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Antioxidant Activity of Basil Leaf Extract (Ocimum basilicum L) to Strengthen the Traditional Balinese Medicine System

I Nyoman Arsana[1)*], Ni Ketut Ayu Juliasih[2)] and A.A.Ayu Sauca Sunia Widyantari[3)]

[1 2 3)]Biology Department, Faculty of Information Technology and Science, University of Hindu Indonesia, Jl Sangalangit Tembau Penatih Denpasar Timur, 80237.

*Corresponding author: arsanacita@gmail.com

Abstract

Antioxidant activity and phytochemical of ethanol extracts of two varieties of basil, Ocimum basilicum L (var.) Bali (OcB) and Ocimum basilicum L (var.) Lombok (OcL) was investigated using standard methods. Morphological characters (stems, leaves, and flowers) are also observed. Antioxidant activity is expressed as IC50. The results showed, in OcB obtained 92 compounds, 10 of which are important compounds that have an antioxidant effect, while in OcL 139 compound components were found, 15 of which are important compounds. Levels of phenols, flavonoids, tannins, as well as IC50 in OcB are; 3526.65 mg GAE/100g, 15841.6795 mg QE/100g, 2402.80 mg/100 g, and 24.9410 mg/L, respectively, while in OcL respectively are; 3297.71 mg GAE/100g, 13242.30 mg QE/100g, 850.71 mg/100, and 33.1105 mg/L. Conclusions, morphologically OcB and OcL showed no difference except leaf width and petiole length. The antioxidant activity of OcB is better, as well as higher levels of phenols, flavonoids, and tannins compared to OcL

Keywords: Active Compound, Antioxidant, Ocimum basilicum L, Usada

Introduction

The system of traditional Balinese medicine is known as Usada. The treatment uses various types of plants as well as other materials such as salt, vinegar, and oil accompanied by certain mantras (prayers). Usada has a long tradition in the life of the Balinese people and this practice of medicine continues today. The procedures for practicing Usada are contained in many ancient manuscripts known as Lontar Usada.

Plants have been recorded as a medicinal ingredient, with no less than 182 types, most of which include members of the families Euphorbiaceae, Moraceae, Fabaceae, and Zingiberaceae. Plant

parts are used in the form of leaves, shoots, bark, roots, fruits, sap, and tubers, as well as other ingredients such as coconut oil, salt, vinegar, arak (traditional alcohol), and whiting(Arsana, 2019).

One type of plant used in traditional Balinese medicine, Usada, is basil (Ocimum basilicum L). This plant is for treating diseases or symptoms of diseases such as stiff muscles, rheumatism, and vomiting blood. Its use is carried out in the form of polyherbal or mixtures of various types of plants as well as other ingredients such as vinegar(Arsana & Suardana, 2020).

Some research shows that basil has been used in medicine around the world such as for the treatment of breast cancer (Torres, 2018), counteracts anxiety and depression in Alzheimer's disease conditions (Gradinariu et al., 2015), (Ayuob et al., 2018), as an antimicrobe especially against Staphylococcus aureus, Klebsiella pneumoniae and Bacillus subtilis (Vadivel, 2011), has cardiotonic and adrenergic effects (Muralidharan & Dhananjayan, 2004). Ethyl-acetate, n-butanol, and aqueous extracts from basil leaves have strong antioxidant activity (Kaurinovic et al., 2011) and have the potential to be anti-malarial (Ntonga et al., 2014). Daily consumption of Ocimum basilicum seeds was able to inhibit the production of cytokine Th2 (Activated T-helper) and goblet cell hyperplasia involved in asthma in model mice (Nasaba, 2020).

Two varieties of basil (OcB and OcL) organoleptically have differences. OcL has a sharper odor than OcB. OcB is very commonly consumed by local Balinese people but not by local Lombok people, and vice versa, OcL is very commonly consumed by the local people of Lombok but not by the people of Bali. OcB is usually used to eliminate the fishy smell in fresh fish and is also used as a fresh vegetable, while OcL is used as a vegetable ingredient. However, there have not been many studies that have revealed the antioxidant activity of the two varieties of basil. This study aims to determine the antioxidant activity of OcB and OcL.

Methods

Materials

OcB samples were obtained from a traditional market in Denpasar, Bali. This plant is commonly traded as a fresh vegetable by local people. OcL is obtained from a traditional market on the island of Lombok where local people generally trade it as vegetable ingredients. Both varieties of basil have been identified at Bali Botanical Garden "Eka Karya" BRIN and identified as Ocimum basilicum L.

Morphological Characters

A total of 19 characters of OcB and OcL were investigated, three of which are quantitative characters and 16 are qualitative. These characters are leaf blade length, leaf blade width, and petiole length. Qualitative characters are the type of stem, the shape of the stem, the shape of the leaf blade, the shape of the tip of the leaf, the shape of the edge of the leaf blade, the upper surface of the leaf, the lower surface of the leaf, the leaf bone, the flesh of the leaf, the type of flower, the location of the flower, the color of the petals, the color of the flower crown, pistils, and stamens. Observations were made using a Magnifier.

Extraction

Basil leaf extract was obtained through a maceration process with 96% ethanol solvent. Basil leaves are thoroughly washed and dried, further, grinding until they become a dry flour form. A total of 300 gr of samples were dissolved with 3L of 96% ethanol for 48 hours and macerated twice. The filtrate is then filtered with filter paper and concentrated with a rotary evaporator at a temperature of 45ºC.

Determination of total phenol

Analysis using folin-cioccalteu phenol reagent. A sample with an initial concentration of 11.80 ppm was taken 0.4 ml, 0.4 folin-cioccalteu phenol reagents were added, then the test tube was gently shaken. 4.2 ml of 20% sodium carbonate was added to the tube and incubated in the open air for 20 minutes, then its absorbance was measured with a spectrophotometer at a wavelength of 760 nm. Gallic acid is used as a calibration curve. The result was expressed as mg gallic acid equivalent (GAE) per 100 grams (g) of the dry weight of the extract. The standard curve of gallic acid is made with a concentration of 2.5 ppm; 5,0; 10,0; 15,0; 20.0, and 25.0 ppm, then measured absorbance with a spectrophotometer at a wavelength of 760 nm (Wrasiati et al., 2011).

Determination of Flavonoid

A total of 11.80 mg of extract samples were dissolved in 5 ml of ethanol, then 20 µL were taken and 2.5 mL of aquades and 0.15 µL NaNO2 5% were added, mixed, and incubated for 5 min. Next, 0.3 µL AlCl3 5% was added and incubated for 5 min. Finally, 1 mL of NaOH 1N and aquades were added until they reached a volume of 5 mL, mixed until homogeneous, and incubated for 30 minutes, then the absorbance was measured with a spectrophotometer at a wavelength of 510 nm. As a standard used quarsetine made with a concentration of 4.0 ppm; 8,0; 12,0; 16.0, and 20.0 ppm, then the absorbance is measured with a spectrophotometer at a wavelength of 510 nm. The result is expressed as mg quarsetine equivalent per 100 grams (g) of the dry weight of the extract (Dalawai, 2021).

Determination of Tannin

A total of 11.80 mg of extract samples were dissolved in 5 mL of ethanol, then 0.1 ml were taken and extracted with 10 mL of hot aquades, then filtered after cooling. Then, a total of 0.25 mL of filtrate was dissolved with 0.25 mL of Folin-Denis reagent, and 2 mL of 5% Na2CO3 solution was added, then mixed and incubated for 30 minutes. Then, its absorbance was measured with a spectrophotometer at a wavelength of 755 nm. The result was expressed as mg tannic acid equivalent per 100g of the dry weight of the extract. Standard tannic acid is made with a concentration of 4.0 ppm; 8,0; 16,0; 24,0; 32.0 and 40.0 ppm and their absorbance were measured by spectrophotometer at a wavelength of 755 nm (Dalawai, 2021).

Determination of Antioxidant activity

Antioxidant activity was analyzed using DPPH as a source of free radicals. A total of 11.80 mg of extract was dissolved in 5 mL of ethanol. The solution was then diluted again with the same solvent to obtain a concentration of 10; 30; 40; and 50 ppm. The solution was then mixed with a 0.04% DPPH solution in a ratio of 1:1, then incubated in dark conditions at room temperature for 30 minutes. After 30 minutes, its absorbance was measured with a spectrophotometer at wavelength

517. The activity of DPPH scavenging is calculated by the equation: % inhibition=(Ao-As)/Ao x 100, where Ao is the control absorbance, and As is the absorbance of the sample. Antioxidant activity is expressed as IC50 i.e. the concentration of the extract required to absorb 50% of DPPH radicals. IC50 is calculated using a linear regression equation (Dowlath, 2020).

Gas chromatography and mass spectrometry (GC-MS) Analysis

The active compounds were analyzed by Gas Chromatography-Mass Spectrophotometry (GC-MS). GCMS instruments include; capillary column HP-5MS UI (30.0 m x 0.25 mm x 0.25 µm). The carrier gas used is Helium with a flow rate of 10 ml per minute. The temperature of the GC is set as follows. The injector temperature is 290oC, the column starting temperature is 60oC, the temperature rise rate is 10oC/min, and the end temperature is 280oC. Identification of compounds was carried out with the Willey7 Library database. GC-MS analysis was carried out at the Integrated Research and Testing Laboratory, Gadjah Mada University.

Data Analysis

Plant morphological quantitative data were analyzed with one-way ANOVA at a 95% confidence interval, while qualitative data were qualitatively analyzed. Phytochemical data (phenols, flavonoids, tannins, antioxidant activity, as well as GC-MS) were analyzed. qualitatively.

Results and Discussion

The results showed that the morphological characters of OcB and OcL did not reveal any differences except for the width of the leaf blade and the length of the petiole. OcB has a wider leaf blade than OcL, while OcL has a longer petiole length than OcB (Table 1). Meanwhile, the qualitative morphological characters showed similar characteristics between OcB and OcL (Table 2 to 4).

Table 1. Quantitative Character of Ocimum basilicum L Leaves. Average expressed as mean ± S.D (n = 30/group). Average with dissimilar superscripts within the same columns differ significantly at a level of 0.05. OcB (Ocimum basilicum L (var.) Bali). OcL (Ocimum basilicum L (var.) Lombok)

Varieties	Leaf blade length (mm)	Leaf blade width (mm)	Petiole length (mm)
OcB	50.29 ± 5.44 [a]	25.27 ± 2.69 [a]	15.98 ± 3.14 [a]
OcL	51.89 ± 3.71 [a]	22.92 ± 1.51 [b]	18.42 ± 2.31 [b]

Table 2. Qualitative Character of Ocimum basilicum L. Leaves. OcB (Ocimum basilicum L (var.) Bali). OcL (Ocimum basilicum L (var.) Lombok)

Varieties	Circumsrciptio	Apex Folii	Basis Folii	Margo Folii	Abaxial	Adaxial	Nervatio	Intervenum
OcB	Ovalis	Acutus	Acutus	Serratus	Nitidus	Nitidus	Penninervis	Membranaceus

OcL	Ovalis	Acutus	Acutus	Serratus	Nitidus	Nitidus	Penninervis	Membranaceus

Table 3. Qualitative Character of the Stem (caulis) of Ocimum basilicum L. OcB (Ocimum basilicum L (var.) Bali). OcL (Ocimum basilicum L (var.) Lombok).

Varieties	The type of stem	The shape of the stem
OcB	Lignosus	Quadrangularis
OcL	Lignosus	Quadrangularis

Table 4. Qualitative Character of Ocimum basilicum L flower. OcB (Ocimum basilicum L (var.) Bali). OcL (Ocimum basilicum L (var.) Lombok).

Varieties	The type of flower	Location of flower	Petals	Flower crown	Stamens	Pistils
OcB	Verticillaster (with 6 flowers).	The tip of the stem. (Terminal)	Green color, hairy, 4-pointed lower petals, tapered upper petals, both petals fused at the bottom (lobatus)	Color white, 2-lip, 4-pointed upper crown, tapered lower crown, feathered outer side, both crowns fused.	Stamens 4, white in color, surrounding the pistil.	Pistil 1, white in color, pistil stalk slightly reddish.
OcL	Verticillaster (with 6 flowers).	The tip of the stem. (Terminal)	Green color, hairy, 4-pointed lower petals, tapered upper petals, both petals fused at the bottom (lobatus)	Color white, 2-lip, 4-pointed upper crown, tapered lower crown, feathered outer side, both crowns fused.	Stamens 4, white in color, surrounding the pistil.	Pistil 1, white in color, pistil stalk slightly reddish.

The results of the GC-MS analysis of OcB extract obtained 92 peak compounds (Figure 1), 10 of which are important main compounds (Table 5). Meanwhile, from OcL extract, 139 peak compounds were obtained (Figure 2), 15 of which are important compounds (Table 6).

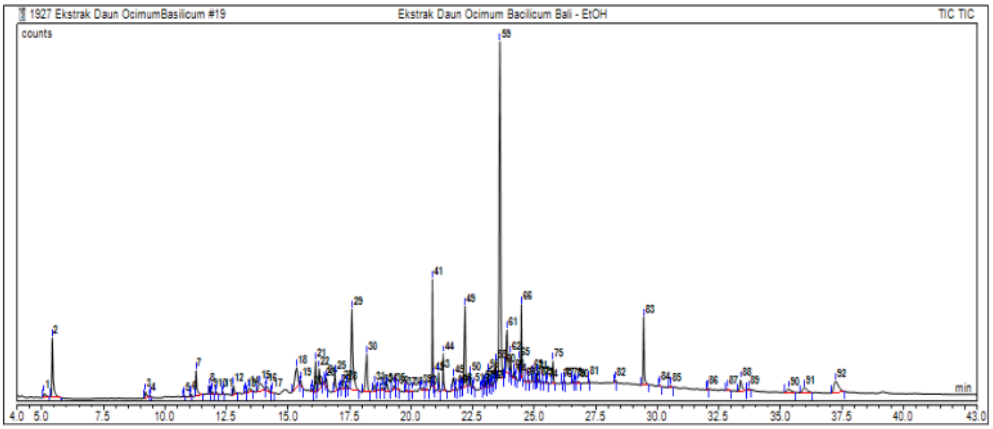

Figure 1. Chromatogram GC-MS of Extract Ocimum basilicum L (var.) Bali. Peak Numbers in Figure According to Compounds in Table 5.

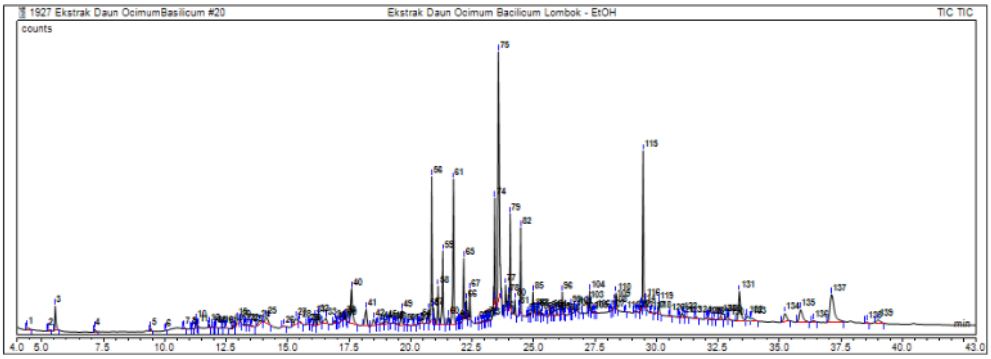

Figure 2. Chromatogram GC-MS of Extract Ocimum basilicum L (var.) Lombok. Peak Numbers in Figure According to Compounds in Table 6

Table 5. Active Compound of Etanol Extract of Ocimum basilicum L (var.) Bali Based on Mass Chromatogram GC-MS.

Peak	RT (minute)	Compound	Rel. area (%)	Mol. Weight	Chemical Formula
2	5.43	Glycolaldehyde dimethyl acetal	3.82	106	C4H10O3
7	11.26	Linalool	1.57	154	C10H18O

29	17.60	Cyclohexene, 4-[(1E)-1,5-dimethyl-1,4-hexadien-1-yl]-1-methyl-	7.88	204	C15H24
30	18.20	Caryophyllene oxide	3.80	220	C15H24O
41	20.86	Neophytadiene	3.40	278	C20H38
49	22.18	n-Hexadecanoic acid	5.13	256	C16H32O2
59	23.60	Phytol	27.67	296	C20H40O
61	23.89	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	3.46	278	C18H30O2
66	24.48	Phytol, acetate	2.17	338	C22H42O2
83	29.45	Squalene	3.60	410	C30H50

Table 6. Active Compound of Etanol Extract of Ocimum basilicum L (var.) Lombok Based on Mass Chromatogram GC-MS.

Peak	RT. (minute)	Compound	Rel. area (%)	Mol. Weight	Chemical Formula
3	5.54	Formamide, N-methoxy-	1.28	75	C2H5NO2
40	17.59	1H-Benzocycloheptene, 2,4a,5,6,7,8,9,9a-octahydro-3,5,5-trimethyl-9-methylene-, (4aS-cis)-	3.16	204	C15H24
41	18.19	Caryophyllene oxide	1.69	220	C15H24O
56	20.86	Neophytadiene	5.38	278	C20H38
61	21.73	Hexadecanoic acid, methyl ester	7.26	270	C17H34O2
65	22.16	n-Hexadecanoic acid	2.45	256	C16H32O2
66	22,26	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl	0.49	578	C16H50O7Si8
67	22.40	Hexadecanoic acid, ethyl ester	1.14	284	C18H36O2
74	23.42	9-Octadecenoic acid (Z)-, methyl ester	3.39	296	C19H36O2
75	23.57	Phytol	15.60	296	C20H40O
79	24.04	Ethyl 9,12,15-octadecatrienoate	3.05	306	C20H34O2
82	24.47	Phytol, acetate	2.52	338	C22H42O2
115	29.45	Squalene	6.45	410	C30H50
131	33.37	Vitamin E	3.26	430	C29H50O2
137	37.11	γ-Sitosterol	6.19	414	C29H50O

Tables 5 and 6 show that Phytol is the most component, reaching 27.6% on OcB, and 15.60% at OcL. Phytol is derived from the hydrolysis of chlorophyll and is a side chain of chlorophyll prenyl bound to ester bonds (Gutbrod, 2019). In plants, phytol is used to synthesize tocopherol (vitamin E), or phylloquinone (vitamin K) (Durrett, 2021). Phytol is known to have anti-anxiety, cytotoxic, metabolism-modulating, antioxidant, apoptosis, antinociceptive, anti-inflammatory, immunomodulatory, and antimicrobial effects (Islam, 2018), antihyperalgesic, anti-inflammatory, and antiarthritis (Carvalho, 2020). Research conducted on rats showed that phytol acts as an antioxidant so that it can improve benzo(a)pyrene-induced pulmonary carcinogenesis through inhibition of oxidative stress and apoptosis (Sakthivel, 2019).

Another important compound is squalene which is an important excipient in pharmaceutical applications, especially for the delivery of vaccines, drugs, and other biological components (Reddy & Couvreur, 2009). In OcB extract, the relative abundance of squalene reaches 3.60%, while in OcL it reaches 6.45%. Squalene is a precursor in the synthesis of cholesterol (animal), ergosterol (fungi), β-sitosterol (plant), 24-methylenecholesterol (Protista), and hopanoids (bacteria) (Chua, 2020). Squalene is a lipophilic biomolecule belonging to the class of triterpenes. Squalene is an odorless, colorless liquid oil (Micera, 2020). The compound was named squalene because it was first isolated from shark liver oil (Squalus milsukurii) by Tsujimoto (Tsujimoto, 1916), However, squalene can also be found in a variety of plants such as olives (Martínez-Beamonte, 2020), (Chira, 2021), green tea leaves (Camellia sinensis) (Park, 2020).

Linalool is also an important compound obtained from OcB ethanol extract with a relative abundance of 1.57%. Linalool is a monoterpene found in many aromatic plants. A total of 0.34 kg of linalool was obtained from the dry biomass of Ocimum basilicum L irrigated by reclaimed water (Melo, 2021). Linalool is proven to be an antioxidant so it has an organ-protective and myeloprotective effect and reduces the occurrence of oxidative stress (Ola, 2021), (Salimi, 2021).

The n-Hexadecanoic acid compound is also found in OcB with a fairly large abundance of 5.13%. Another name for this compound is palmitic acid. While in OcL there is in the form of methyl ester, namely Hexadecanoic acid, methyl ester with a relative abundance reaching 7.26%. Hexadecanoic acid is also found in abundant amounts (reaching 56.84%) of palm oil and is widely used in cosmetics and coloring (Masyithah, 2021). Neophytadiene compounds were also found to be quite abundant, reaching 3.40% in OcB and 5.38% in OcL. Neophytadiene isolated from brown algae, Turbinaria ornata, is known to act as an anti-inflammatory, antioxidant, and cardioprotective (Bhardwaj et al., 2020).

Caryophyllene oxide are compound was found with an abundance of up to 3.80% in OcB, while in OcL it was 1.69%. Caryophyllene oxide is an oxygenated sesquiterpenoid. Caryophyllene oxide is the main component in the Salvia verticillata plant and has proven to be an anticholinesterase and antioxidant (Karakaya, 2020). Caryophyllene oxide is also found as a major component in the leaves of Hymenaea courbaril and is a safe anti-proliferative agent against prostate cancer cells (PC-3), as well as induces apoptosis with low toxicity in normal cells (Delgado, 2021). Caryophyllene oxide is also the main compound of the Chromolaena odorata plant and can induce sedative activity in mice (Dougnon, 2021).

The use of basil in medicine is inseparable from the presence of secondary metabolite compounds such as alkaloids, diterpenes, glycosides, saponins, steroids, terpenoids, tannins, phenols, and flavonoids (Akoto, 2020). The results of this study found that the total phenols, flavonoids, and tannins in OcB were higher compared to OcL (Table 7). This indicates that secondary metabolites are influenced by cultivars and morphology (Bajomo, 2022). OcB and OcL organoleptically have slight differences. OcL has a sharper odor and is usually used as a vegetable ingredient, while OcB has a softer smell and is usually used to eliminate fishy odors in fresh fish and is also used as a fresh vegetable. However, the results of this study showed that of the 19 morphological characters observed there was no difference except for the width of the leaf blades and the length of the petioles. OcB has wider leaf blades compared to OcL, while OcL has longer petioles compared to OcB (Table 1).

Phenols show a strong association with antioxidants (Zhao et al., 2014). The antioxidant activity of polyphenols is related to their ability to neutralize free radicals by donating electrons or their hydrogen atoms to free radicals so that no further initiation or propagation reactions occur (Olszowy, 2019). The results of this study showed that the antioxidant activity (IC_{50}) of OcB was 24.9410 mg / L, meaning that to reduce 50% of free radicals, an extract with a concentration of 24.9410 mg / L. was needed. The antioxidant activity of OcB is slightly better than OcL because to reduce 50% of free radicals, more OcL extract is needed, namely 33.1105 mg / L (Table 7). Other studies have also shown that basil leaf extract has a high antioxidant capacity with IC_{50} of 285.36 mg / mL against DPPH, so it can be said that basil is a promising source of phenolic compounds and basil leaf extract is a potential antioxidant agent for pharmaceutical use (Do, 2020). In another study, basil extract contains a considerable total phenolic and exhibits a high scavenging capacity of DPPH radicals as well as a high correlation between antioxidant activity and total phenolic content (Ahmed, 2019). The antioxidant activity of basil leaves is stronger when compared to the leaves, flowers, stems, and roots of the plant Isotoma longiflora (Egarani et al., 2020), stronger than the seeds of Simmondsia chinensis (Siahaan et al., 2020), and stronger than Microalgae extract Cosmarium sp. (Agustini et al., 2022).

Table 7. Phytochemical, Antioxidant Activity Two Varieteies of Basil. (OcB: Ocimum basilicum L (var.) Bali. OcL: Ocimum basilicum L (var.) Lombok)

Varieties	IC_{50} (ppm)	Phenol (mg/100g	Flavonoid (mg/100g)	Tannin (mg/100g)
OcB	24.9410	3526.65	15841.6795	2402.80
OcL	33.1105	3297.71	1324.30	850.71

Antioxidant activity is associated with the presence of active compounds. The results of the GC-MS analysis of OcB ethanol extract obtained 92 peak compounds, 10 of which are the main compounds that have an antioxidant effect. These main compounds include; Glycolaldehyde dimethyl acetal; Linalool; Cycloheptene,4-[(1E)-1,5-dimethyl-1,4-hexadien-1-yl]-1-methyl-; Caryophyllene oxide; Neophytadiene; n-Hexadecanoic acid; Phytol; 9,12,15-Octadecatrienoic acid,(Z,Z,Z)-; Phytol, acetate; and Squalene (Tabel 5). Meanwhile, the results of the GC-MS analysis of OcL ethanol extract obtained 139 compound components, 1 5 of which are the main compounds, namely: Formamide, N-methoxy-; 1H-Benzocycloheptene, 2,4a,5,6,7,8,9,9a-octahydro-3,5,5-trimethyl-9-methylene-, (4aS-cis)-; Caryophyllene oxide; Neophytadiene; Hexadecanoic acid, methyl ester; n-Hexadecanoic acid; Octasiloxane,

1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl; Hexadecanoic acid, ethyl ester; 9-Octadecenoic acid (Z)-, methyl ester; Phytol; Ethyl 9,12,15-octadecatrienoate; Phytol, acetate; Squalene; Vitamin E; and γ-Sitosterol (Table 6).

The results of this study have provided scientific information so that the use of basil, especially OcB, in traditional medicine becomes more optimal. Bali's traditional medicine system, known as *Usada*, utilizes plants either in singular form or in polyherbal form, as well as added other ingredients such as vinegar, *arak* (traditional alcohol). These medicinal materials are used in the form of herbal medicine (loloh), scrub (boreh), sprayed (sembar), drops (*tutuh*), paste (*tampel*), and wound-washing liquid (*ses*) (Arsana, 2019), and even herbal medicine (*loloh*) has been produced and drunk exclusively (Sujarwo et al., 2015).

Conclusion

The conclusions of the study are; morphologically OcB and OcL do not have much difference except for the width of the leaf blade and the length of the petiole. OcB has wider leaf blades, while petiole length is shorter compared to OcL. OcB antioxidant activity is better, with higher levels of phenols, flavonoids, and tannins compared to OcL. Phytol is the dominant active compound in both varieties of basil studied. This result can be strengthening the use of basil in traditional medicine.

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