INTRODUCTION

The genus Syzygium is known as the general basis of complementary medicines because of its abundant bioactive compounds (Chua et al. 2019). This genus also has many benefits for human health such as antioxidant, anticancer, anti-diabetic, anti-inflammatory, anti-fungal, antibacterial (Annadurai et al. 2012), antiviral, anti-HIV, anti-diarrheal (Abera et al. 2018), and reduce the blood triglyceride (Nugroho et al. 2012). Various metabolites have been found that act as important exogenous antioxidants, including phenolic compounds (e.g. phenolic acids, coumarins, flavonoids, lignans, stilbenes, tannins), terpenoids (e.g. carotenoids), and vitamins (e.g. vitamin C, vitamin E) (Baiano and Del Nobile 2016). The genus Syzygium is reported to contain phenolic compounds with antioxidant activity such as chalcones, flavonoids, lignans, alkyl phosphogluconols, hydroxylable tannins, and chromone derivatives (Memon et al. 2015). In addition, many terpenoid compounds that have antioxidant properties such as oleanolic acids and betulinic acids are also found in the genus Syzygium (Chua et al. 2019). Previous studies showed that several species of the genus Syzygium have been reported as potent antioxidants and anticancer (Twilley et al. 2017; Mahomoodally et al. 2020). The ethanolic extract of Syzygium aromaticum flower buds has high antioxidant activity, which is almost the same as ascorbic acid activity (Singh et al. 2018).

Syzygium zollingerianum was first discovered by Miquel in 1855 on a riverside of Sumbawa Island, Indonesia. Based on the recent report, S. zollingerianum (Miq.) Amshoff has only been discovered in Indonesia and distributed in Sumatra and the Lesser Sunda Islands such as Java, Bali, and Sumbawa. This plant is found at the altitudes between 400-475 MASL on the island of Sumatra, Java (e.g. Mount Slamet) at the altitudes of 700-1000 MASL, and on Bukit Tapak Bali at the altitudes higher than 1000 MASL (Widodo et al. 2011; Dharma et al. 2017). In Sumatra, it is found in Sibolangit, Lampung, Mount Reti Berenong, and Simeulue Island (Widodo et al. 2011), and it is also found in the western part of Central Java (Backer and Brink 1963), and Kalimantan (Royyani and Efendy 2015). However, studies on phytochemical profiles and potential bioactivity of S. zollingerianum have not been carried out in any previous research. Therefore, this study was carried out to determine the phytochemical content of ethanolic extract of S. zollingerianum leaves, antioxidant properties, and its cytotoxic effects on Vero cell lines.

MATERIALS AND METHODS

Preparation of sample and extraction

Mature leaves of S. zollingerianum were collected from the top of hills in Sepang Village, Busungbiu District, Buleleng Regency, Bali, Indonesia. The leaves were
cleaned with water and air-dried at room temperature. The dried leaves were ground with a blender to obtain leaf powder. The leaf powder was macerated with 70% ethanol (1:10 w/v) and stirred with an orbital shaker for 3 days. The solution was filtered using Whatman filter paper No.1 and the filtrate was evaporated using a rotary evaporator (50-65°C) for about 4 hours. The dark brown concentrated extract was dried using an incubator at a temperature of 50°C and stored in a refrigerator.

**Determination of total phenolics content (TPC)**

50 mg of sample extract was mixed with 0.5 mL of Folin-Ciocalteu reagent and 7.5 mL of distilled water. The mixture was allowed to stand at room temperature for 10 minutes. Afterward, 1.5 mL of 20% Na₂CO₃ was added. Distilled water was added until it reached 10 mL of volume. The absorbance was measured at a wavelength of 760 nm (Kumar et al. 2017). A standard curve was obtained from various concentrations of gallic acid (6.25, 12.5, 25, 50 µg/mL).

**Determination of total flavonoids content (TFC)**

50 mg of sample extract was mixed with 0.3 mL of 5% NaNO₂, left for 5 minutes and as much as 0.6 mL of 10% AlCl₃ was added and left for 5 minutes. Two mL of 1 M NaOH was added, followed by the addition of distilled water until it reached 10 mL of volume (Lim et al. 2019). The absorbance was measured at a wavelength of 510 nm. A standard curve was obtained from various concentrations of quercetin (0.5, 1, 2, 5, 10, 25, 50, 75, 100 µg/mL).

**Determination of total alkaloids content (TAC)**

100 mg of extract was added with 5 mL of 2N HCl. The solution was washed with 10 mL of chloroform 3 times in a funnel separator and then the chloroform phase was discarded. The solution was neutralized by adding 0.1 N NaOH. After that, 5 mL of bromocresol green solution and 5 mL of phosphate buffer were added. The solution was extracted with 5 mL of chloroform 2 times and stirred using a magnetic stirrer at 500 rpm for 15 minutes. The chloroform phase was collected and evaporated with nitrogen gas. The extract was added with chloroform until it reached 10 mL of volume and diluted 5 times. The absorbance was measured at a wavelength of 470 nm (Tabasum et al. 2016). A standard curve was obtained from various concentrations of quinine (3.125, 6.25, 12.5, 25, 50, 100, 200, 400 µg/mL).

**Determination of total tannins content (TTC)**

50 mg of sample extract was extracted with 10 mL of diethyl ether for 20 hours by maceration. The extract was filtered and diethyl ether was evaporated. Distilled water was added until it reached 10 mL of volume. One mL of sample solution was added with 0.1 mL of Folin Ciocalteu reagent and vortexed for 5 minutes. After that, 2 mL of 20% Na₂CO₃ was added and vortexed for 5 minutes, followed by the addition of distilled water until the volume reached 10 mL. The solution was diluted 20 times and incubated for 30 minutes at room temperature. The absorbance was measured at a wavelength of 760 nm (Wahyuni et al. 2020). A standard curve was obtained from various concentrations of tannic acid (0.125, 0.25, 0.5, 1, 2, 4, 8, 16 µg/mL).

**Antioxidant activity assay**

The antioxidant activity assay was carried out by the DPPH method based on Zhou et al (2020) with slight modifications. The extract was made with various concentrations (1, 3, 6, 9, 12, 15 µg/mL). Vitamin C (ascorbic acid) was used as a standard at the concentrations of 0.5, 1, 2, 4, 6, 8 µg/mL. One mL of each concentration of extract and the ascorbic acid solution was mixed with 1 mL of 0.1 mM DPPH solution in methanol (1:1 v/v). Methanol was used as a blank and 0.1 mM DPPH solution was used as a control. The absorbance was measured at a wavelength of 517 nm after incubating at room temperature in dark conditions for 30 minutes. Analysis was carried out in triplicate for each concentration of extract and standard. The radical scavenging activity was calculated using formula [1]. The 50% inhibitory concentration (IC₅₀) of extract that can scavenge 50% of DPPH radicals was calculated by linear regression. The antioxidant activity of the extract was expressed as the antioxidant activity index (AAI) which was calculated by the formula [2].

\[
\text{Scavenging activity (\%)} = \left(\frac{\text{A}_{\text{control}} - \text{A}_{\text{extract}}}{\text{A}_{\text{control}}}\right) \times 100\%
\]

Where: \(\text{Ac}\) was the absorbance of DPPH control, \(\text{As}\) was the absorbance of DPPH mixed with extract or ascorbic acid solution

\[
\text{AAI} = \frac{\text{IC}_{50} (\mu g/mL)}{\text{Concentration (\mu g/mL)}}
\]

**MTT assay for cytotoxicity assessment**

Vero cell lines were cultured with Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS) and 1% antimycotic antibiotic solution (10000 units/mL penicillin, 10 mg/mL streptomycin, and 25 µg/mL amphotericin B). Vero cell lines were seeded in 96 well plates and incubated at 37°C, 95% humidity, and 5% of CO₂. Treatment with *S. zollingerianum* extract and doxorubicin was performed when the cells had reached 80-90% confluence. Concentrated extracts (5000 µg/mL) were serially diluted in methanol. MTT solution (0.5 mg/mL) was added after the cells were exposed to the test solution for 24 hours. The cells were incubated for 4 hours. After incubation, 10% SDS in 0.01 N HCl was added and incubated overnight in dark conditions. The absorbance was measured at a wavelength of 595 nm. The percentage of viable cells was calculated using formula [3]. The cytotoxicity of the extract was expressed as IC₅₀ as the concentration of the extract that caused inhibition of metabolic activity in 50% of the cell.

\[
\text{Viable Cells (\%)} = \left(\frac{\text{A}_{VC} - \text{A}_{IC}}{\text{A}_{VC} - \text{A}_{MC}}\right) \times 100\%
\]

Where: \(\text{A}_{TC}\) is the absorbance of the medium containing treated cells, \(\text{A}_{IC}\) is the absorbance of the medium containing untreated cells, \(\text{A}_{MC}\) is the absorbance of the medium without cells.
Data analysis
Quantitative data were statistically analyzed using SPSS version 17 and Microsoft Office Excel Professional Plus 2016. IC₅₀ values were calculated by linear regression using Microsoft Excel. The differences of IC₅₀ values between extracts and positive controls were statistically analyzed using unpaired t-test analysis at significance levels of p < 0.05. The differences in phytochemical contents were analyzed using one-way ANOVA followed by the Duncan test at a significance level of p < 0.05. Data were presented as mean ± standard deviation (SD) based on 3 replications.

RESULTS AND DISCUSSION
Phytochemical content of S. zollingerianum leaves extract
The quantitative analysis of phytochemical content focused on total phenolic, flavonoid, alkaloid, and tannin content using spectrophotometric methods. Statistical analysis showed that the chemical compounds were significantly different (p < 0.05) (Table 1). The total tannin content of ethanolic extract of S. zollingerianum leaves was significantly higher (p < 0.05) compared to total phenolic, flavonoid, and total alkaloids content.

Antioxidant activities
The results of the DPPH free radical scavenging method showed that S. zollingerianum leaves extract possesses antioxidant activity. The IC₅₀ value of ethanolic extract of S. zollingerianum was not significantly different (p > 0.05) from ascorbic acid as a standard antioxidant. Antioxidant Activity Index (AAI) value of extract and standard were > 2 (Table 2) indicates strong antioxidant activity.

Cytotoxic activities
The cytotoxic activity of S. zollingerianum leaves extract was evaluated on Vero cell lines. Figure 1 shows the viability of Vero cells based on the log dose series. The IC₅₀ values for cytotoxic activity of S. zollingerianum leaves extract were 101.42 ± 6.823 µg/mL. This value was significantly higher than doxorubicin (23.79 ± 3.659 µg/mL). This indicated that S. zollingerianum leaves extract was significantly less toxic (p < 0.05) compared to doxorubicin on the Vero cell line (Figure 2).

Discussion
The bioactive potential of plant extracts is closely related to their phytochemical composition. Quantitative analysis of bioactive compounds from ethanolic extract of S. zollingerianum leaves showed a significant difference in content (p < 0.05) of the analyzed chemical compounds (Table 1). Several factors affected the distribution and accumulation of bioactive compounds in plants, i.e., environmental factors (e.g., air temperature, intensity and quality of light, rainfall, humidity, soil type, and composition), the stage of plant development (Li et al. 2020), the presence of microbes, the threat of predators, etc. (Mohiuddin 2019). However, the composition of bioactive compounds is also closely related to a taxon (i.e. genes, enzymes) (Li et al. 2020). Chemical compounds in the plant may be more common or unique in certain genera and species and can be similar within genus and family (Liu et al. 2017). On the other hand, the composition of compounds obtained from plant extracts is also determined by the part of the plant being used as extract (Li et al. 2020), the type of solvent, and the extraction method (Hosyar et al. 2016).

Our study showed that the total tannin content (23.71 ± 0.076% w/w) was significantly higher (p < 0.05) than the other analyzed compounds (Table 1). Based on research by Rezende et al. (2015), high levels of tannins in S. jambos have a negative correlation with temperature. A high level of phenolics, including tannins in the leaves of S. jambos, may be associated with the increase of phenylalanine ammonia-lyase (PAL) activity at low temperatures (Rezende et al. 2015). Similar to this study, the leaves of S. zollingerianum were collected from a tree that grew on hilltops (more than 700 MASL) with an air temperature range of 17-26°C at the time of collecting samples, therefore phenolic and tannin content were also increased along with increasing CO₂ levels and greater light intensity (Kraus et al. 2003). However, investigations into other abiotic and biotic factors are still needed.

Phenolic compounds have been known to have excellent antioxidant abilities through the number and position of phenolic hydroxyls, methoxy, and carboxylic acid groups (Chen et al. 2020). Phenolic compounds that have been found in the genus Syzygium include gallic acid and this compound is widely used as a positive control for antioxidant activity tests with an IC₅₀ value of 25.0 ± 0.1 µM by DPPH method (Simirgiotis et al. 2008). The total phenolic content in the leaves extract of S. zollingerianum (5.97 ± 0.284% w/w) (Table 1) was similar to S. samarangense (5.99±0.061% w/w), but higher than leaves extract of S. aequum (3.91 ± 0.055% w/w) and lower than S. cumini (8.52 ± 0.055% w/w), S. jambos (7.59± 0.040% w/w), and S. malaccense (6.11± 0.083% w/w) (Sheela and Cheenickal 2017). Several studies have shown a significant positive correlation between the total phenolic contents and the antioxidant activity in several plant tissues in the genus Syzygium (Sultana et al. 2014; Sathyaranayanan et al. 2018). So, the antioxidant activity of the leaves extracts of S. zollingerianum seems to have a positive correlation with the total phenolic content.

Table 1. Phytochemical content of ethanolic extract of S. zollingerianum leaves

| Compound | Content (% w/w) |
|----------|-----------------|
| Phenolics | 5.97 ± 0.284a   |
| Flavonoids| 8.00 ± 0.515b   |
| Alkaloids | 1.44 ± 0.014c   |
| Tannins  | 23.71 ± 0.076d   |

Note: All data expressed as mean ± SD; Differ significantly by one-way ANOVA and followed by Duncan test to compare the mean difference between each group (p < 0.05). Mean ± SD with different letters showed significantly different values.
Table 2. The IC₅₀ value and AAI of ethanolic extract of S. zollingerianum leaves for antioxidant activity

| Sample             | IC₅₀(µg/mL) | AAI     |
|--------------------|-------------|---------|
| S. zollingerianum  | 0.57 ± 0.211| 68.75 ± 31.386 |
| Ascorbic acid      | 0.70 ± 0.423| 56.06 ± 36.580 |

Note: All data expressed as mean ± SD; Not significantly different by unpaired t-test analysis at p-value < 0.05. Mean ± SD with the same letter showed no significantly different value.

Figure 1. The effect of S. zollingerianum leaves extract on cell viability of Vero cell line. The data represented 3 independent experiments.

Figure 2. The IC₅₀ value for the cytotoxic effect of S. zollingerianum leaves extract compared to currently used chemotherapy agent Doxorubicin on Vero cell line. All data expressed as mean ± SD based on 3 replicates (n=3); significantly different by unpaired t-test analysis at p-value <0.05*

The leaves extract of S. zollingerianum has the highest total flavonoid content (8.00 ± 0.515% w/w) (Table 1) than other Syzygium species that were previously reported by Sheela and Cheenickal (2017), including the leaves extract of S. aqueum (0.423± 0.021% w/w), S. cumini (0.468± 0.005% w/w), S. jambos (0.496± 0.014% w/w), S. malaccense (1.044± 0.007% w/w), and S. samarangense (1.117± 0.006 w/w). Several studies have confirmed that several compounds belonging to the flavonoid group are found in the Syzygium genus including quercetin (Batista et al. 2017) and myricetin, (Nguyen et al. 2016). Quercetin is a compound with very strong antioxidant activities (IC₅₀ 0.87 µg/mL) on DPPH assay (Meda et al. 2005) and it was used as a standard to determine the flavonoid content in this study. According to Eshwarappa et al. (2014), the antioxidant activity of S. cumini leaf gall extracts has a strong correlation to both the total phenolic and flavonoid contents. In this study, the total flavonoid content may also have a positive correlation with the antioxidant activity of the S. zollingerianum extract.

The total alkaloid content in the extract of S. zollingerianum leaves (1.44 ± 0.014% w/w) (Table 1) was lower than that of S. cumini leaves extract (3.326 ± 0.235% w/w) (Zahra et al. 2019). The antioxidant activity of alkaloids may be determined by the number of aromatic hydroxyl groups. At the cellular level, the antioxidant effects of alkaloids can influence oxidative stress pathways through several possible mechanisms described as follows: inhibition of synthesis, activation, or translocation of NADPH-oxidase subunits; activation of the nuclear factor Nrf2; activation of transcription factors FOXOs and PPARs; epigenetic effects (influence on histone acetylation/methylation, DNA methylation, or expression of microRNA); and directly inhibits the myeloperoxidase (Macáková et al. 2019). The alkaloids compounds such as sanguinarine that have been isolated from S. aromaticum (Batiha et al. 2020) have potential as antioxidants, anti-inflammatory, proapoptotic, and growth inhibitory agents on various cancer cells, and have antiangiogenic and anti-invasive properties (Fu et al. 2018).

The total tannin content from S. zollingerianum leaves extract (23.71 ± 0.076% w/w) (Table 1) was three times higher than in the leaves extract of S. cumini (7.968 ± 0.164% w/w) (Silva et al. 2021). Medini et al. (2018) reported that biological factors, environmental conditions, extraction methods, and solvent will affect different metabolic content and amount. The tannin content of S. guineense showed very strong antioxidant activity with an IC₅₀ 4.5 ± 0.3 µM (Nguyen et al. 2016). Therefore, we suspect that the presence of tannins has a major influence on the results of the antioxidant activity of the leaves extract of S. zollingerianum as shown in Table 2 (AAI 68.75 ± 31.386, IC₅₀ 0.57 ± 0.211 µg/mL).

The antioxidant activity of S. zollingerianum leaves extract was classified as very strong (AAI > 2.0) according to Scherer and Godoy (2016). The AAI value of S. zollingerianum leaves extract was not significantly different (p>0.05) with ascorbic acid (56.06 ± 36.580). It explains that the leaves extract of this plant has a very strong antioxidant activity and even higher than ascorbic acid. This activity is closely related to the compounds contained in the leaves of this plant. Although the abundance of tannins may have a major influence on the antioxidant activity of S. zollingerianum leaves extract, however, the accumulative or synergistic effects of the other compounds should be considered.
We also found that the leaves extract of *S. zollingerianum* had moderate cytotoxic activity (IC₅₀ 101.42 ± 6.823 µg/mL) compared to doxorubicin on the Vero cell. Based on U.S. National Cancer Institute (NCI) and Geran protocol, the cytotoxic activity of the compound was classified as follows; very high cytotoxic activity if the IC₅₀ value is <20 µg/mL, moderate cytotoxic if IC₅₀ is ranged between 21 and 200 µg/mL, weak cytotoxic if IC₅₀ is ranged between 201 and 500 µg/mL, and not toxic if IC₅₀ >501 µg/mL (Sajjadi et al. 2015). However, it should be considered that compounds in plants might have synergistic, antagonistic, or accumulative effects (Martin et al. 2021) when they act as bioactive agents.

This study showed that the leaves extract of *S. zollingerianum* has the potential to be a promising source of natural exogenous antioxidants agents due to its strong antioxidant activity and moderate cytotoxic effect on Vero cell lines. Therefore, further studies are needed to isolate the potential chemical compounds and determine the antioxidant potential from pure compounds or fractions from leaves extract of *S. zollingerianum*.

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REFERENCES

Abera B, Adane L, Mamo F. 2018. Phytochemical investigation the root extract of *Syzygium guineense* and isolation of 2, 3, 23-trihydroxy methyl oleanate. J Pharmacogn Phytochem 7 (2): 3104-3111.

Amadurzai G, Masilla J, Jothiramalekar S, Palanisami E, Puthayparayil S, Parida AK. 2012. Antimicrobial, antioxidant, anticancer activities of *Syzygium caryophyllatum* (L.) Alston. Int J Green Pharm 2012 (6): 285-288. DOI: 10.4103/0973-8258.108210.

Bucker CA, Bakhuizen van der Brink RC, 1963. Flora of Java I. NVP Noordhoff, Groningen.

Buano A, Del Nobile MA. 2016. Antioxidant compounds from vegetable matrices: Biosynthesis, occurrence, and extraction systems. Crit Rev Food Sci Nutr 56 (12): 2053-2068. DOI: 10.1080/10408398.2013.812059.

Bathia GES, Alkaazmi LM, Wasef LG, Beshbishi AM, Nadwa EH, Rashawaa EK. 2020. *Syzygium aromaticum* L.(Myrtaceae): Traditional uses, bioactive chemical constituents, pharmacological and toxicological activities. Biomol 10: 202. DOI: 10.3390/biom10020202.

Batista A, da Silva JL, Cazarin C, Biasoto A, Sawaya A, Prado MA, junior MRM. 2017. Red-jambo (*Syzygium malaaccense*): Bioactive compounds in fruits and leaves. LWT-Food Sci Tech 76 (2017): 284-291. DOI: 10.1016/j.lwt.2016.05.013.

Chen J, Yang J, Ma L, Li J, Shahzad N, Kim CK. 2020. Structure-antioxidant activity relationship of methoxy, phenolic hydroxyl, and carboxylic acid groups of phenolic acids. Sci Rep 10: 2611. DOI: 10.1038/s41598-020-59451-z.

Chua IK, Lim CL, Ling APK, Chye SM, Koh RY. 2019. Anticancer potential of *Syzygium* species: A review. Plant Foods Hum Nutr 74 (1): 18-27. DOI: 10.1007/s11130-018-0704-z.

Dharma IDP, Solihah SM, Kuswantoro F, Yuzammi. 2017. Koleksi Kebun Raya Lombok: Tumbuhan Sunda Kecil. LIPI Press, Jakarta. [Indonesian]

Eshwarappa RSB, Iyer RS, Subbaramaiyah SR, Richard SA, Dhananjaya BL. 2014. Antioxidant activity of *Syzygium cumini* leaf gall extracts. BioImpacts 4 (2): 101-107. DOI: 10.5681/bi.2014.018.

Fu C, Guan G, Wang H. 2018. The anticancer effect of sanguinarine: A review. Curr Pharm Des 24 (24): 2760-2764. DOI: 10.2174/138161226666670829100601.

Hoshyar R, Mahbob Z, Zarban A. 2016. The antioxidant and chemical properties of *Berberis vulgaris* and its cytotoxic effect on human breast carcinoma cells. Cytotechnol 68 (4): 1207-1213. DOI: 10.1007/s10616-015-9880-y.

Kraus TE, Dahlgren RA, Zuebski, RJ. 2003. Tannins in nutrient dynamics of forest ecosystems-a review. Plant Soil 256 (1): 41-66. DOI: 10.1023/A:1026206511084.

Kumar S, Yadav A, Yadav M, Yadav JP. 2017. Effect of climate change on phytochemical diversity, total phenolic content and in vitro antioxidant activity of *Aloe vera* (L.) Burm. f. BMC Res Notes 10: 60. DOI: 10.1186/s13104-017-2385-3.

Li Y, Kong D, Fu Y, Sussman MR, Wu H. 2020. The effect of developmental and environmental factors on secondary metabolites in medicinal plants. Plant Physiol Biochem 148: 80-89. DOI: 10.1016/j.plaphy.2020.01.006.

Lim YP, Pang SF, Yusoff MM, Mundalip SA, Gimbin J. 2019. Correlation between the extraction yield of mangiferin to the antioxidant activity, total phenolic and total flavonoid content of *Platelia macrocarpa* fruits. J Appl Res Med Aromat Plants 14: 1000224. DOI: 10.1016/j.jar-nat.2019.1000224.

Liu K, Abdullah AA, Huang M, Nishioka T, Alatt-UL-Amin M, Kanaya S. 2017. Novel approach to classify plants based on metabolite-content similarity. BioMed Res Intl 2017: 5296729. DOI: 10.1155/2017/5296729.

Macakovi K, Afonso R, Saso L, Mladěnka P. 2019. The influence of alkaloids on oxidative stress and related signaling pathways. Free Radical Biol Med 134: 429-444. DOI: 10.1016/j.freeradbiomed.2019.01.026.

Mahmoodally MF, Ugurlu A, Llorent-Martínez EJ, Nagamootoo M, Picot-Allam MCN, Baloglu MC, Atungho YC, Hosseynali M, Zengin, G. 2020. *Syzygium coriaceum* Bosser & J. Guêho-Andic plant potentiates conventional antibiotics, inhibits clinical enzymes and induces apoptosis in breast cancer cells. Ind Crops Prod 143: 111948. DOI: 10.1016/j.indcrop.2019.111948.

Martin O, Scholze M, Ermel S, McPhie J, Bopp SK, Kienzler A, Parissis Y, Kortenkamp A. 2021. Ten years of research on synergism and antagonisms in chemical mixtures: A systematic review and quantitative reappraisal of mixture studies. Environ Int 146: 106206. DOI: 10.1016/j.envint.2020.106206.

Meda A, Larmen CE, Romito M, Millogo J, Naoctula OG. 2005. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chem 91 (3): 571-577. DOI: 10.1016/j.foodchem.2004.10.006.

Medini F, Fellah H, Ksouri R, Abdelly C. 2014. Total phenolic, flavonoid and tannin contents and antioxidant and antimicrobial activities of organic extracts of shoots of the plant *Limonum delticulatum*. J Taibah Univ Sci 8 (3): 216-224. DOI: 10.1016/j.tusci.2014.01.003.

Memon AH, Ismail Z, Al-Sude FSR, Aisha AF, Hamil MSR, Saeed MAA, Laghari M, Majid AMSA. 2015. Isolation, characterization, crystal structure elucidation of two flavanones and simultaneous RP-HPLC determination of five major compounds from *Syzygium campanulatum* Korth. Molecules 20 (8): 14212-14233. DOI: 10.3390/molecules200814212.

Miquel FAW. 1855. *Jambosa zollingeri* Miq. Flora of Nederland Indies 1 (1): 413.

Mohuiddin AK. 2019. Impact of various environmental factors on secondary metabolism of medicinal plants. J Pharmacol Clin Res 7 (1): 555704. DOI: 10.19080/JPCR.2019.07.555704.

Nguyen TL, Rusten A, Bugge MS, Bakhuizen van den Bergr BA, Dalsø A, Bugge MS, Malterud KE, Diallo Z, Zarban A. 2016. The antioxidant and chemical properties of *Berberis vulgaris* and its cytotoxic effect on human breast carcinoma cells. Cytotechnol 68 (4): 1207-1213. DOI: 10.1007/s10616-015-9880-y.

Nugroho AA, Hikmatiyani NH, Djumaga S. 2012. Effect of salam (*Syzygium polyanthum*) leaf extract to decrease blood triglyceride
level on white rats. Asian J Nat Prod Biochem 10: 40-45. DOI: 10.13057/biofar/100202.

Rezende WPD, Borges LL, Santos DLD, Alves NM, Paula JRD. 2015. Effect of environmental factors on phenolic compounds in leaves of Syzygium jambos (L.) Alston (Myrtaceae). Mod Chern Appl 3 (2): 1000157. DOI: 10.4172/2329-6798.1000157.

Ryu B, Kim HM, Woo JH, Choi JH, Jang DS. 2016. A new acetophenone glycoside from the flower buds of Syzygium aromaticum (cloves). Fitoterapia 115: 46-51. DOI: 10.1016/j.fitote.2016.09.021.

Sajadi SE, Ghanadian M, Haghighi M, Moushebat L. 2015. Cytotoxic effect of Coussinia verbasculifolia Bunge against OVCAR-3 and HT-29 cancer cells. J HerbMed Pharmacol 4 (1): 15-19.

Sathyaranayanan S, Chandran R, Thanikaran S, Abrahamse H, Thangaraj P. 2018. Phytochemical composition, antioxidant and anti-bacterial activity of Syzygium calophyllifolium Walp. fruit. J Food Sci Technol 55 (1): 341-350. DOI: 10.1007/s13197-017-2944-6.

Scherer R, Godoy HT. 2009. Antioxidant activity index (AAI) by the 2,2-diphenyl-1-picrylhydrazyl method. Food Chem 112 (3): 654-658. DOI: 10.1016/j.foodchem.2008.06.026.

Sheela D, Cheemical M. 2017. Total phenolics and flavonoids among the selected species of Syzygium, Gaertn. Res J Pharmacogn Phytochem 9 (2): 101-104. DOI: 10.5958/0975-4385.2017.00018.8.

Silva CC, Gomes CL, Danda LJ, Roberto AE, Carvalho A, Ximenes EC, Silva RM, Angelos MA, Rolim LA, Rolim Neto P. 2021. Optimized microwave-assisted extraction of polyphenols and tannins from Syzygium cumini (L.) Skeels leaves through an experimental design coupled to a desirability approach. An Acad Bras Cienc 93 (2): e20190632. DOI: 10.1590/0004-273020120190632.

Simirgiotis MJ, Adachi S, To S, Yang H, Reynertson KA, Basile MJ, Gil RR, Weinstein IB, Kennelly EJ. 2008. Cytotoxic chalcones and antioxidants from the fruits of Syzygium samarangense (Wax Jambu). Food Chem 107 (2): 813-819. DOI: 10.1016/j.foodchem.2007.08.086.

Singh V, Pahuja C, Ali M, Sultana S. 2018. Analysis and bioactivities of essential oil of the flower buds of Syzygium aromaticum (L.) Merr. et LM Perry. J Med Plants Stud 6 (6): 79-83.

Sultana B, Anwar F, Mushitaq M, Aslam M, Ijaz S. 2014. In vitro antimutagenic, antioxidant activities and total phenolics of clove (Syzygium aromaticum L.) seed extracts. Pak J Pharm Sci 27 (4): 893-899.

Tabasum S, Khare S, Jain K. 2016. Spectrophotometric quantification of total phenolic, flavonoid, and alkaloid contents of Abrus precatorius L. seeds. Asian J Pharm Clin Res 9 (2): 371-374.

Tiwlely D, Langhanová L, Palaniswamy D, Lall N. 2017. Evaluation of traditionally used medicinal plants for anticancer, antioxidant, anti-inflammatory and anti-viral (HPV-1) activity. S Afr J Bot 112: 494-500. DOI: 10.1016/j.sajb.2017.05.021.

Wahyuni R, Wignyanto W, Wijana S, Sucipto S. 2020. Optimization of protein and tannin extraction in Moringa oleifera leaf as antioxidant source. Food Res 4 (6): 2224-2232.

Widodo P, Chikawati T, Wibowo DN. 2011. Distribusi dan status konservasi Syzygium zollingerianum (Miq.) Amsh. (Myrtaceae). Prosiding Seminar Nasional “Konservasi Tumbuhan Tropika: Kondisi Terkini dan Tantangan ke Depan”. UPT Balai Konservasi Tumbuhan Kebun Raya Cibodas – LIPI, 7 April 2011. [Indonesian].

Zahra N, Nadir M, Malik A, Shaukat A, Parveen A, Tariq M. 2019. In vitro phytochemical screening and antioxidant activity of jaman (Eugenia jambolana Linn) plants parts collected from Lahore, Pakistan. Biochem Mod Appl 2 (1): 20-23. DOI: 10.33805/2638-7735.118.

Zhou W, He Y, Lei X, Liao L, Fu T, Yuan Y, Huang X, Zou L, Liu Y, Ruan R, Li J. 2020. Chemical composition and evaluation of antioxidant activities, antimicrobial, and anti-melanogenesis effect of the essential oils extracted from Dalbergia pinnata (Lour.) Prain. J Ethnopharmacol 254: 112731. DOI: 10.1016/j.eph.2020.112731.