharir S, Priyati A (2016) Analysis of heat energy utilization in onion (*Allium ascalonicum* L.) drying using green houses gasses (GHG) dryer. *J Ilmiah Rekayasa Pertanian dan Biosistem* 4(2):264–274
- Metcalf W (2003) Metcalf and Eddy wastewater engineering: treatment and reuse. In: *Wastewater engineering: treatment and reuse*. McGraw Hill, New York, NY, p 384
- Giusti MM, Wrolstad RE (2010) Characterization and Measurement of Anthocyanin by UV-Visible Spectroscopy. John Wiley and sons Inc., London
- Ariyadi T, Sintadewi S (2009) The effect of ultraviolet light on the growth of *Bacillus* sp as contaminant bacteria. *J Ilmu Kesehatan (health science journal)* 2:2
- DanYunianta A (2014) Effects of power lights and time ultraviolet-C irradiation on microbial population of snake fruit Pondoh (*Salacca edulis*) fruit juice. *J Food Agroind* 3(4):1337–1344
- Kissinger K, Yamani A, Thamrin GA, Muhayah R (2016) Bio-prospecting of Kerangas forest as natural medicine material sources: screening phytochemistry compound of Kerangas forest tree species. *J Wetlands Environ Manag (JWEM)* 4(2):13–19
- Prasetyo P, Inoriyah E (2003) Management of medicinal plant cultivation (*Material of Simplicia*). Publishing Agency of the Faculty of Agriculture, Universtiy of Bengkulu
- Harborne JB (1998) *Phytochemical methods a guide to modern techniques of plant analysis*. Published by Chapman & Hall, an imprint of Thomson Science, 2–6 Boundary Row, London SE18HN, UK
- Wati R (2018) Effect of heating plate count agar (PCA) repeated on total plate count (TPC) test in microbiology laboratory results technology. *J Temapela* 1(2):44–47. <https://doi.org/10.25077/temapela.1.2.44-47>
- Imra I, Tarman K, Desniar D (2016) Antioxidant and antibacterial activities of Nipah (*Nypa fruticans*) against *Vibrio* sp isolated from Mud Crab (*Scylla* sp.). *JPHPI* 19(3):241–250
- Sukadana IM (2010) Isolation of flavonoid antibacterial compounds from Awar-awar root skin (*Ficus septica* Burm F). *J Kimia* 4(1):63–70
- Mariajancyrani J, Chandramohan G, Dan Elayaraja A (2013) Isolation and antibacterial activity of Terpenoid from *Bougainvillea glabra* Choisy leaves. *Asian J Plant Sci Res* 3(3):70–73
- Doss A, Mubarak M, Dan Dhanabalan R (2009) Antibacterial activity of tannins from the leaves of *Solanum trilobatum* Linn. *Ind J Sci Technol* 2(2):41–43. <https://doi.org/10.17485/ijst/2009/v2i2.5>

22. Parubak AS (2013) Antibacterial flavonoids from akway (*Drimys beccariana* Gibbs). Chem Progress J 6(1):34–37
23. Detha A, Dan Datta FA (2016) Phytochemical of sopi and make as a potential antimicrobial agent. J Kajian Veteriner 4:12–14
24. Dewi CSU, Soedharma D, Kawaroe M (2012) Phytochemical components and toxicity of bioactive compounds from seagrass *enhalus acoroides* and *thalassia hemprichii* from Pramuka Island, DKI Jakarta. J Fish Marine Technol 3(2):23–27
25. Akinpelu BA, Igbeneghu OA, Awotunde AI, Iwalewa EO, Dan Oyedapo OO (2014) Antioxidant and antibacterial activities of saponin fractions of *Erythropheleum suaveolens* (Guill. and Perri.) Stem Bark Extract. Sci Res Essays 9(18):826–833
26. Pfoze NL, Kumar Y, Myrboh B, Bhagobaty RK, Joshi SR (2011) In vitro antibacterial activity of alkaloid extract from stem bark of *Mahonia manipurensis* Takeda. J Med Plants Res 5(5):859–861
27. Sulastri NH, Yogeswara IBA, Nursini NW (2017) The effectiveness of ultraviolet light against contamination of pathogenic bacteria in soude liquid food for immune-compromised patients. Ind Nutrition J 5(2):112–211
28. Ahmad NS, Ghani MN, Ali AM, Johari SA, Harun MH (2012) High performance liquid chromatography (HPLC) profiling analysis and bioactivity of *Baeckea frutescens* L. (Myrtaceae). J Plant Studies 1(2):101–108
29. Sartini S (2019) *Isolation of secondary metabolites and antibacterial bioactivity test from the dichloromethane fraction of Laban leaves (Vitex pubescens Vahl)*. University of Andalas, Indonesia

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