Yield and Quality Extract of *Clitoria ternatea* Accessions with Different Drying Methods

Kusumiyati^{a,*}, Nita Yuniati^a, Yusuf Eka Maulana^a, Agung Karuniawan^a

^a Department of Agronomy, Faculty of Agriculture, Universitas Padjadjaran, Jatinangor, Kab. Sumedang 45363, Indonesia Corresponding author: ^{*}kusumiyati@unpad.ac.id

Abstract— *Clitoria ternatea* has well-known potential roles for medicinal purposes because it is a source of antioxidants. Drying is important for obtaining *C. ternatea* extract by removing the moisture from the fresh materials. However, it might also influence bioactive compounds and alterations of sensory, nutritional, and physical qualities. This study aimed to determine the best combination of drying method and *C. ternatea* accession for yield and quality of extract without damaging its bioactive compounds and antioxidant activities. A completely randomized block design with nine combination treatments was used in this study. Each treatment was replicated three times. Drying methods consisted of shade drying, sun drying, and oven drying, while *C. ternatea* accessions were white single petal, blue single petal, and blue double petal. The LCMS analysis of single blue petal of *C. ternatea* in methanol extract showed that the dominant metabolites found were flavonoid groups; quercetin, kaempferol, and luteolin. Total phenolic content was recorded higher in blue double petal accession of *C. ternatea* combined with the sun drying method. Moreover, shade drying combined with white single petal of *C. ternatea* achieved the best total extraction yield, flavonoid, antioxidant activity (IC₅₀), kaempferol, and quercetin yields. Due to its affordability, shade drying is a potential best option for drying C. ternatea, particularly for white single petal accession.

Keywords— Antioxidant activity; Clitoria ternatea; drying; flavonoid; phenolic.

Manuscript received 22 Feb. 2021; revised 21 May 2021; accepted 11 Jul. 2021. Date of publication 31 Oct. 2022. IJASEIT is licensed under a Creative Commons Attribution-Share Alike 4.0 International License.



I. INTRODUCTION

Clitoria ternatea is a perrenial plant in the Fabacaea family. Its flower size is large, so it is commonly used as an ornamental flower. The flower comes in several colors: dark blue, blue, white, and mauve. The double petal flower varieties are more enticing than single petal flowers, particularly for ornamental purpose, and the plant's petals can be used as a source of natural dyes and food additives.

Recent evidence has shown that *C. ternatea* also has potential medicinal uses. The extract of *C. ternatea* has been found to contain bioactive compounds that play key roles as anti-inflammatories, antimicrobials, antipyretics, antidiabetics, and more [1]. It has also been reported that the petals of *C. ternatea* contain phenolic compounds, particularly those in the flavonoid groups, such as kaempferol and quercetin [2]. The presence of the flavonoids suggests that *C. ternatea* may be used as a source of antioxidants because it has a phenolic chemical structure. Antioxidants are substances that can prevent certain illnesses, such as cardiovascular disease, neuronal illness, and diabetes [3]

Drying, a vital process for producing *C. ternatea* extract, decreases deteriorative biochemical reactions by removing the moisture from the fresh materials [4]. By drying the material, alterations due to enzymatic or metabolic reactions can be retarded, thus maintaining the quality of extracts.

Generally, there are two types of drying methods that can be used, natural drying and modern drying. Natural drying uses the sun or air (shade) as the source of drying energy, while modern drying uses equipment to heat the materials. Farmers often use natural drying because it is very cheap, particularly if there is a large quantity of material. However, this method is less efficient than modern drying and is relatively prone to contamination by microorganisms due to the direct exposure to the environment. Conversely, modern drying offers a faster and more effective process that also protects the material from various contaminants. With these methods, the drying process can be done without considering the weather and drying temperatures can be adjusted as desired. Oven drying is the most commonly used process among many modern drying methods.

Although drying is important for the extraction process, it might also influence bioactive compounds and its alterations of sensory, nutritional, and physical qualities [5]. Drying of stevia leaves using sun and oven at 60 °C resulted in a decrease in antioxidant activity when compared to fresh samples [6]. A decline in phenolic content has also been found in purple perilla leaves dried in the shade and the sun [7]

Based on these prior studies, it is necessary to find appropriate methods for obtaining extracts from plant materials. In this study, we investigated appropriate drying methods to use before the extraction process with several *C. ternatea* accessions. The objective of this study was to determine the best combination of drying method and *C. ternatea* accession for extraction yield and quality without damaging bioactive compounds and antioxidant activities.

II. MATERIALS AND METHOD

A. Plant Material

Fresh *C. ternatea* accessions (white single petal, blue single petal, and blue double petal) were collected from Universitas Padjadjaran, Indonesia. The *C. ternatea* was packaged and kept in closed paper bags until the samples were cleaned and then dried according to the drying method being tested.

B. Drying Treatment

Three different methods—shade drying, sun drying, and oven drying—were used with 100 g samples of cleaned *C. ternatea*. For shade drying, *C. ternatea* was placed in the laboratory at room temperature (23-27 °C) for 7 days. With sun drying, *C. ternatea* was kept under direct sunlight from 9 AM to 3 PM for 2 days. Finally, for oven drying, the *C. ternatea* samples were dried in an oven (Memmert BM-500; Memmert GmbH, Schwabach, Germany) at low temperature (50 °C) for 6 hours.

C. Extraction Process

Before the extraction process, the dried material was ground into a powder using an electric blender, packaged inside flip plastic and kept in a freezer at 0 °C. Maceration using a 96% ethanol solvent was selected for the extraction method, during which 25 g of *C. ternatea* dried powder was blended with solvent at a ratio of 1:30 w/v, then put into a 500 ml Erlenmeyer flask and left for 24 hours without stirring. The liquid extract obtained from maceration was filtered and then concentrated using a rotary evaporator (BÜCHI Rotavapor R-100; BÜCHI Labortechnik AG, Flawil, Switzerland) at 50 °C. This evaporation process was conducted until a paste-like sample was obtained.

D. Experimental Design

This research used a randomized block design, with nine treatments and three replications. The treatments were combinations of drying method (shade, sun, and oven drying) and three accessions of *C. ternatea* (white single petal [WSP], blue single petal [BSP], and blue double petal [BSP]).

E. Determination of Extraction Yield

The extract obtained was weighed to determine the extraction yield, calculated as a ratio of milligrams of dried extract to grams of dried *C. ternatea* sample, represented as a percentage (%).

F. Determination of Total Phenolic Content

measure the total phenolic content using То spectrophotometry, 10 mg of C. ternatea extract was dissolved in methanol 10 mL. Then, 0.5 mL of the extract was blended with Folin-Ciocalteu reagent and sodium bicarbonate 7.5% for each 2.5 mL and stored for 3-8 minutes at 25 °C. Prior to blending with the extract, the Folin-Ciocalteu reagent was diluted tenfold with distilled water. A standard calibration curve was made by making a standard gallic acid solution at several concentrations. Methanol was mixed with 10 mg of gallic acid and then was diluted to 2, 4, 8, 16, and 32 mg/L at 765 nm for both the sample and the standard. The total phenolic content was determined by milligrams of gallic acid equivalent (GAE) to grams of the dried extract [8]. The equation used to calculate total phenolic contents can be expressed as in the following formula.

Total Phenolic GAE =
$$\frac{C \times V}{DE}$$
,

where C = the concentration of total phenolics after measurement (mg/L), V = the sample volume in liters, and DE = the weight in grams of dried *C. ternatea* extract.

G. Determination of Total Flavonoid Content

10 mg of C. ternatea extract was dissolved in 10 mL of methanol. Next, 0.5 mL of methanol and the C. ternatea extract were separated and the C. ternatea was mixed into a solution of 0.5 mL methanol, 0.1 mL aluminum chloride, 0.1 mL sodium acetate, and 2.8 mL distilled water. A blank solution was also prepared using the same steps except that the aluminum chloride was replaced with distilled water. Both the sample solution and the blank solution were filtered using filter paper and then measured at 415 nm absorbance [8]. Standard quercetin solutions were prepared in several concentrations (5, 10, 20, 40, and 80 mg/L) to make a standard calibration curve by dissolving and methanol and 10 mg of quercetin and then diluting to obtain each of the concentrations at 415 nm. Total flavonoid contents were determined by milligrams of quercetin equivalent (QE) to grams of dried extract. The equation used to calculate total flavonoid content is

Total Flavonoid QE =
$$\frac{C \times V}{DE}$$
,

where C = concentration of total flavonoids after measurement (mg/L), V = sample volume in liters, and DE =weight in grams of dried *C. ternatea* extract.

H. Determination of Kaempferol and Quercetin Content

High-performance liquid chromatography (HPLC) was used to analyze 10 mg/L samples of kaempferol and quercetin [9]. The mobile phase consisted of methanol and orthophosphate 0.1% v/v (49:51) at a flow rate of 1 mL/minute and wavelength of 254 nm. The stationary phase used a C18 column (150 mm × 4.6 mm; 5 μ) to determine the retention time of each compound. Methanol under sonication was used to dissolve 50 mg of viscous extract of *C. ternatea* for 5 minutes at room temperature. Next, the dissolved extract was hydrolyzed with H₂SO₄ 7% at 80 °C for 30 minutes and then put into a 10 mL volumetric flask, diluted with methanol, and then filtered using a 0.45 μ m PTFE syringe filter. Finally, the sample was analyzed under condition above.

I. Determination of Antioxidant Activity (IC50)

Antioxidant activity (IC₅₀) was carried out using DPPH method. A stock solution of DPPH (1 mg/L in methanol) was prepared and stored at 18 °C. Next, 8 mL of the solution was diluted with 43 mL of methanol to achieve an absorbance of 1.1 at 515 nm. The extract of C. ternatea was made in five concentrations to determine the 50% inhibitory concentration (IC₅₀). For the DPPH reaction, 0.5 mL of C. ternatea extract solution, 0.5 mL of DPPH solution, and 1 mL methanol were mixed in an amber glass tube. This was necessary to make the control solution of a DPPH reaction by blending 0.5 mL methanol and 0.5 mL DPPH solution. This control solution then was mixed with 1 mL of methanol. Both solutions were incubated for 30 minutes at room temperature in a dark room. The mixture was calculated using а UV-VIS (UVmini-1240 spectrophotometer mini, Shimadzu Corporation, Kyoto, Japan) at 515 nm absorbance. A decline in absorbance represents an increase in radical scavenging activity, which can be expressed as

% Inhibition = $\frac{(absorbance of control - absorbance of test solution)}{absorbance of control}$

J. Identification Compounds of Clitoria ternatea Extract with Liquid Chromatography Mass Spectrometry Q-TOF

0,5 g of blue single petal of *C. ternatea* extract was dissolved in methanol and sonicated for 5 minutes at room temperature. Liquid extract was filtered using 0.45 μ m PTFE syringe filter. Extract was analyzed by UHPLC system connected with QTOF mass spectrometer [10]. The analysis conditions used were non-polar column C18 (150x4.6 mm), pore particle size 1.8 μ m and column temperature at 40°C. The mobile phase used were 0.1% formic acid in acetonitrile: 0.1% formic acid in 50:50 v/v aqua bides with a gradient elution system. Mobile phase flow rate was 0.6 mL / min. The screening process of LCMS-QTOF was carried out with UNIFI software.

K. Statistical Analysis

All data were subjected to analysis by one-way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) at the 5% significance level using SPSS v21 software.

III. RESULTS AND DISCUSSION

A. Extraction Yield

Differences in the drying methods and *C. ternatea* accessions had significant effects on extraction yield, as shown in Fig. 1. The different drying treatments with the WSP accession were not significantly different with shade drying at 44.23%, sun drying at 44.29%, and oven drying at 44.55%. However, the WSP accession had significantly greater yields with all of the drying methods than the other accessions. The BSP accession had the lowest extraction yield with all the drying treatments compared to the other accessions.

The extraction yields of ginger were not significantly different after drying with sun, oven, and freeze methods [11]. Moreover, they reported that the drying process caused the samples to become weak and easier to break down. The thermal processing weakened the integrity of the cell walls, making them more brittle. As a consequence, the homogenization process during extraction is more effective. Bioactive compounds are easily transferred into the solvent during extraction because the drying step has broken the cell wall. This result confirms that with WSP accessions, shade and sun drying have the advantage because they use less energy than drying with an oven

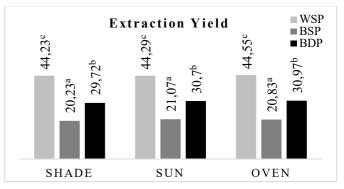


Fig. 1 Extraction yield (%) with different drying methods for three *Clitoria ternatea* accessions. When the average is followed by a letter, it means the results were significantly different according to Duncan's multiple range test (DMRT) at a 5% significance level.WSP = White single petal; BSP = blue single petal; BDP = blue double petal.

B. Total Phenolic Content

It can be seen from Fig. 2 that the treatment of sun drying, the BDP accession resulted in significantly higher total phenolic content (50.98 mg/g) than the other treatments, except for shade drying with BDP (50.84 mg/g) and sun drying with BSP (49.57 mg/g). Otherwise, the BSP accession oven drying (40.32 mg/g) resulted in the lowest total phenolic content of *C. ternatea*.

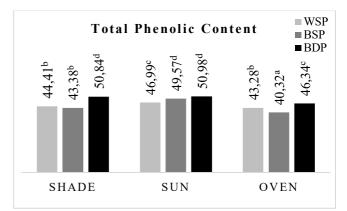


Fig. 2 Total phenolic content (mg GAE/g dried extract) with different drying methods for three *Clitoria ternatea* accessions. When the average is followed by a letter, it means the results were significantly different according to Duncan's multiple range test (DMRT) at a 5% significance level. WSP = White single petal; BSP = blue single petal; BDP = blue double petal.

In accordance with the present results, a previous study on spearmint demonstrated that a drying process using the combination of air and sun exposure resulted in less reduction of total phenolic content than drying by oven or drying in air without sun exposure [12]. In that study, drying spearmint in the sun resulted in higher temperatures than drying by air, leading to the inactivation of the polyphenol oxidase enzyme. Polyphenol oxidase is an enzyme that contributes to the degradation of phenolic compounds. Moreover, polyphenol oxidase activity decreased polyphenol levels at higher temperatures (55 °C), which color changes could see in apricot pulp [13]. Pineapple slices dried in a convective oven at 60 °C has also resulted in the lowest total phenolic content [14].

The BDP accession of *C. ternatea* showed higher total phenolic content than the other accessions, which might be caused by the anthocyanin contained in this accession. Anthocyanins are one of the phenolic compounds classified as polyphenols. The research showed that the BDP accession of *C. ternatea* has large amounts of ternatins, which are (poly)acylated anthocyanins [15]. Moreover, they revealed that different from the blue petaled accessions, white petaled *C. ternatea* had a lack of anthocyanins.

C. Total Flavonoid Content

The different drying methods and accessions of *C. ternatea* showed significant differences with respect to total flavonoid content, as shown in Fig. 3. The treatment of shade drying with WSP resulted in higher total flavonoid content (9.70 mg/g) than the other treatments, but there was no significant difference from the shade treatment with BDP (9.33 mg/g). Conversely, BSP treated in an oven resulted in the statistically significant lowest quantity of total flavonoids (7.03 mg/g).

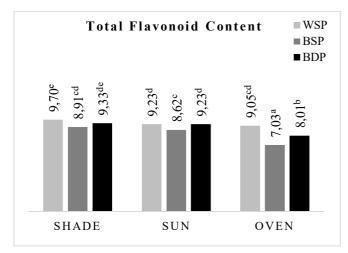


Fig. 3 Total flavonoid content (mg QE/g dried extract) with different drying methods for three *Clitoria ternatea* accessions. When the average is followed by a letter, it means the results were significantly different according to Duncan's multiple range test (DMRT) at a 5% significance level.WSP = White single petal; BSP = blue single petal; BDP = blue double petal.

The present findings seem consistent with another study that showed that shade drying resulted in slower water loss rates, causing higher total flavonoid levels than other drying methods, such as freeze drying, sun drying, and oven drying [16]. On the other hand, cocoa beans treated with oven drying had lower total flavonoid levels than sun drying or freeze drying [17].

This result may also be explained by the fact that flavonoids are sensitive to heat. The drying temperatures of natural methods are relatively lower than oven drying and may lead to less reduction of flavonoid content. Loss of flavonoids due to heating can cause harsh effects in samples and was significantly affected by the duration and temperature of the drying process. Heating processes could influence the phytochemical compounds in materials and alter cell walls' integrity. Furthermore, heating might result in the thermal breakdown of material components due to chemical reactions involving light, oxygen, and enzymes, causing a loss of some components [18].

D. Kaempferol and Quercetin Content

Kaempferol and quercetin are included in the polyphenol group, specifically, flavonoids, mainly found in fruits and vegetables. They are commonly embedded in sugar molecules, and the attachment type determines the bioavailability and bioactivity of those compounds and specific sugar molecules involved [19].

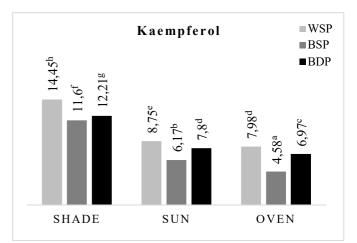


Fig 4. Total kaempferol (mg/g dried extract) with different drying methods for three *Clitoria ternatea* accessions. When the average is followed by a letter, it means the results were significantly different according to Duncan's multiple range test (DMRT) at a 5% significance level. WSP = White single petal; BSP = blue single petal; BDP = blue double petal.

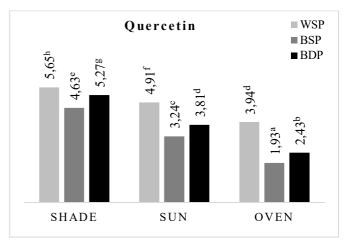


Fig. 5 Total quercetin (mg/g dried extract) with different drying methods for three *Clitoria ternatea* accessions. When the average is followed by a letter, it means the results were significantly different according to Duncan's multiple range test (DMRT) at a 5% significance level. WSP = White single petal; BSP = blue single petal; BDP = blue double petal.

Differences in the drying method and *C. ternatea* accession had significant effects on the quantities of kaempferol (Fig. 4) and quercetin (Fig. 5). The treatment of shade drying with the WSP accession showed the statistically significant highest levels of kaempferol (14.45 mg/g) and quercetin (5.65 mg/g), while oven drying with BSP had the lowest levels (4.58 mg/g and 1.93 mg/g, respectively).

The highest levels of kaempferol and quercetin when using shade drying with WSP accession in this study might be attributed to the drying temperature. Shade drying generated the lowest temperature of the drying methods because the drying force was obtained only from the air. Another research investigated the kaempferol levels in several fruits, and the results showed that with a higher drying temperature, there were lower kaempferol levels [20]. This trend could be seen on Eureka lemon seeds and kaempferol was even difficult to detect in Orlando oranges at 70 °C and 80 °C, respectively. Apple slices dried in an oven also showed the lowest quercetin content [21].

E. Antioxidant Activity

Antioxidant activity can be represented by the IC_{50} , which describes the sample concentration that can be used against 50% of free radicals using the DPPH method. Lower IC_{50} values indicate greater antioxidant activity in the measured sample.

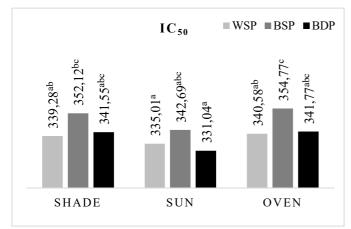


Fig. 6 Antioxidant activity (IC_{50} ; mg/L dried extract) with different drying methods for three *Clitoria ternatea* accessions. When the average is followed by a letter, it means the results were significantly different according to Duncan's multiple range test (DMRT) at a 5% significance level.WSP = White single petal; BSP = blue single petal; BDP = blue double petal.

In the present study, there was a significant effect according to the drying methods, and *C. ternatea* accessions

on IC₅₀ (Fig. 6). The treatment of oven drying with the BSP accession resulted in higher IC₅₀ values (354.77 mg/L), but no significant difference was found for shade drying with BSP (352.12 mg/L) and BDP (341.55 mg/L); sun drying with BSP (342.69 mg/L); or oven drying with BDP (341.77 mg/L). The treatment of sun drying with the BDP accession had significantly lower IC₅₀ values (331.04 mg/L) than shade drying with BSP (354.77 mg/L), while no significant difference was found in comparison to other treatments.

The treatment of sun drying with BDP showed better IC_{50} values. This result was in line with previous study that *Pimpinella anisum* L. seeds treated by sun drying had lower IC_{50} levels than those dried in an oven [22]. One of the factors that influence antioxidant activity is temperature, and phenolic compounds and antioxidants are relatively sensitive to thermal treatments

Total phenolic and flavonoid contents were closely related to antioxidant activity [23]. However, the level of relationship between antioxidant activity and total phenolic content is greater than with flavonoids due to their redox properties. Based on the total phenolic content shown in Fig. 5, the treatment of sun drying for the BDP accession resulted in higher total phenol content (50.98 mg/g), leading to the lowest IC₅₀ level, which indicates high levels of antioxidant activity. However, the IC₅₀ value for this treatment was not statistically significantly different from shade drying with WSP and BDP, sun drying with WSP and BSP, or oven drying with WSP and BDP.

F. Identification Compounds of Clitoria ternatea Blue Single Petal Extract

An earlier study revealed that the difference between white single petal (WSP) and blue single petal (BSP) or blue double petal (BDP) of *C. ternatea* was the content of ternatins, one of anthocyanin compounds [15]. All the petals had similar flavonoid compounds, while WSP had no ternatins. LCMS identification chromatogram and the metabolite compounds of BSP using methanol extract can be found in Fig. 7 and Table 1, respectively.

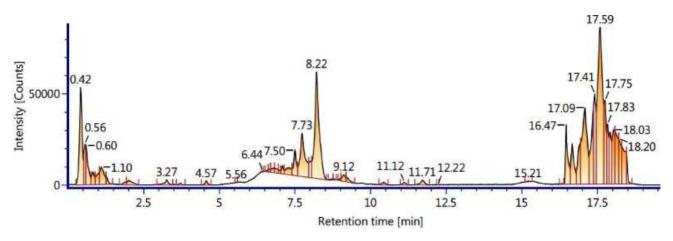


Fig. 7 LCMS blue single petal C. ternatea extract

TABLE I
LCMS COMPOUNDS DATA OF BLUE SINGLE PETAL OF C. TERNATEA EXTRACT

No	Compound	Formula	Retention Time (min)	Observed Mass (m/z) ([M-H] ⁻)	Response
1	2"-O-Acetyl-3'-O-methylrutin	C ₃₀ H ₃₄ O ₁₇	0.40	666.58076	254
2	Trigonelline	C7H7NO2	0.41	138.05577	29010
3	2α,3β,6exo-Trihydroxy nortropane	C7H13NO3	0.81	159.09292	3340
4	Adenosine	C10H13N5O4	1.28	268.10557	23749
5	Gentiatibetine	C9H11NO2	1.88	166.08683	6288
6	Nevadensin-7-O-[α -L-rhamnosyl(1 \rightarrow 6)]- β -D-glucoside	C30H36O16	4.96	651.19531	3008
7	3-O-[β -D-Glucopyra-nosyl-(1 \rightarrow 2)]- β -D-glucopyranosyl-7-O- α -Lglucopyranosyl-kaempferol	C33H40O20	5.50	755.20807	3654
8	Cyclolaudenol	C33H40O21	6.82	771.2086	17290
9	7-α-L Rhamnosyl kaempferol 3-O-β-D- glucopyranosyl(1 \rightarrow 6)-β-Dglucopyranoside	$C_{33}H_{40}O_{20}$	7.48	755.20525	325032
10	Isorhamnetin-3-gentiobioside-7-glucoside	C34H42O22	7.48	791.18041	11041
11	Quercetin-3-galactoside-7-glucoside	C ₂₇ H ₃₀ O ₁₇	7.49	625.14803	22565
12	6-Hydroxykaempferol-7-O-glucoside	$C_{21}H_{20}O_{12}$	7.51	465.10446	28900
13	Cyanidin 3,5-diglucoside 1	C ₂₇ H ₃₀ O ₁₆	7.51	611.16061	54828
14	Robinetin	C15H10O7	7.51	303.05035	77982
15	Isohyperoside	$C_{21}H_{20}O_{13}$	7.59	479.08791	10717
16	Luteolin	C15H10O6	7.74	287.05506	121617
17	3-O-[β-D-Glucopyra-nosyl- $(1\rightarrow 2)$]-β-D-glucopyranosyl- kaempferol	C ₂₇ H ₃₀ O ₁₆	7.90	609.14648	407779
18	Cyanin	$C_{27}H_{31}C_1O_{16}$	7.90	645.12231	20309
19	Grosvenorine	C33H40O19	8.12	740.21361	615365
20	Luteolin-7-O-β-D-glucopyranoside	C ₂₁ H ₂₀ O ₁₁	8.22	449.10851	88230
21	Isorhamnetin-3-O-β-gentiobioside	C ₂₈ H ₃₂ O ₁₇	8.40	639.15631	36895
22	7-O-α-L-Rhamnosyl-3-O-β-D-glucopyranosyl kaempferol	C27H30O15	8.61	593.15105	639661
23	Cyanidin-3-rutinoside	C ₂₇ H ₃₁ C ₁ O ₁₅	8.61	629.13432	47510
24	2"-O-Acetylrutin	C29H32O17	8.66	651.16278	19456
25	Quercetin-3-O-β-D-glucopyranoside	C21H20O12	8.70	463.08873	132289
26	Limocitrin-3,7-O-β-D-glucopyranoside	C ₂₉ H ₃₄ O ₁₈	8.81	669.16671	3314
27	Luteolin-7-O-[β -D-apiofuranosyl(1 \rightarrow 6)] β -D-glucopyranoside	$C_{26}H_{28}O_{15}$	8.85	579.14028	5142
28	Quercetin-3-O-(2G-α-L-rhamnosyl)-rutinoside	C33H40O20	8.95	755.21223	34722
29	Luteolin 7-beta-neohesperidoside	$C_{27}H_{30}O_{15}$	9.08	593.15499	108337
30	Quercetin-3-O-(6"-O-acetyl)-β-D-glucopyranoside	$C_{23}H_{22}O_{13}$	9.26	505.10296	7933
31	Apigenin-6,8-di-C-glucoside	$C_{27}H_{30}O_{15}$	9.40	593.15122	65986
32	Viscumneoside IV	$C_{29}H_{32}O_{16}$	9.50	635.16354	60392
33	Schaftoside	$C_{26}H_{28}O_{14}$	9.76	563.15477	3595
34	Luteolin 7-O-β-D-(6"-acetyl)-glucopyranoside	$C_{23}H_{22}O_{12}$	10.39	489.1082	4325
35	Quercetin	C15H10O7	12.10	301.03571	57973
36	Menthoside	C36H36O17	12.10	739.1973	4386
37	5,7,8,4'-Tetrahydroxy flavone	$C_{15}H_{10}O_6$	14.01	286.04644	13017
38	3'-Methoxydaidzein	$C_{16}H_{12}O_5$	16.48	283.10376	774

Identification compounds of blue single petal of C. ternatea in methanol extract showed that dominant metabolites found were flavonoid groups; quercetin (Quercetin-3-O-β-D-glucopyranoside, 7-α-L Rhamnosyl kaempferol 3-O- β -D-glucopyranosyl(1 \rightarrow 6)- β -Dglucopyranoside), kaempferol (3-O-[β-D-Glucopyra-nosyl- $(1\rightarrow 2)$]- β -D-glucopyranosyl-kaempferol), 7-O-α-L-Rhamnosyl-3-O-β-D-glucopyranosyl kaempferol), and luteolin (Luteolin 7-beta-neohesperidoside). Quercetin could be utilized as anti-oncology and cardiovascular protection and anti-immunosuppression treatment; and had potential to prevent toxicity of mycotoxins [24]. Kaempferol also was known as anti-inflamatory [25]. On the other hand, luteolin had potential effects as anti-cancer under both in vitro and in vivo conditions [26]. Grosvenorine, one of flavonoid glycoside compounds, also dominant found on our study. Meanwhile, the pigment compounds caused blue colour on C.

ternatea petals were Cyanidin 3,5-diglucoside_1, Cyanin, and Cyanidin-3-rutinoside.

IV. CONCLUSION

This study was designed to determine the best combination of drying method and *C. ternatea* accession for producing the best quality and highest levels of extracts. The LCMS analysis of blue single petal of *C. ternatea* in methanol extract showed that dominant metabolites found were flavonoid groups; quercetin, kaempferol, and luteolin. The results showed that the treatment of shade drying with the WSP accession of *C. ternatea* resulted in the best extraction yield and total flavonoid, kaempferol, and quercetin levels. Although this treatment had lower total phenolic content than sun drying of the BDP accession, the antioxidant activity was as high as sun drying with BDP. Shade drying could be good alternative for drying *C. ternatea*, particularly the WSP accession, due to its affordability.

ACKNOWLEDGMENT

The authors sincerely appreciate Virda, Ine, Diana, who contributed to providing materials for this research.

REFERENCES

- N. V. Kalmankar, R. Venkatesan, P. Balaram, and R. Sowdhamini, "Transcriptomic profiling of the medicinal plant *Clitoria ternatea*: identification of potential genes in cyclotide biosynthesis," *Sci. Rep.*, vol. 10, no. 1, pp. 1–20, 2020, doi: 10.1038/s41598-020-69452-7.
- [2] P. Chayaratanasin *et al.*, "*Clitoria ternatea* flower petal extract inhibits adipogenesis and lipid accumulation in 3T3-L1 preadipocytes by downregulating adipogenic gene expression," *Molecules*, vol. 24, no. 10, pp. 1–16, 2019, doi: 10.3390/molecules24101894.
- [3] B. Salehi *et al.*, "Plant-derived bioactives and oxidative stress-related disorders: A key trend towards healthy aging and longevity promotion," *Appl. Sci.*, vol. 10, no. 3, pp. 1–26, 2020, doi: 10.3390/app10030947.
- [4] M. Saifullah, R. Mccullum, A. Mccluskey, and Q. Vuong, "Effects of different drying methods on extractable phenolic compounds and antioxidant properties from lemon myrtle dried leaves," *Heliyon*, vol. 5, no. 8, pp. 1–8, 2019, doi: 10.1016/j.heliyon.2019.e03044.
- [5] R. P. F. Guiné, "The drying of foods and its effect on the physicalchemical, sensorial and nutritional properties," *Int. J. Food Eng.*, vol. 4, no. 2, pp. 93–100, 2018, doi: 10.18178/ijfe.4.2.93-100.
- [6] A. A. Halim, Z. M. Zain, A. Mubarak, and F. T. Ahmad, "Effect of different drying methods on antioxidant properties, stevioside and rebaudioside A contents of stevia (*Stevia rebaudiana* bertoni) leaves," *Asian. J. Agric. Biol*, vol. 7, no. 1, pp. 61–68, 2019.
- [7] Y. Xing, H. Lei, J. Wang, Y. Wang, J. Wang, and H. Xu, "Effects of different drying methods on the total phenolic, rosmarinic acid and essential oil of purple perilla leaves," *J. Essent. Oil Bear. Plants*, vol. 20, no. 6, pp. 1594–1606, 2017, doi: 10.1080/0972060X.2017.1413957.
- [8] O. Sytar, I. Hemmerich, M. Zivcak, C. Rauh, and M. Brestic, "Comparative analysis of bioactive phenolic compounds composition from 26 medicinal plants," *Saudi J. Biol. Sci.*, vol. 25, no. 4, pp. 631– 641, 2018, doi: 10.1016/j.sjbs.2016.01.036.
- [9] J. Makasana, B. Z. Dholakiya, N. A. Gajbhiye, and S. Raju, "Extractive determination of bioactive flavonoids from butterfly pea (*Clitoria ternatea* Linn.)," *Res. Chem. Intermed.*, vol. 43, no. 2, pp. 783–799, 2017, doi: 10.1007/s11164-016-2664-y.
- [10] C. Cortés-Herrera, G. Artavia, A. Leiva, and F. Granados-Chinchilla, "Liquid chromatography analysis of common nutritional components, in feed and food," *Foods*, vol. 8, no. 1, pp. 1–63, 2019, doi: 10.3390/foods8010001.
- [11] I. Mustafa, N. L. Chin, S. Fakurazi, and A. Palanisamy, "Comparison of phytochemicals, antioxidant and anti-inflammatory properties of sun-, oven- and freeze-dried ginger extracts," *Foods*, vol. 8, no. 10, pp. 1–11, 2019, doi: 10.3390/foods8100456.
- [12] A. Orphanides, V. Goulas, and V. Gekas, "Effect of drying method on the phenolic content and antioxidant capacity of spearmint," *Czech J.*

Food Sci., vol. 31, no. 5, pp. 509–513, 2013, doi: 10.17221/526/2012-CJFS.

- [13] M. A. Madrau *et al.*, "Effect of drying temperature on polyphenolic content and antioxidant activity of apricots," *Eur. Food.Res. Technol*, vol. 228, pp. 441–448, 2009, doi: 10.1007/s00217-008-0951-6.
- [14] N. Izli, G. Izli, and O. Taskin, "Impact of different drying methods on the drying kinetics, color, total phenolic content and antioxidant capacity of pineapple," *CyTA - J. Food*, vol. 16, no. 1, pp. 213–221, 2018, doi: 10.1080/19476337.2017.1381174.
- [15] K. Kazuma, N. Noda, and M. Suzuki, "Flavonoid composition related to petal color in different lines of *Clitoria ternatea*," *Phytochemistry*, vol. 64, no. 6, pp. 1133–1139, 2003, doi: 10.1016/S0031-9422(03)00504-1.
- [16] X. Zhang, X. Wang, M. Wang, J. Cao, J. Xiao, and Q. Wang, "Effects of different pretreatments on flavonoids and antioxidant activity of *Dryopteris erythrosora* leave," *PLoS One*, vol. 14, no. 1, pp. 1–17, 2019, doi: 10.1371/journal.pone.0200174.
- [17] H. Majid and H. A. Rining, "The effect of drying techniques on the antioxidant capacity, flavonoids and phenolic content of fermented local cocoa bean," *J. Adv. Res. Appl. Mech.*, vol. 47, no. 1, pp. 11–19, 2018.
- [18] M. K. M. Zainol, A. Abdul-Hamid, F. A. Bakar, and S. P. Dek, "Effect of different drying methods on the degradation of selected flavonoids in *Centella asiatica*," *Int. Food Res. J.*, vol. 16, pp. 531–537, 2009.
- [19] W. M. Dabeek and M. V. Marra, "Dietary quercetin and kaempferol: bioavailability in humans," *Nutrients*, vol. 11, no. 10, pp. 1–19, 2019, doi: 10.3390/nu11102288.
- [20] F. Al Juhaimi, M. M. Özcan, N. Uslu, and K. Ghafoor, "The effect of drying temperatures on antioxidant activity, phenolic compounds, fatty acid composition and tocopherol contents in citrus seed and oils," *J. Food Sci. Technol.*, vol. 55, no. 1, pp. 190–197, 2018, doi: 10.1007/s13197-017-2895-y.
- [21] B. Schulze, E. M. Hubbermann, and K. Schwarz, "Stability of quercetin derivatives in vacuum impregnated apple slices after drying (microwave vacuum drying, air drying, freeze drying) and storage," *LWT - Food Sci. Technol.*, vol. 57, no. 1, pp. 426–433, 2014, doi: 10.1016/j.lwt.2013.11.021.
- [22] I. B. Rebey *et al.*, "On the effect of initial drying techniques on essential oil composition, phenolic compound and antioxidant properties of anise (*Pimpinella anisum* L.) seeds," *J. Food Meas. Charact.*, vol. 14, pp. 220–228, 2019, doi: 10.1007/s11694-019-00284-4.
- [23] B. Kunarto and E. Y. Sani, "Antioxidant activity of extract from ultrasonic-assisted extraction of durian peels," *J. Appl. Food Technol.*, vol. 5, no. 2, pp. 25–29, 2018, doi: 10.17728/jaft.3309.
- [24] D. Yang, T. Wang, M. Long, and P. Li, "Quercetin: its main pharmacological activity and potential application in clinical medicine," Oxid. Med. Cell. Longev., vol. 2020, pp. 1–13, 2020, doi: 10.1155/2020/8825387.
- [25] W. Alam, H. Khan, M. A. Shah, O. Cauli, and L. Saso, "Kaempferol as a dietary anti-inflammatory agent: current therapeutic standing," *Molecules*, vol. 25, no. 18, pp. 1–12, 2020, doi: 10.3390/molecules25184073.
- [26] M. Imran *et al.*, "Luteolin, a flavonoid, as an anticancer agent: A review," *Biomed. Pharmacother.*, vol. 112, pp. 1–10, 2019, doi: 10.1016/j.biopha.2019.108612.