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The flavonol glycoside- and antioxidant alterations during the flowering stages of cloves (*Syzygium aromaticum* (L.) Merr

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Tripama, B., Soeparjono, S., Dewanti, P., Nugraha, A. S. and Siswoyo, T. A. (2022). The Changes of Flavonol Glycosides and Antioxidant Properties During Flowering Stages of Cloves (*Syzygium aromaticum* (L.) Merr. International Journal of Agricultural Technology 18(6):2689-2702.

Abstract The flavonol glycosides belong to a large group of flavonoids diverse in chemical structures and properties. In plants, these compounds are usually in *O*-glycosides with common quercetin or myricetin aglycon. Quercetin is generally the most abundant and prominent component. Flavonols and flavone glycosides were common cloves chemotypes with potential natural antioxidants. The changes and antioxidant capacities of flavonol glycosides during the flowering stages of clove (FS-1 to FS-4) were investigated. Identifications of flavonol glycosides constituent, total phenolic, flavonoids, and antioxidant capacities were evaluated the extracts generated in every stage of clove flowers formation. The experiment showed various concentrations of flavonols (quercetin, quercetin-3-*O*-glycoside, myricetin, myricetin-3-*O*-glycoside) and flavones (luteolin-7-*O*-glycoside, apigenin-7-*O*-glycoside), as the highest concentration of quercetin-3-*O*-glycoside was found during the bloom stage (FS-4) at 110.27 µg/g. The blooming stage also had a significantly higher *in-vitro* antioxidant capacity than FS-1, FS-2, and FS-3. Antioxidant activity was based on 1,1-diphenyl-2-picrylhydrazyl (DPPH), hydroxyl radical (OH•), and superoxide radical (•O₂) scavenging. It is suggested as the best stage in harvesting the cloves for the natural antioxidant source.

Keywords: Antioxidant, Flavonols, Flavones, Flower Cloves, Glycosides

Introduction

Broadly known as clove, *Syzygium aromaticum* (L.) Merr. is a Myrtaceae family species that has been one of the most ancient and valuable herbs in the world. This plant has been used for culinary, pharmaceutical, and aromatherapy purposes. Several scientific studies have discovered cloves to contain active

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compounds with pharmacological properties, including anesthetic and analgesic, antimicrobial, antioxidant, anti-inflammatory, anticonvulsant, anti-cancer, and antimutagenic effects (Miyazawa and Hisama, 2001; Harborne and Williams, 2000; Hamad *et al.*, 2017). These medicinal plants provide a potential source for antioxidant compounds such as flavonoids (Neveu *et al.*, 2010). Flavonoids are the largest group of natural antioxidants in which this polyphenol is present in most land-based plants. Arrays of biological activities of flavonoids have been reported in flavanols from several different sources, such as in the process of inhibiting the reactive radical formation and scavenging several species from several different radical types, enhancement antioxidant enzyme activity, inhibiting platelet aggregation, increasing blood circulation, reducing inflammation and growth inhibition, and accelerating the cancer cell apoptosis (Cheng *et al.*, 2007; Nichenametla *et al.*, 2006; Bestwick *et al.*, 2007; Ramadhan *et al.*, 2020; Fernandes *et al.*, 2017).

Flavanols are the dominant group in flavonoids, especially in *O*-glycoside form. The sugar moiety influence flavanols bioavailability as the diet in the form of glycosidic is partially absorbed in the stomach compared to aglycone form, which remains unabsorbed (Crespy *et al.*, 2002). The speed and absorption efficiency of flavanol glycosides depend on the species size and the location of the sugar moieties (Chang *et al.*, 2005). For example, the peak concentration of quercetin in plasma is higher and achieved faster after intake with some quantity of quercetin-3-*O*-glucoside than quercetin-3-*O*-rutinoside. Quercetin is a specific flavonoid found in various fruits, vegetables, and other industrial crops, including cloves which its antioxidant effects benefit human health.

In previous reports, chemotype exploration of clove flowers was limited to the mature flower age, without a detailed information regarding the chemotypes changes in different flowering stages. In this study, each flowering stage of clove flowers was studied for their glycosidic flavanol changes quantitatively and evaluated their antioxidant activities.

Materials and methods

Materials

Clove flowers at various stages: the first stages of baby clove (FS-1), second stages of young clove flower (FS-2), clove flower maturation (FS-3), and fourth stages of flower clove bloom (FS-4) were collected from an experimental farm at the University of Jember, East Java, Indonesia, in April, 2018 (Figure 1). Chemical reagents, 1,1-diphenyl-2-picrylhydrazyl (DPPH),

trinitrobenzene sulfonic acid (TNBS), 2-deoxy-D-ribose, pyrogallol, 3,5-dinitrosalicylic acid (DNS), ferric chloride solution (FeCl_3), hydrogen peroxide (H_2O_2), tertiary butyl alcohol (TBA), folin-ciocalteu's phenol reagent, trichloroacetic acid (TCA) ethylenediaminetetraacetic acid (EDTA) were procured from *Sigma-Aldrich, Singapore*.

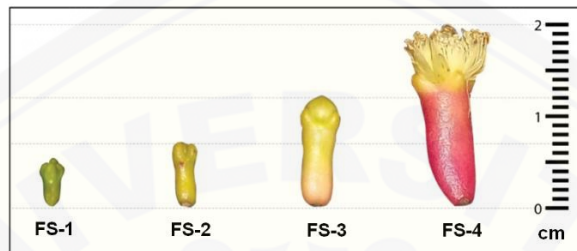


Figure 1. Clove flower in a different stage of maturation. FS-1 (4 months); FS-2. (5.5 months); FS-3 (6.5 months) and FS- 4 (7.5 months)

Extraction of clove samples

Clove flower (5 g) was mashed with a mortar, and methanol (50% in water, 15 ml) was added with a ratio of 1:3 (w/v). The mixture was left for 24 hours at room temperature, followed by centrifugation at 10,000 rpm for 10 min. The supernatant was collected and stored in a freezer ($-20\text{ }^{\circ}\text{C}$) for further analyses of total phenolic, total flavonoids, DPPH, anion superoxide, and hydroxyl scavenging.

Total phenolic analysis

Total phenolic measurement was based on a standard method by Ratnadewi *et al.* (2020). To a small amount of extract of clove flower (5 μl), methanol (80 % in water, 45 μl), Na_2CO_3 (2 % in water, 1 ml), Folin Ciocalteu (50 % in water, 50 μl) were added. The mixture was then homogenized using a vortex before incubation at room temperature for 30 min. The absorbance value was measured at λ 750 nm, and quantification was carried out based on the gallic acid standard, and the result was expressed in μg GAE/g sample.

Total flavonoid analysis

The flavonoid content with AlCl_3 was determined using a standard protocol by Ratnadewi *et al.* (2020), with several modifications. The clove

flower extract (10 μ l) was mixed with methanol (40 μ l), aquadest (400 μ l), and NaNO₂ (5% in water, 30 μ l), until homogenous before incubation at room temperature (27 °C) for 5 min. AlCl₃ (10% in water, 30 μ l) was added to the mixture, followed by incubation at room temperature for 6 min. Then, the mixture was added with NaOH (1 N, 200 μ l) and aquadest (240 μ l). The absorbance was recorded at λ 415 nm, and quercetin quantification was carried out based on the quercetin standard, as the result was expressed in μ g QE/g sample.

HPLC analysis

The analysis of flavonoids was carried out using a *Thermo Accela 1250 U-HPLC* system (*Thermo Fisher Scientific, San Jose, CA, USA*) equipped with a quarter solvent pump, column oven, auto-sampler, and UV detector. A 1 μ l aliquot of each sample solution was injected and analyzed on a *Hypersil Gold (50mm x 2.1mm x 1.9 μ m)* column, *ThermoFisher Scientific, USA*. The separation was conducted at 30 °C (column temperature) using a gradient elution method with 0.1% formic acid in acetonitrile (solvent A) and 0.1 % formic acid in distilled water (solvent B). The solvent gradient in volumetric ratios was set for: 0.0–1.0 min at 80 % B; 1.0–2.0 min at 20 % B; 2.0–3.0 min at 20 % B; 3.0–3.5 min at 80 % B. The flow rate was set at 300 μ l/min, and the effluents were monitored from λ 200 to 600 nm, while a selected chromatogram was collected at λ 275 and 330 nm.

MS/MS analysis

Flavonoids were identified using a *Thermo Accela 1250 U-HPLC* system with a UV detector coupled to a *TSQ Quantum Access MAX mass spectrometer (Thermo Fisher Scientific)*. The electrospray ionization (ESI) was applied in the negative ion mode (NI) for the MS analysis. The operation conditions of mass analysis were set at: capillary temperature, 300 °C; vaporizer temperature, 250 °C; sheath gas (argon) pressure, 40 psi; auxiliary gas (argon) pressure, 10 psi; spray voltage, 2.5 kV. The mass spectra were recorded in the mass range from m/z 150 to 1500. The MS/MS spectra were obtained using the Data-Dependent mode, and the collision energy was set as follows: collision energy (CE), 10 V; collision energy grad (CE grad), 0.035 V/m.

Antioxidant activity

The clove flower extract in each stage was determined the antioxidant activity based on their scavenging capability against 1,1-diphenyl-2-

picrylhydrazyl (DPPH), superoxide, and hydroxyl radicals. The antioxidant activity against DPPH was determined according to Galvez *et al.* (2005) and Nugraha *et al.* (2021). Briefly, a portion of extract (100 μ l) was mixed with methanol (100 μ l) and DPPH (0.5 mM, 800 μ l). The mixture was incubated for 20 min at 27 °C. The reduction absorbance was recorded at λ 517 nm. The following formula determined the percentage of DPPH radical reduction: Radical Scavenging Activity (%) = $(A_0 - A_s)/A_0 \times 100$ %, with A_0 was the Blank Absorbance and A_s is the Sample Absorbance.

Superoxide anion scavenging was analyzed by Tang *et al.* (2010) and Supriyadi *et al.* (2019). The analysis of superoxide anion scavenging activity was conducted by mixing the clove flower extract (200 μ l) with Tris-HCl buffer (50 mM, pH 8.2, 1.7 ml), pyrogallol (10 mM in 10 mM HCl, 100 μ l) followed by incubation at room temperature for 10 min. The reaction slope was determined from pyrogallol autoxidation at 4 min, in which the absorbance was recorded at λ 320 nm. The reduction percentage of superoxide anion radicals using generic clove organ extract was compared to ascorbic acid scavenging (0.1 μ g/ μ l).

Hydroxyl radical scavenging activity was analyzed based on Siswoyo *et al.* (2017). The flower clove extract (150 μ l) mixed with 2-deoxy-D-ribose (28 mM in 20 mM phosphate buffer (pH 7.4), 50 μ l) was added to clove generative supernatants, EDTA (1 mM in water, 100 μ l), FeCl₃ (10 mM in water, 100 μ l), H₂O₂ (1 mM in water, 50 μ l) and ascorbic acid (1 mM, 50 μ l) in a microtube, followed by incubated for 1 hour at 37 °C. The TBA (1 % in water, 500 μ l) and TCA (2.8 % in water, 500 μ l) were added to the mixture and homogenized using a vortex, before incubation for 30 mins at 80°C to produce a pink color. The absorbance was measured at λ 532 nm, and the percentage of hydroxyl radical inhibition was compared against the ascorbic acid inhibition at 0.1 μ g/ μ l concentration.

Statistical analysis

Data are presented as mean \pm standard deviation. The analysis of variance was applied to testify the significant difference between the mean values. If there was a significant different effect, the Duncan's Multiple Range Test (DMRT) was further applied at the 0.05 confident level using the SPSS software.

Results

Total phenolic and flavonoids

The results of the phenolic and flavonoid contents are summarized in Table 1. The total number of phenolic and flavonoid compounds was expressed as micrograms gallic acid equivalent (GAE) per gram of dry weight. The concentrations of phenolic compounds increased along with flowering stages, namely the first stage (FS-1) (45.95 μg GAE/g), second stage (FS-2) (69.20 μg GAE/g), clove flower maturity (FS-3) (76.10 μg GAE/g), and fourth stage (FS-4) (79.97 μg GAE/g). This was shown on the color changes in from green to yellowish-red. Furthermore, the concentration increase of flavonoid constituents tends to increase from the F-1 to F-3 and decrease at FS-4. The flavonoid compounds in FS-2 (8.09 μg QE/g), FS-3 (9.24 μg QE/g), and FS-4 (8.99 μg QE/g) increased significantly compared to FS-1 (4.82 μg QE/g) (Table 1).

Table 1. Total phenolic and flavonoid content on each flowering stage

Flowering Stages	Phenolic (μg GAE/g)	Flavonoid (μg QE/g)	Ratio Flavonoid/Phenolic (%)
FS-1	45.95 \pm 9.50c	4.82 \pm 0.50b	10.64 \pm 1.07a
FS-2	69.20 \pm 1.17b	8.09 \pm 1.49a	11.68 \pm 2.21a
FS-3	76.10 \pm 3.59ab	9.24 \pm 1.25a	12.18 \pm 1.89a
FS-4	79.97 \pm 10.11a	8.99 \pm 0.87a	11.24 \pm 1.43a

Note: The mean value followed by the same letter in the same column shows a significant different ($p < 0.05$).

The flavonoid/phenolic ratio parameters increased from the FS-1 (10.64 %), FS-2 (11.68 %), to FS-3 (12.18 %), but the decreased ratio occurred in the FS-4 (11.24 %). All clove flower stages were not significantly different. The phenolic compounds increased along with the clove flower stage. The highest concentration was found in the fourth stage (79.97 μg GAE/g) (Table 1). In comparison, the highest concentration of flavonoid parameters was found in the third stage as clove flower maturity stage (9.24 μg QE/g). Although there was the most significant concentration difference in each stage, it can be referred that the concentration of the two compounds tends to increase with the increasing flowering stage. Both phenolic and flavonoid compounds are widely distributed in fruit, vegetable, and cereal crops, primarily on flowers.

HPLC-MS/MS

The analysis results using HPLC-MS/MS showed varying concentrations. The six components were identified as bioactive compounds of flavonol glycoside in four flowering stages, namely quercetin, quercetin-3-*O*-glycoside, myricetin, myricetin-3-*O*-glycoside, luteolin-7-*O*-glycoside, and apigenin-7-*O*-

glycoside, as shown in Figure 2. Quercetin compound increased from the FS-1 (0.42 $\mu\text{g/g}$), FS-2 (2.17 $\mu\text{g/g}$), FS-3 (2.25 $\mu\text{g/g}$) to the FS-4 (3.03 $\mu\text{g/g}$). The increased quercetin in the FS-2 and FS-3 did not differ significantly, but showing a significant different in the FS-4 (Table 3). Increased concentration with an increasing trend in each stage of clove flower also occurred in the quercetin-3-*O*-glycoside, where the highest concentration was found in the FS-4 (110.27 $\mu\text{g/g}$).

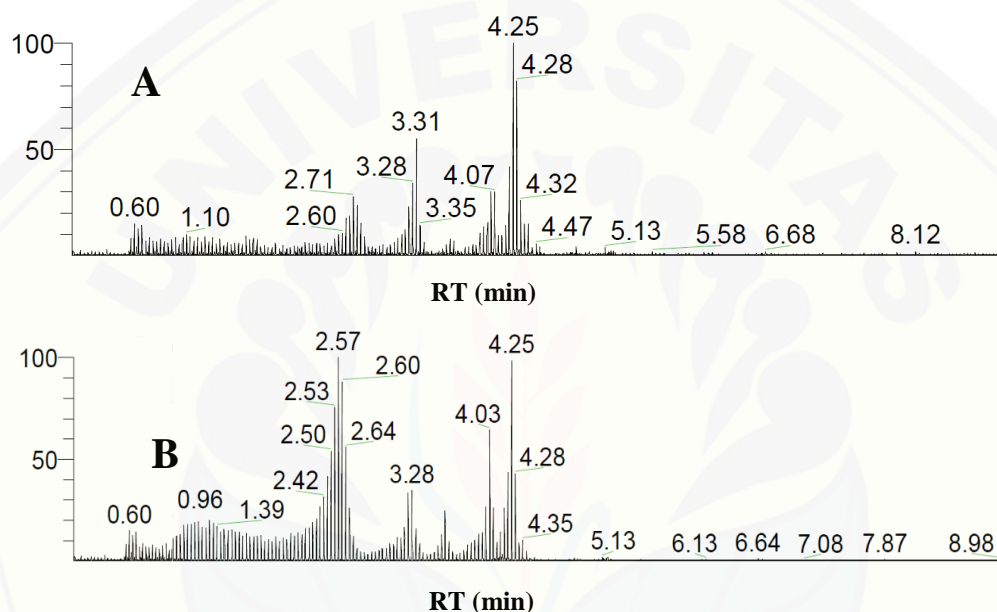


Figure 2. The MS profile of clove flower related to flavonol constituents. A. FS-1 (at 4 months); B. F-4 (at 7.5 months). RT. 3.28 min = Luteolin-7-*O*-glycoside; RT. 5.13 min = Myricetin; RT. 3.03 min = Myricetin-3-*O*-glycoside; RT. 6.64 min = Apigenin-7-*O*-glycoside; RT. 4.20 min = Quercetin; Rt. 4.25 min = Quercetin-3-*O*-glycoside

The myricetin content increased in the FS-1 (0.20 $\mu\text{g/g}$), FS-2 (1.02 $\mu\text{g/g}$), FS-3 (1.16 $\mu\text{g/g}$), to FS-4 (1.34 $\mu\text{g/g}$). The increased concentration in the FS-1 and FS-2 was not significantly different from the FS-2 and FS-3. A significant increase in myricetin concentration occurred in the FS-4. Furthermore, the myricetin-3-*O*-glucoside compound increased significantly in the FS-1 (21.98 $\mu\text{g/g}$), the FS-2 (28.97 $\mu\text{g/g}$), and the FS-3 (41.37 $\mu\text{g/g}$). However, an increase in the FS-4 (71.09 $\mu\text{g/g}$) was not significantly different from the FS-3 (Figure 3).

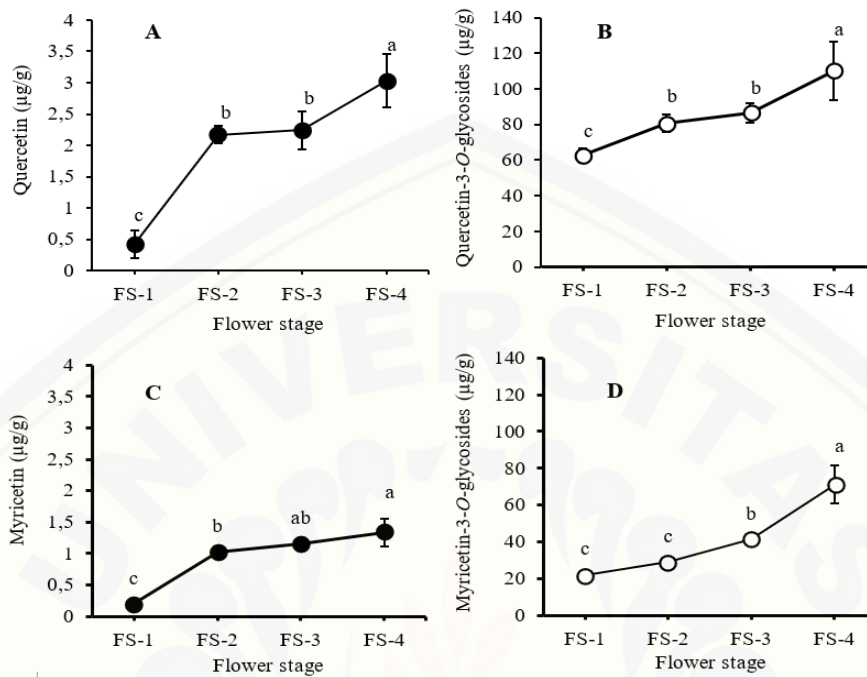


Figure 3. The changes in the content of flavonols in each flowering stage of cloves. A. Quercetin; B. Quercetin-3-*O*-glycoside; C. Myricetin; D. Myricetin-3-*O*-glycoside. Bars that display different letters are shown as significantly different ($p < 0.05$).

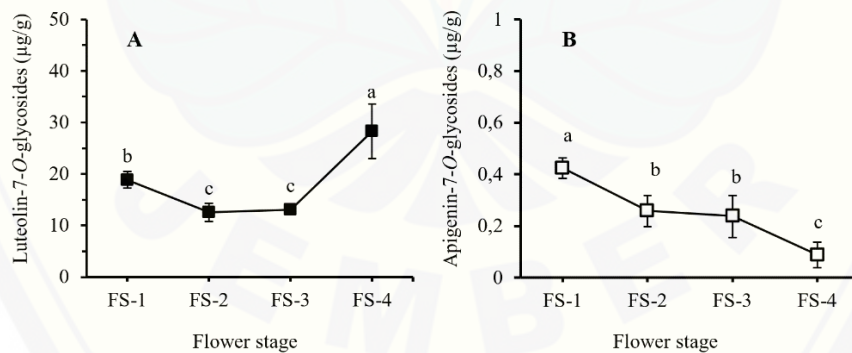


Figure 4. The changes in the content of flavones in each flowering stage of cloves. A. Luteolin-7-*O*-glycoside; B. Apigenin-7-*O*-glycoside. Bars that display different letters are shown as significantly different ($p < 0.05$).

The luteolin-7-*O*-glucoside compound has a fluctuating decrease and increases from the FS-1 (18.85 $\mu\text{g/g}$), FS-2 (12.56 $\mu\text{g/g}$), FS-3 (13.11 $\mu\text{g/g}$) to FS-4 (28.29 $\mu\text{g/g}$). A significant decrease occurred from the FS-1 to the FS-2, then there was an increase in concentration in the FS-3, but a significant increase occurred only in the FS-4. The content of apigenin-7-*O*-glucoside in all stages of clove flower decreases with the increase of the FS-1 (0.42 $\mu\text{g/g}$), the FS-2 (0.26 $\mu\text{g/g}$), the FS-3 (0.23 $\mu\text{g/g}$), and FS-4 (0.08 $\mu\text{g/g}$). The significant decrease in apigenin-7-*O*-glucoside concentration occurred in the first and FS-2, but the subsequent decrease was not significant. The lowest decrease in apigenin-7-*O*-glucoside occurs significantly in the FS-4 (Figure 4).

Free radical scavenging activity assay

Percentages of DPPH antioxidants, hydroxyl, and superoxide scavenging activity in each flowering stage of clove are presented in Table 2. The percentage of DPPH decreased from FS-1 to FS-4, which was then increased at FS-4 with an inhibition value of 72.60 %. In addition, hydroxyl scavenging showed a linear pattern within flowering stages in which FS-1, FS-2, FS-3, and FS-4 percentage of inhibition as 28.37, 29.60, 30.90, and 31.60%, respectively. Statistical analysis indicated that these value differences were significant. Fluctuate data was also shown in superoxide scavenging activity in which FS-1, FS-2, FS-3, and FS-4 have a percentage of inhibition value of 23.08, 21.17, 26.43, and 17.83%, respectively. The further statistical test showed no significant differences among all flowering stages of clove.

Table 2. Antioxidant activity, DPPH, hydroxyl, and superoxide on each flowering stage of cloves

Flowering Stages	DPPH (%)	Hydroxyl (%)	Superoxide (%)
FS-1	61.74 \pm 1.65c	28.37 \pm 0.75c	23.08 \pm 8.71a
FS-2	67.95 \pm 2.77b	29.60 \pm 0.45bc	21.17 \pm 0.91a
FS-3	68.83 \pm 0.16b	30.90 \pm 1.34ab	22.43 \pm 2.83a
FS-4	72.60 \pm 2.46a	31.60 \pm 1.41a	19.83 \pm 0.60a

Note: the average followed by the same letter in the same column shows significantly different ($p < 0.05$).

Discussion

Plants synthesize and gather various secondary metabolites, which have been proven to possess antioxidant capacity (Quideau *et al.*, 2011; Kasote *et al.*, 2015). These biologically active compounds belong to different chemical

groups, such as phenols, flavonoids, anthocyanins, diterpenes, and isoflavones (Saxena *et al.*, 2013). Both phenolic and flavonoid compounds are widely distributed in fruit, vegetable, and cereal crops, primarily in flowers (Li *et al.*, 2015; Kaisoon *et al.*, 2011). Our study on the flowering stage showed that phenolic compounds increased with the clove flowering stage. The highest concentration was found in the fourth stage of the clove flower bloom (79.97 µg GAE/g) (Table 1). In comparison, the highest concentration of flavonoid parameters was found in the third stage, namely the optimum clove flower maturity (9.24 QE/g). Although there was a significant concentration difference in each stage, it can be referred that the concentration of the two compounds tends to increase with the increasing flowering stage. In accordance with our findings, several researchers reported an increased concentration of flavonoid content in different plants, such as *Nigella sativa* (Zribi *et al.*, 2014), *Aquilaria beccariana* (Anwar *et al.*, 2017), *Ziziphora clinopodioides* (Ding *et al.*, 2014) and *Celosia argentea* L (Adegbaaju *et al.*, 2020) during their flowering stage. As the plant prepares for flowering, more flavonoids are likely synthesized. Moreover, the biosynthesis of flavonoids during the flowering stage can also become a defensive mechanism against pests that may attack the flowers and a potential pollinator attractant (Petruzza *et al.*, 2013). The specific pattern of the formation of flavonoids in each plant species is determined by a complicated system with a genetically controlled way, regulating the synthesis and distribution in plant organisms (Kozłowska and Wegierek, 2017). Thus, in addition to intrinsic factors, the content of flavonoids in plants is strongly influenced by extrinsic factors, such as variations in type and growth of plants, season, climate, level of maturity, and processing (Ewald *et al.*, 1999; Stewart *et al.*, 2000; Vuorinen *et al.*, 2000).

The identified flavonoid compounds increased along with an increasing flowering stage (Figure 3 and 4). In this study, the highest concentration of each flowering stage was found in quercetin-3-*O*-glucoside compounds, followed by myricetin-3-*O*-glycoside, myricetin, quercetin, apigenin-7-*O*-glucoside, and luteolin-7-*O*-glycoside. Furthermore, Sarangowa *et al.* (2014) reported that quercetin 3-*O*-glucoside and kaempferol 3-*O*-glucoside were the main flavonols in the petals of five *R. gallica* cultivars and three *R. damascene* cultivars. Quercetin *O*-glycosides are quercetin derivatives with at least one *O*-glycosidic bond that are widely distributed in plants. Each phase of *M. malabathricum* L. flowering is characterized by different levels of quercetin. The research results showed that the highest level of quercetin derivatives was found in the blooming flowers (F-4). This condition corresponded to Isnaini *et al.* (2018) and Schmitzer *et al.* (2009), who reported that the highest quercetin level in *M. malabathricum* L. and rose flower hybrid was found in the blooming flowers.

Quercetin is a flavanol found in fruits and vegetables as a food component proven beneficial for health (Kaur and Kapoor, 2001). Many

biochemical activities were reported that the compound was one of the most potent antioxidants among polyphenols. Quercetin has also been shown to have antiviral, antibacterial, anticarcinogenic, and anti-inflammatory effects (Rauf *et al.*, 2018). Quercetin inhibits the oxidation and cytotoxicity of low-density lipoprotein *in vitro* and reduces the coronary heart disease or cancer (Yoshida *et al.*, 1990; De Whaley *et al.*, 1990). The *in vitro* oxidation model shows quercetin, myricetin, and routines to be more potent antioxidants than traditional vitamins (Vinson *et al.*, 1995). Flavonols and flavones have antioxidants and free radical activity in food and significant sparing vitamin C activity, with myricetin as one of the most active compound (Middleton and Kandaswami, 1992).

The most studied chemical properties of phenolic compounds (phenolic and flavonoid) were their antioxidant activities. Several active phytochemicals of phenolic compounds have been discovered in *S. aromaticum* L, such as quercetin, luteolin, caffeic acid, kaempferol, and catechin (Adefegha *et al.*, 2016). Several flavonoids have been successfully identified from *S. aromaticum* L, including rutin and quercetin, which are well known to have significant antioxidants. In the present study, the antioxidant potential of *S. aromaticum* L was investigated through 2,2-diphenyl-1-picrylhydrazil (DPPH), hydroxyl scavenging, and superoxide scavenging activity assays. Protective antioxidant by plant products properties such as *S. aromaticum* may contribute as therapeutic drugs for free radical-induced pathologies. The antioxidant properties of phenolic compounds are associated with their ability to transfer hydrogen or electrons, chelation of metal ions, and oxidation activity inhibition (Rice-Evans *et al.*, 1997; Kasote *et al.*, 2015). Also, phenolic compounds act as an antioxidant, preventing free radical peroxidation and injury to cell membranes. Typically, many metabolic alterations occur during the flower-bud formation and opening and secondary metabolites due to well-defined sequences, such as cell division, cellular differentiation, membrane permeability, and cell elongation (Sood and Nagar, 2003). In the present study, the radical scavenging capacity (DPPH, hydroxyl, and superoxide) showed that antioxidant activity in the first stage of baby clove was significantly different from the fourth stage of clove flower bloom. Thus, clove flowers in the first and fourth blooming stages can be utilized as a source of antioxidants. The absorption and metabolism of phenolic compounds such as quercetin and its derivatives attract much attention to the health aspect's value. The total intake of flavonoids from food sources is estimated from several hundred milligrams to one gram per day (Formica and Regelson, 1995; Hertog *et al.*, 1993). Quercetin derivatives, especially glycosides, represent a large portion of the food content. Quercetin is a derivative with many metabolic conversions and

occurs in body tissues, mainly in glucuronate, sulphated-, and methylated-forms (Graf *et al.*, 2006; Scalbert *et al.*, 2002; Williams *et al.*, 2004). This study concludes that the highest quercetin-3-*O*-glycosides can be harvested in the clove flower blooming stage. The quercetin-3-*O*-glycosides have the potential as a source of natural antioxidants for various uses in the future.

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