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ORIGINAL ARTICLE

Plant secondary metabolism and flower color changes in damask rose at different flowering development stages

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Abstract

Secondary metabolites content as well as petal color changes was quantified in flower extract of *Rosa damascena* Mill. during four flower development stages [(pre-anthesis; PA), (partially open flower; POF), (full open flower; FOF), and (senescent flower; SF)]. Petal brightness was increased at the last stage of flower opening compare to the PA level. An increase in *b** value and decrease in *a** value were recorded during damask rose flower development. The result of analysis by HPLC revealed that the main secondary metabolites of *Rosa damascena* Mill. flowers are phenolic acids [(gallic (GA), caffeic (CA), chlorogenic (CG), and coumaric (CO)] and flavonoids (rutin, quercetin, kaempferol, and apigenin). The content of most phenolic acids were the highest at SF stage, while the flavonoids content was the highest at FOF stage. The apigenin content was the highest at PA stage and then declined. Enzymatic activity of phenylalanine ammonia-lyase (PAL) showed a positive correlation with some flavonoids production. Results indicated that based on the need for specific phenolic compounds in the therapeutic process, flower harvesting at FOF stage or SF stage is suggested.

Keywords Flavonoid · Human health · Kaempferol · PAL · Quercetin

Introduction

Secondary metabolites (SMs) are low molecular weight compounds and occur in plants in a high structural diversity (Wink 2015). Secondary metabolites are low molecular weight compounds and play important functions such as species interactions and protection, but are not necessary for survival (Bourgaud et al. 2001; Schäfer et al. 2016). Plant secondary metabolites are not essential for the plant to survive, however can promote many morpho-physiological activities within plants. Defense mechanism is one of the most important roles on SMs in plants. They are used to protect against herbivores, pests, and pathogens (Wink and Schimmer 2018). An enormous variety of SMs which have important roles in plant defense mechanisms as well as human health are derived from shikimic acid (phenylpropanoid pathway) (Hendrich and Murphy 2016) or aromatic amino acids and many other compounds which have important roles in plant defense mechanisms as well as human health.

For instance, flavonol quercetin, along with some other antioxidant compounds such as vitamin C, E, and carotenoids, can protect human body tissues against oxidative stresses (David et al. 2016). However, it seems possibly that it has a role in improving physical/mental performance and reducing infection risk during intense exercise in athletes (Davis et al. 2009). Likewise, the risk of chronic diseases, especially cancer, can be reduced via kaempferol treatment as another SM (Liao et al. 2016). Several therapeutic properties of kaempferol such as positive effect on the treatment of cardiovascular diseases, cancers, and neurodegenerative diseases has been reported (Devi et al. 2015). In addition, some other SMs such as caffeic acid and chlorogenic acid (5-caffeoylquinic acid), are polyphenol compounds which absorb in human diet and play an important role in body health (Shahidi and Ambigaipalan 2015). Damask rose (*Rosa damascena* Mill.) from *Rosaceae* family, has been longley cultivated in India, Turkey, Bulgaria, and Iran due to its nutritional and medicinal properties (as a source of polyphenols) as well as a shrub in gardening (Tabaei et al.

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2006; Pal et al. 2014; Mahboubi 2016). Polyphenols possess a high range of biochemical activities such as antidepressant (Hussain et al. 2016), anti-inflammatory and antimutagenic (Kumar et al. 2008), antioxidants, free radical scavengers (Kolodziejczyk-Czepas et al. 2015), anticancer agents (Niedzwiecki et al. 2016), and is important in improving the cardiovascular system (Kwon et al. 2009). Due to the many above-mentioned therapeutic properties of polyphenols, it is vital to determine their content in medicinal plants to be used in human health. Polyphenol-enriched extract of damask rose is a food ingredient. The antioxidative effect of *Rosa damascena* extract has been reported by many researchers (Kovatcheva-Apostolova et al. 2008; Shikov et al. 2012; Pal et al. 2014; Davoodi et al. 2017). Shikov et al. (2012) added damask rose petal extract in 250–500 ppm concentrations to the canned strawberries and reported that petals extract accelerated the antioxidant capacity of the canned strawberries, even after 12 weeks of storage.

Flower opening can be effect by many factors, including petal movements, reversible expansion and contraction of petal cell growth, combination of sugar uptake and degradation of polysaccharides, or may be due to temperature, water status, and light (van Doorn and Kamdee 2014). Cell enlargement is one of the main reason of petal growth (Sood et al. 2006). Further, genes expression level in biosynthesis pathway and corresponding metabolites synthesis showed a positive correlation (Muhlemann et al. 2012). These processes may affect plant metabolism and let to the modification in production of primary and secondary metabolites in plant cells. Oka et al. (1999) indicated that the total amount of aromas of *R. damascena* changed during flower development stages and reached the highest value at flower opening time. Polyphenols content, including quercetin, catechin, anthocyanins, and phenolic acids of Rose 'KORcrisett' was changed during flower open development (Schmitzer et al. 2009a, b). Further, the content of phenolics and antioxidant compounds of Safflower (*Carthamus tinctorius* L.) was changed during flower open development (Salem et al. 2011). Few reports are available on phenolic content of damask rose flowers and, in most cases, the available data are inconsistent, therefore it needs more investigation to identify the metabolite profile of *R. damascena*. Considering the value of damask rose polyphenols as a source of antioxidant and anticancer compounds, this study was done to determine the content of secondary metabolites at four different stages of flower development to define the best stage of harvest for pre-specified compound.

Materials and methods

Rosa damascena samples were selected from plants were cultivated based on completely randomized design with three blocks (including three plants per block). A homogenized flower sample was supplied at four flowering development stages [(Pre-Anthesis; PA), (Partially Open Flower; POF), (Full Open Flower; FOF), and (Senescent Flower; SF)] (Fig. 1) and freeze-dried with liquid nitrogen for subsequent uses.

Flower color measurement

Middle part of each *Rosa damascene* petal was used for color measurement (three points on both petal surfaces selected randomly), by a portable Minolta chromameter (CR-300; Minolta Ramsey, NJ, USA). The color change was determined as L^* [(brightness-darkness; 0 = black − 100 = white)]. Color parameters a^* and b^* extend from − 60 to + 60 [− a^* = green and + a^* = red; − b^* = blue and + b^* = yellow]. A white standard plate was used to calibrate the chromameter before use. The hue angle ($h°$) is expressed in degrees from 0° to 360°

Fig. 1 Stages of flower open development: **a** pre-anthesis (PA); **b** partially open flower (POF); **c** fully open flower (FOF); and **d** senescent flower (SF)

(0° = red, 90° = yellow, 180° = green, and 360° = blue) (Schmitzer et al. 2010).

PAL (EC: 4.3.1.5) enzyme activity assay

To quantify the content of phenylalanine ammonia lyase (PAL), 0.5 g of freeze-dried petals of damask rose was homogenized in 6.5 mL Tris–Hcl 50 mM buffer (pH 8.8) containing 15 mM β-mercaptoethanol. Samples were centrifuged at 50,000 g for 30 min at 4 °C, after grounding in a cold mortar and pestle. PAL enzyme assay was done using the collected supernatant. PAL activity was measured according to the rate of cinnamic acid production, based on the method of Wang et al. (2006) with some modifications. Briefly, 1 mL of extraction buffer, 0.5 mL of 10 mM *L*-phenylalanine, 0.4 mL of deionized water, and 0.1 mL of enzyme extract were mixed and incubated at 37 °C for 1 h. The reaction was terminated by adding 0.5 mL of HCl (6 M), and the product was extracted in 15 mL ethyl acetate. The product was left at room temperature until the extracting solvent had evaporated, after which the solid residue was suspended in 3 mL of 0.05 M NaOH. Cinnamic acid concentration was measured using a spectrophotometer at an absorbance of 290 nm. A unit of enzyme activity was defined as the amount of PAL that produced 1 µM of cinnamic acid in 1 min.

Chemicals

The standards chemicals of phenolic acids and flavonoids were purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA) and solvents were purchased from Merck company (Darmstadt; Germany).

Preparation of standard solutions

The stock solution (1 mg/mL) was prepared via dissolving 1 mg of purchased polyphenols in 0.5 mL HPLC-grade methanol and then the extract was sonicated for 10 min. Afterwards, the extract was made up to 1 mL with the solvent for the mobile phase (acetonitrile and 1% aq. acetic acid 1: 9), and standard curve (Fig. 2) was obtained.

Extraction of plant samples

Flower petal samples were freeze-dried in liquid nitrogen. Then, 500 mg of petal samples were extracted with 2 mL methanol containing 3% (v/v) formic acid (HCOOH) and 1% (w/v) 2, 6-di-*tert*-butyl-4-ethylphenol (BHT) in an ultrasonic bath for 1 h. Afterwards, samples were centrifuged at 12,000 rpm for 7 min. Supernatant was filtered using the chromafil AO-20/25 filter (Macherey–Nagel Düren, Germany) and prepared for injection into the HPLC (Schmitzer et al. 2009a, b).

HPLC analysis of phenolic compounds

HPLC system (Agilent 1100, USA) with a diode array detector at 250, 272, and 310 nm according to absorption maxima of analyzed compounds were applied for sample analyzing. A Nova pack C18 (250*4.6; 4 mm) HPLC column at 25 °C was used. The injection volume was regulated as 20 µL and the flow rate was 1 mL.min^{-1}. The elution solvents were aqueous 1% acetic acid (A) and acetonitrile (B). The mobile phase involved 1% aq. The gradient elution was modified from 10 to 40% B for duration of 28 min, from 40 to 60% B in 39 min, from 60 to 90% B in 50 min. The mobile phase composition back to initial condition (solvent B: solvent A: 10: 90) in 55 min and allowed to run for another 10 min, before the injection of another sample. Total analysis time per sample was 65 min. Each compound was identified by its

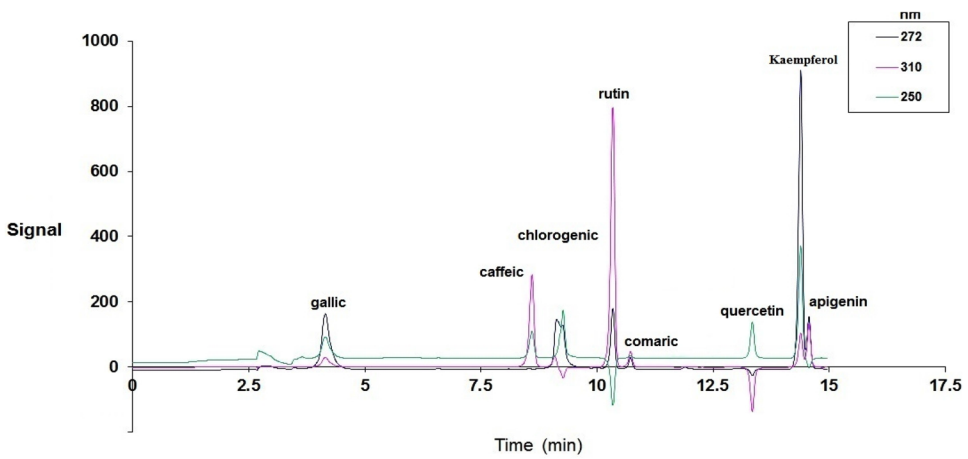

Fig. 2 Standard chromatogram of secondary metabolites. PA (**a**), POF (**b**), FOF (**c**) SF (**d**)

retention time and by spiking with standards under the same conditions. The sample content was measured by calculation of the integrated peak area and the content was calculated using the calibration curve by plotting peak area against concentration of the respective standard sample.

Statistical analysis

Data analysis was done using SAS V9.2 and means were compared using Duncan's multiple range tests at $P \leq 0.01$ and $P \leq 0.05$.

Results and discussion

Flower color

The L^* value of petal color was increased as flower opening developed and a significant difference ($P \leq 0.01$) was observed between four stages of flower development. Petal brightness (an increase in L^* value) was enhanced at the last stage of flower development compared to the PA stage. The increase in b^* value and decrease in a^* value implied the middle range of brightness and darkness (Table 1). As flower opening developed, the $h°$ value increased and reached the 34.4° which indicated the petal brightening.

A range of physio-biochemical changes and genetic processes happen during flower development that contributes to the visual changes in petal color (Schmitzer et al. 2010). The main reason for petal brightness is change in cell sap pH and sugar content in petal cells. The pH addition was done due to increasing free cellular ammonia accompanying proteolysis (Diaz-Mendoza et al. 2016) and decline in sugar content during flower development by affecting the soluble and storage carbohydrates (Eason et al. 1997), led to the brightening of petals.

Hydrolysis of stored starch during *R. damascena* flower development, led to the decrease in water potential and affect petal water status (Sood et al. 2006) and water deficiency causes petal color change. Our results is consistent with Rasouli et al. (2018), which reported the decrease in a^* and L^* value for three *R. damascena* landraces during flower development. The same results have been reported by Kazaz et al. (2010) on *R. damascena* petal color change during cold storage which is in line with our findings.

Secondary metabolites

Petals metabolic profile indicate a significant changes as flower opening stages are developing (Schmitzer et al. 2010). HPLC analysis of *Rosa damascena* flower extract during flower development indicated the presence of many polyphenols. The obtained chromatogram of four flower development stages (Fig. 3) indicated various compounds. The peaks were identical to gallic acid, caffeic acid, chlorogenic acid, rutin, coumaric acid, quercetin, kaempferol and apigenin.

Gallic acid (GA: 3, 4, 5-trihydroxybenzoic acid) concentration was the lowest at PA stage and then start to increase. A sharp increase in GA concentration was observed from POF stage to FOF, where the highest concentration of GA was produced (95.12 ± 2.67 µg. g^{-1}FW) (Table 2). As flower development was progressed, the concentration of GA was declined.

The caffeic acid content (CA) was increased as flower opening stages were developed and reached the highest level at SF stage (50.03 ± 1.44 µg. g^{-1}FW a; Table 2). However, the concentration of chlorogenic acid (CG) start to decrease as flower opening developed up to FOF stage and then suddenly increased to the highest level at SF stage (743.92 ± 24.43 µg. g^{-1}FW a; Table 2). Coumaric acid (CO) content showed the same trend as chlorogenic acid and firstly decreased as flower opening started and then increased and reached the highest level at SF stage (128.3 ± 3.2 µg. g^{-1}FW). Results implied that the content of total phenolics was reduced at POF stage and then started to increase and reached the highest level at SF stage (Table 2). Considering the amount of phenolic acids during four flower development

Table 1 Color parameters value of *Rosa damascena* at different flower development stages (Mean ± SE, $n = 3$)

Flower development stages	†Colorimetric parameter				Sap cell pH
	a^*	b^*	L^*	$h°$	
††PA	32.1 ± 1.23 d†††	− 3.0 ± 0.08 a	57.5 ± 1.12 a	16.1 ± 0.72 a	5.1 ± 0.05 d
POF	26.0 ± 0.85 c	0.0 ± 0.0 b	60.7 ± 1.46 b	25.1 ± 0.53 b	5.66 ± 0.03 c
FOF	20.2 ± 1.33 b	1.2 ± 0.06 c	61.4 ± 1.73 b	27.7 ± 0.44 c	5.85 ± 0.06 b
SF	15.6 ± 1.45 a	2.8 ± 0.06 d	65.5 ± 2.01 c	34.3 ± 0.33 d	5.98 ± 0.03 a

†Colorimetric parameters base on CIE (International Commission on Illumination) system for color representation: (L^*: Lightness, a^*: green (−) to red (+), b^*: blue (−) to yellow (+), $h°$: huge angle)

††*PA* pre-anthesis, *POF* partially open flower, *FOF* full open flower, and *SF* senescent flower

†††Different letters in each column indicate significant differences determined using a Duncan's multiple range test ($P < 0.01$)

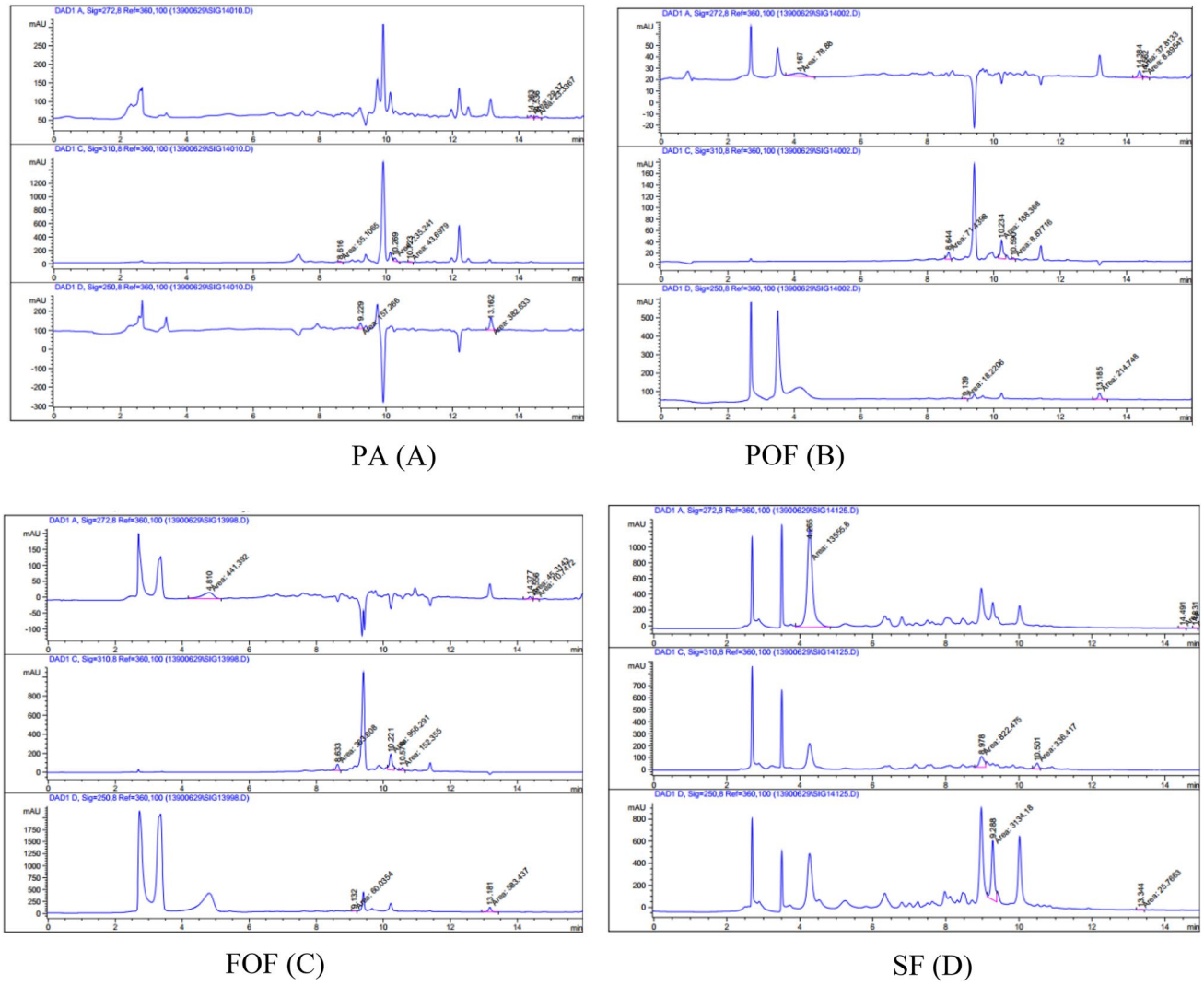

Fig. 3 HPLC chromatogram of secondary metabolites in *Rosa damascena* at four flower development stages. *PA* pre-anthesis, *POF* partially open flower, *FOF* full open flower, and *SF* senescent flower

stages, the most abundant compounds were chlorogenic acid, coumaric acid, gallic acid, and caffeic acid, respectively.

One of the key intermediate substrates for production of plant tannins is gallic acid. Gallic acid is also a vital precursor for many plant SMs. The shikimate dehydrogenase (SDH) enzyme in phenylpropanoid pathway is the direct responsible enzyme for biosynthesis of GA in plants, where this enzyme was cloned from *Juglans regia* and its direct role in GA synthesis was confirmed. 3-dehydroshikimate (3-DHS), the main substrate for production of gallic acid in plants, is the fifth intermediate in phenylpropanoid pathway (Muir et al. 2011). High radical scavenging activity in petals is mainly due to phenolics with dominant role of free gallic acid (Vinokur et al. 2006; Badhani et al. 2015).

Flower development involves a number of processes including growth, development, hormones change, senescence, and abscission (Sood and Nagar 2003). Few reports are available on phenolics characterization by HPLC during flower development and data available are inconsistent in some cases. Therefore, it needs more investigation to manifest the phenolic compounds in *Rosa damascena* flowers during development. Different gene expression takes place during flower development. For instance, in *Rhododendron pulchrum*, the expression of *FCA*, *FPA*, *FT*, *PHYB*, and *SOC1* genes were enhanced during PA stage, however, during POF stage, the expression of *DELLA*, *APETALA*1, and *AGAMUS* genes were increased. At FOF stage, the expression of *CRY*, *FLC*, *CONSTANT* (*CO*), and some MADS-box family genes were enhanced (Wang et al. 2018). Different gene expression during flower development can affect metabolite production (Sood et al. 2006), and led to the increase or

Table 2 Comparison of the effects of different stages of flowering on secondary metabolites production content (μg.g^{-1}FW) in damask rose (Mean ± SE, $n = 3$)

Compounds	Flower development stages			
	†PA	POF	FOF	SF
Gallic acid	0.98 ± 0.003 d††	12.02 ± 0.44 c	95.12 ± 2.67 a	29.14 ± 0.88 b
Caffeic acid	4.66 ± 0.21 c	5.46 ± 0.04 c	22.08 ± 0.46 b	50.03 ± 1.44 a
Chlorogenic acid	35.2 ± 0.45 b	4.4 ± 0.24 b	14.09 ± 0.47 b	743.92 ± 24.43 a
Coumaric acid	18.7 ± 0.52 c	4.3 ± 0.28 d	61.51 ± 2.02 b	128.3 ± 3.2 a
Total phenolic acids (μg.g^{-1}FW)	59.54	26.18	192.8	951.39
Rutin	7.19 ± 0.18 b	5.61 ± 0.26 b	22.95 ± 1.13 a	0.98 ± 0.003 c
Quercetin	57.60 ± 1.31 b	32.18 ± 1.20 c	91.92 ± 1.79 a	6.06 ± 0.34 d
Kaempferol	153.0 ± 1.67 b	161.85 ± 1.97 b	216.53 ± 3.31 a	118.90 ± 1.12 c
Apigenin	3.07 ± 0.078 a	1.72 ± 0.161 b	1.78 ± 0.110 b	1.39 ± 0.075 b
Total flavonoids (μg.g^{-1}FW)	220.86	201.36	333.18	127.33

†*PA* pre-anthesis, *POF* partially open flower, *FOF* full open flower, and *SF* senescent flower

††Different letters in each row indicate significant differences determined using a Duncan's multiple range test ($P < 0.01$)

decrease in amount of phenolics. It seems that up-regulation of responsible genes at early stages of flower development in *R. damascena* and down-regulation of these genes at SF stage of damask rose flower development is in charge of changes in gallic acid production over flower development. Hence, down-regulation of responsible genes at early stages of petal development and up-regulation at FOF and SF stages are responsible in chlorogenic and coumaric acids production.

Gallic acid was detected in hydroalcoholic extract of *R. damascena* (Tabriz and Kashan landraces), which is consistent with our results, however caffeic acid and coumaric acid did not detect (Memariani et al. 2015). Baydar and Baydar (2013) reported the presence of gallic acid in hot and cold methanolic extracts of *R. damascena*. However, they did not detect caffeic, coumaric, and chlorogenic acids which is inconsistent with our finding. Moreover, Kumar et al. (2008) identified gallic acid, rutin, and quercetin in fresh flowers of *R. damascena*, however, they did not detect caffeic, coumaric, and chlorogenic acid as well as apigenin. These differences may be due to different extraction method, different analysis method, and different genetic resources. The earlier studies in *Arabidopsis thaliana* (Wagstaff et al. 2009) and *Antirrhinum majus* (Muhlemann et al. 2012) have shown the similar results on metabolites production and transcriptome changes over flower development which is consistent with our results. Moreover, Change in phenolic pattern of Christmas rose (*Helleborus niger*) over flower development was reported by (Schmitzer et al. 2013). Additionally, metabolic profile in tea (*Camellia sinensis*) leaves and flowers was changed during flower development (Jia et al. 2016), which are in line with our results.

Flavonoids

The FOF stage of *R. damascena* contained the highest level of rutin and quercetin and it is found that as flower senescence progressed, the concentration of both metabolites dropped. Moreover, the concentration of kaempferol steadily increased up to FOF stage and then decreased. However, PA stage led to the production of the highest level of apigenin (Table 2). The content of apigenin remain low, although there was a slight increase during FOF stage. Further, PAL enzyme activity was reduced as flower opening started (POF stage) and then increased and reached the highest level at FOF stage (Fig. 5).

Flavonoids are polyphenols with diphenylpropanes (C_6–C_3–C_6) skeletons. Rutin is an important flavonoid of medicinal value, along with some of its related flavonoids. Quercetin is a flavonoid (as aglycone of rutin) ubiquitously found in nature (D'Andrea 2015). Quercetin could be one of the main parts of the medicines for the treatment against protease, skin cancer, as well as tumorigenic cells (Nguyen et al. 2017). Further, oxidation of HDL cholesterol can be highly inhibited via quercetin treatment (Bondonno et al. 2015). Kaempferol is a natural flavonol, a type of flavonoid, found in a variety of plants. Kaempferol exhibits many therapeutic and antioxidant activities and play an important role as an anticancer activities agent. The rutin, quercetin, and kaempferol synthesized via phenylpropanoid pathway from phenylalanine amino acid as precursor with catalyzing activity of some enzymes, such as PAL (phenylalanine ammonia-lyase), C4H (cinnamate 4-hydroxylase), 4CL (4-coumarate: coenzyme A ligase), CHS (chalcone synthase), and FLS (flavonol synthase) (Fig. 4).

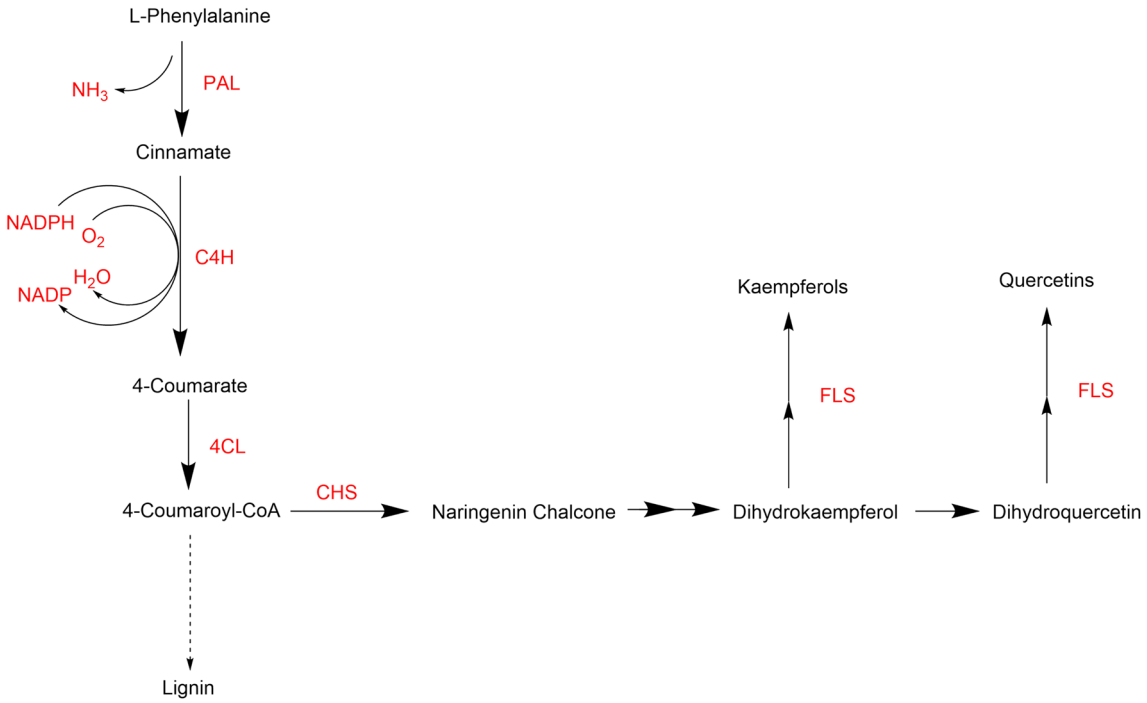

Fig. 4 Simplified scheme of phenylpropanoid synthesis in *Arabidopsis* with pathways leading to lignin, kaempferols, and quercetins. Key enzymes marked are *PAL* phenylalanine ammonia-lyase, *C4H* cinnamate 4-hydroxylase, *4CL* 4-coumarate: coenzyme A ligase, *CHS* chalcone synthase, and *FLS* flavonol synthase

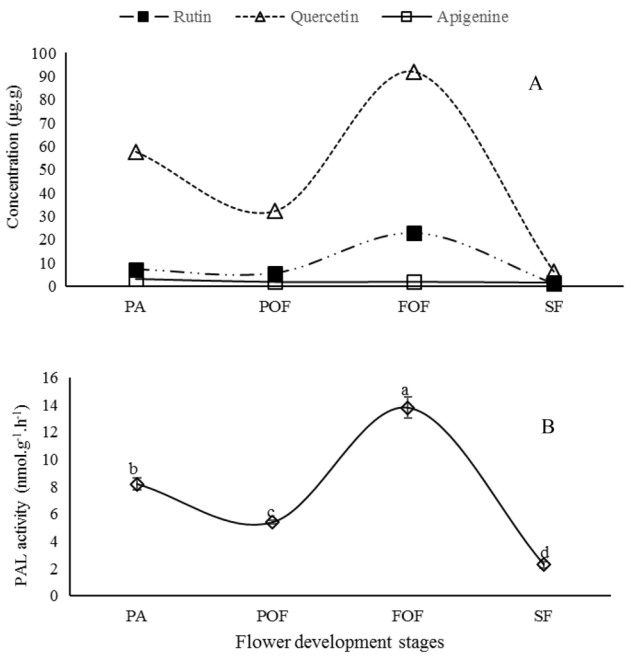

Fig. 5 Concentration of some secondary metabolites (**a**) and PAL enzyme activity (**b**) in *Rosa damascena* petals during flower open development

Apigenin is a natural product belonging to the flavone class and found in many plants. Apigenin is probably the responsible product for attracting pollinators during flowering. (Repčák and Krausová 2009).

Modified PAL enzyme activity, the key enzyme in phenylpropanoid pathway, can directly impact on synthesis of downstream secondary metabolites (Ferrari 2010; Kanani and Nazarideljou 2017). Change in PAL enzyme activity was recorded during flower development stages of *Rosa damascena* (Fig. 5).

Results manifested that PAL enzyme activity was declined from PA stage to POF and then started to increase up to FOF stage. Afterwards, PAL activity was reduced and reached the lowest level at SF stage. Modifications in PAL enzyme activity trend during flower development may be due to different environmental conditions and subsequently plant defense reactions (Ganapathy et al. 2016), which have directly affected downstream metabolites production. Change in PAL enzyme activity directly affected the synthesis of rutin and quercetin and a positive correlation between PAL enzyme activity with rutin ($R^2 = 0.9372$) and quercetin ($R^2 = 0.9907$) was observed (Fig. 6). Different in PAL enzyme activity during different growth stages of *Fagopyrum esculentum* and *Fagopyrum tataricum* and its effect on rutin, quercetin, and kaempferol synthesis has already been reported by Gupta et al. (2011) which is consistent with our finding. Further, differential expression of *PAL1*

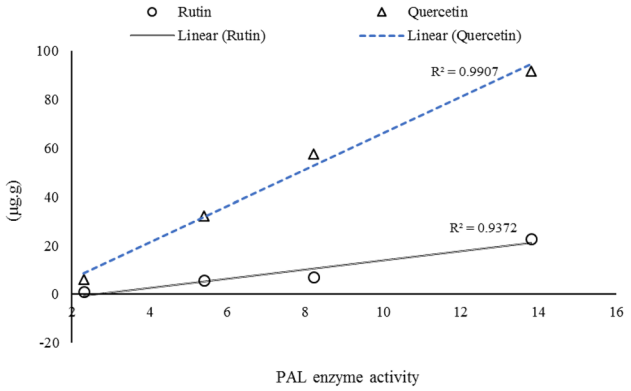

Fig. 6 Correlation between PAL enzyme activity with rutin and quercetin biosynthesis in *Rosa damascena* in different flower development stages

and *PAL2* genes in *Arabidopsis* plant, modified PAL enzyme activity and affected quercetin and kaempferol synthesis (Olsen et al. 2008), which is consistent with our findings. Change in PAL activity affected flavonoid synthesis, but this effect was not apparent in apigenin production. Furthermore, modified PAL enzyme activity via UV-C irradiation, affected rutin and quercetin production in tomato fruits (Liu et al. 2018). A significant positive correlation between PAL activity over three apple fruit developmental stages and the total flavonoids synthesis was reported by Sarkate et al. (2017).

PAL enzyme, as the starter enzyme in phenylpropanoid pathway, plays an important role in biosynthesis of flavonoids (Chang et al. 2009). Taguchi (2016) reported that formation of flavonoids in plants depend on PAL activity. The level of coumaric acid (as precursor for lignin synthesis) in FOF stage was lower compare to SF stage, where the amount of all flavonoids was higher in FOF stage compare to SF stage, which could be due to competition between flavonoid and lignin synthesis for precursor. This phenomenon has already been reported in *Arabidopsis* by Besseau et al. (2007).

Conclusions

Sustainable changes happen during flower development in *Rosa damascena* accompanied by changes in concentration of phenolic compounds and petal color. Active degradation of anthocyanin, petal expansion during flower development, gene expression modifications during flower development and senescence during flower development may be the main reasons for changes in polyphenols content during flower development in *R. damascena*. Enzymatic cleavage of glucose and releasing of aglycone may explain the higher level of quercetin to rutin in *R. damascena* at all flower development stages. During the FOF stage, the highest content of rutin, quercetin, and kaempferol were produced, which indicate that the FOF is the best stage for obtaining above-mentioned flavonoids to be used in therapeutic processes, pre-clinical, and clinical studies targeting the production of antimutagenic, antidepressant, and anticancer drugs. Caffeic, chlorogenic, and coumaric acids content were the highest at SF stage, therefore senescent flowers of *R. damascena* are nominate as a good source of most phenolic acids and can be used as a valuable source of antioxidants to promote the body health. Taken together, secondary metabolites production change during *R. damascena* flower development and based on demand for special compound, the harvesting at FOF stage or/and SF stage is recommended.

Author contribution statement M.K. performed the experiment, collected data, and wrote the paper. E.C. designed and performed the experiment and co-wrote the paper, and also supervised the research. A.A.S. analyzed data. M.T-G. revised the paper.

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