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ARTICLE

# Antioxidant activity of *Chelidonium majus* extract at phenological stages

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**Abstract** *Chelidonium majus*, from Papaveraceae family, is a rich source of different antioxidants with a range of medicinal activities including antispasmodic and diuretic properties. In this study, antioxidant potential of extracts from leaves during different phenological stages was measured by ferric-reducing power (FRAP) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity. Factors affecting antioxidant activity, i.e., total phenols, flavonoids, anthocyanin and carotenoids, were then investigated. Soluble sugar and total protein contents of samples were also determined. According to the results, maximum DPPH radical scavenging activity was 408/88 ± 24/83 g/g DW at growing stage, and the FRAP value reached maximum during fruiting stage (1.75 ± 0.04 mg/g FW). The leaves of flowering stage contained the most content of total phenol (17.8 ± 1.59 mg/g DW), flavonoid (69.7 ± 0.86 mg/g DW), anthocyanin (0.233 ± mg/g DW) and soluble sugar (0.338 ± 0.009 mg/g DW). However, the highest value for carotenoid (2.083 mg/g DW) and protein (0.27 ± 0.034 mg/g DW) was found at the vegetative stage.

**Keywords** Antioxidants · *Chelidonium majus* · Flowering · Fruiting · Phenological stages · Vegetative

The original version of this article was revised.

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## Introduction

Production of chemically active molecules including reactive oxygen species (ROS) is a general response to stressful conditions that living organisms may encounter every day. Oxidative stress has been identified as the main cause of the development and progression of several diseases [1–4]. Plants have an innate ability to biosynthesize a wide range of antioxidants capable of reducing ROS-induced oxidative damage [5–8]. Antioxidants are able to scavenge ROS and establish a balance between naturally produced radicals and antioxidant status. It has been suggested that the use of natural antioxidant may also induce the apoptosis in cancer cells [9–11].

*Chelidonium majus*, commonly known as greater celandine or tetterwort, belongs to Papaveraceae family. Greater celandine is a perennial herb with an erect habit and can reach 30–120 cm high [12]. The leaves are pinnate with lobed and wavy-edged margins, up to 30 cm long. When injured, the plant exudes a yellow to orange latex. The flowers consist of four yellow petals, each about 1 cm long, with two sepals [12]. The plant is considered an aggressive invasive plant in natural areas, and it can be controlled by pulling or spraying the plant before seed dispersal. It has been suggested that the whole plant is toxic in moderate due to the presence of a range of isoquinoline alkaloids. However, there are a number of therapeutic uses when taken at the controlled dosage [13]. *Chelidonium majus* (Papaveraceae) is well known as a medicinal herb distributed in Europe, Asia and Northern Africa. Traditionally, it is widely used to treat ulcer, cancer, oral infection, liver disorders, chronic bronchitis and asthma [14].

Significant antitumor, antiinflammatory, antiviral and antimicrobial properties have been reported for extracts of *Chelidonium majus* (CM) [15]. The effectiveness of CM extract in treatment of cancer is also experienced in the course of clinical investigations [16]. The extracts of CM are able to effectively modulate the strength of immune system leading to treatment of different cancers. The immunostimulant activity of CM extract has been reported to be related to alkaloid derivatives from the plant in patients with various cancers [17].

Based on a scientific report, extract of *C. majus* L. has a strong antioxidant potential and exerts the antiproliferative activity via apoptosis on leukemia cells. This could be related to the presence of the isoquinoline alkaloids and the flavonoid components that play an important role in both cancer chemoprevention through its antioxidant activity and modern cancer chemotherapy as cytotoxic and apoptosis-inducing agent [9, 18].

The main alkaloids identified in *C. majus* L. include chelidonine, berberine, coptisine, sanguinarine and chelerythrine, which are present in different parts of the plant [19]. Some of these alkaloids are also present in other plant types. For example, coptisine and berberine have been reported to be present in *Coptidis Rhizoma*. The study has shown the activity of many alkaloids including and berberine to scavenge peroxy nitrite, $ONOO^-$, formed from NO·and $O_2$· radicals. Peroxy nitrite is a highly reactive oxidizing and nitrating agent, leading to oxidize cellular components, such as proteins, lipids, carbohydrates and DNA. They evaluated alkaloids, harboring $ONOO^-$ scavenging and ROS inhibitory effects, as potential anti-Alzheimer disease candidates. It was found that coptisine and berberine exhibited significant $ONOO^-$ scavenging effects in a dose-dependent manner, with $IC_{50}$ values of 17.73 and 28.70 mM, respectively [20]. Considerable changes in oxidative stress markers have also been reported due to antioxidant activity of berberine alkaloid when it was used for treatment of diabetes [20]. It is worth reminding that the antioxidant defense mechanisms help to maintain the balance of redox in organisms and could be damaged in the pathogenesis of diabetes mellitus.

Therefore, the present research was designed aiming to determine the antioxidant activity and amount of total protein and soluble sugars in extracts of plant leaves during various stages of growth. In practice, total phenol, total flavonoids, anthocyanins, carotenoids, proteins and sugars were measured in the different extracts from the plant parts. The results are expected to show the stage when the greatest amount of antioxidant compounds is produced.

## Materials and methods

### Plant collection and preparation of extracts

Plant leaves were freshly collected during various stages of growth from Asalem forests, Gilan province, North of Iran. The samples were washed with water, dried in the shadow and ground into soft powder. Some portions of fresh leaves were kept frozen at −70 °C.

To prepare the plant extracts, 10 g of various powdered samples was separately soaked in 250 mL of 80% methanol and left at room temperature for 48 h and filtered. Another 250 mL 80% methanol was then added to the extracted powder, mixed and left at room temperature for 24 h and filtered. The filtrates were added together mixed and solvent evaporated at temperature lower than 40 °C on a rotary. The extract was stored in a refrigerator at 4 °C. For each test, the extracts were diluted with an appropriate volume of solvent.

### Measurement of total phenols

The extract solution (100 µL) was mixed with 2.8 mL of distilled water. Folin–Ciocalteu's phenol reagent 100 µL was then added to each test tube. Two mL of saturated sodium carbonate solution was added to the mixture. The mixture was kept at room temperature for 30 min. The absorbance was measured at 720 nm on a UV–visible spectrophotometer. A dose–response linear regression was generated using the gallic acid standard, and the levels in the samples were expressed as gallic acid equivalent [21, 22].

### Measurement of total flavonoids

The extract solution (100 µL) was mixed with 100 µL potassium acetate 1 M, 100 µL aluminum chloride 10%, 1.5 mL 80% methanol and 2.8 mL of water. The absorbance was measured at 415 nm on a UV–visible spectrophotometer after 40 min. A dose–response linear regression was generated using the quercetin standard absorbance, and the levels in the samples were expressed as quercetin equivalent [23].

### Measurement of total carotenoids

0.05 g of dried leaves was added to 5 mL acetone and pulverized in a porcelain mortar in ice bath. One gram of anhydrous sodium sulfate was added and the solution mixed slowly followed by increasing the volume of acetone to 10 mL. The mixture was centrifuged at 26,000 rpm after 10 min. The supernatant was then removed and the

absorbance measured at 662, 645 and 470 nm. The amount of carotenoid (mg/g dry) was calculated for each extract [24].

$$C_a\ (\mu g/mL) = 11.24A_{662} - 2.04A_{645}$$
$$C_b\ (\mu g/mL) = 20.13A_{645} - 4.19A_{662}$$
$$C_t\ (\mu g/mL) = (1000A_{470} - 1.9C_a - 63.14C_b)/214$$

where $C_a$ stands for chlorophyll *a*, $C_b$ for chlorophyll *b* and $C_t$ is total carotenoid content. $A_{470}$ is absorption at 470 nm (related to carotenoids), $A_{645}$ at 645 nm (related to chlorophyll *a*) and $A_{662}$ at 662 nm (related to chlorophyll *b*).

### FRAP test

0.1 g fresh tissue was added to 5 mL of distilled water and pulverized in a porcelain mortar in ice bath and stored at room temperature for 30 min. The FRAP reagent 1.5 mL was then added to 50 µL of each extract. The absorbance was measured at 593 nm and compared to control. A mixture of 50 µL distilled water and 1.5 mL FRAP reagent was used as blank. A dose–response linear regression was generated by using the FRAP standard absorbance, and the levels in the samples were expressed as ferrous ion equivalent, Mmol $Fe^{2+}$/gr of fresh weight, FW [25].

### DPPH radical scavenging assay

A solution of 0.004 g 1,1-diphenyl-2-picrylhydrazyl (DPPH) in 100 mL methanol was prepared, and 2 mL of this was mixed with 2 mL different concentrations of the methanol extract. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm against methanol as blank. The control was a mixture of 2 mL of methanol and 3 mL of DPPH solution. The ability to scavenge DPPH radical was calculated using the following equation [26]

$$\text{DPPH radical scavenging activity}\ (\%) = [(A_{\text{control}} - A_{\text{sample}})/(A_{\text{control}})] \times 100$$

The value of $IC_{50}$ in mg/mL, i.e., concentrations of the test sample and standard and antioxidants that provided 50% inhibition of the DPPH radical, was calculated from the DPPH absorption curve at 517 nm using ascorbic acid as positive control.

### Measurement of anthocyanins

0.02 g of dry tissue was added to 4 mL of 1% hydrochloric acid containing methanol and pulverized in a porcelain mortar. Solution was kept for 24 h in the refrigerator, followed by centrifugation for 10 min at 13,000 rpm. The absorbance of supernatant was measured at 530 and 657 nm and compared to the control. The blank solution was 4 mL of 1% hydrochloric acid solution containing methanol. The amount of anthocyanins (mg per g dry) was calculated for each extract [27].

$$\text{Anthocyanin}\ (\text{mg}) = A_{530} - (0.25 \times A_{657})$$

where "*A*" stands for absorbance.

### Measurement of soluble sugar

To 5 mL of 70% ethanol, 0.05 g of dry tissue was added, mixed well and kept in the refrigerator (about 4 °C) for 1 week. The mixture was later centrifuged at 1000 rpm for 15 min. The supernatant was separated and used for measurement of soluble sugar. The extract (0.5 mL) was diluted to a final volume of 2 mL followed by addition of 1 mL 5% phenol and 5 mL sulfuric acid. The prepared mixture was then stored at room temperature for 30 min, and its absorbance was measured at 485 nm. A dose–response linear regression was generated using the standard sugar, and the value was expressed as mg/g dry weight [28].

### Measurement of total protein

Bradford reagent (100 mg Color Kumasi, 50 mL 95% ethanol, 100 mL 85% phosphoric acid) was first added to 100 µL of extract and mixed. The final volume was increased to 100 mL with distilled water and the solution stored at room temperature for 20 min. The absorbance was measured at 595 nm. A dose–response linear regression was generated by using the Bradford standard.

## Results and discussion

### Plant samples

The collected leaves samples of various stages were shadow dried, ground into powder and kept at 4 °C until each measurement, and some fresh samples were also kept frozen for the FRAP test as recommended by the reference used [25].

### Total phenols

Figure 1 shows total phenol content of leaves extracts at various stages of growth using gallic acid as standard [19]. The highest value was observed at flowering stage (17.8 ± 1.59 mg/g DW).

Polyphenols are among the most abundant antioxidant species found in various parts of plants [5, 9]. Examining a

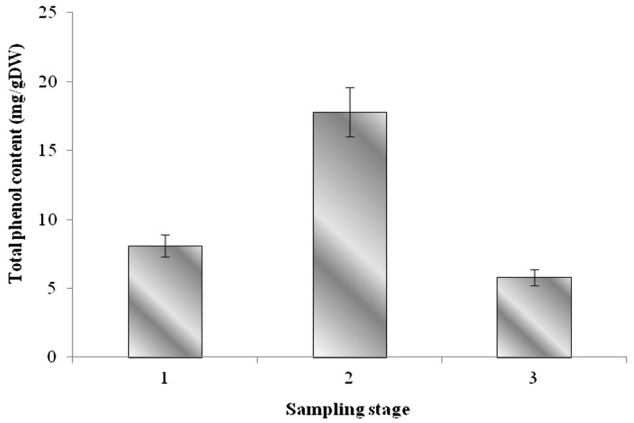


**Fig. 1** Total phenol content of different *Chelidonium majus* leaves extracts at *1* vegetative, *2* flowering and *3* fruiting stages

number of edible wild fruits from Burkina Faso, it has been reported that the polyphenol content is highly dependent on the fruit type and determines the antioxidant activity of the related wild fruit [20, 29]. Besides, in support of our findings, it is reported that flavonoids and phenolic acids are among the major constituents of *C. majus* extracts [30].

Among its other pharmaceutical effects, *C. majus* is also used in traditional medication for liver diseases [31]. The phenolic fraction of *C. majus* can mainly contribute to the effect of plant extract on liver disorders. It has been reported that both phenolic and alkaloidal fractions of *C. majus* exhibit choleretic activity. This is confirmed by the increase in bile acid flow in rats and human with liver diseases [32].

In support of our finding for the polyphenol antioxidant activity, it has been reported that oral use of the polyphenol fraction from different parts of *C. majus* could effectively scavenge various free radicals [33].

More than 8000 polyphenolic compounds have been identified in various plant species. All plant phenolic compounds arise from a common intermediate, phenylalanine, or a close precursor, shikimic acid. Polyphenols may be classified into different groups as a function of the number of phenol rings that they contain and on the basis of structural elements that bind these rings to one another. The main classes include phenolic acids, flavonoids, stilbenes and lignans [34].

## Total flavonoid content

Total flavonoids content was measured using quercetin as standard, and the levels in the samples were expressed as quercetin equivalent. It can be observed from this figure that leaves of flowering stage contained the most content of flavonoid (69.7 ± 0.86 mg/g DW).

In scientific literature, isolation and identification of at least 70 compounds from *Chelidonium majus* is reported [35]. These phytochemicals consist of flavonoids, polyphenols, saponins, vitamins, mineral elements, sterols, acids and their derivatives [36].

It is found that most flavonoids are poorly absorbed in the human body and about 5% which are absorbed are metabolized and excreted very quickly [37, 38]. It is, therefore, suggested that the increase in antioxidant capacity of blood seen after consumption of flavonoid-rich foods is not caused directly by flavonoids, but is due to production of uric acid resulting from flavonoid depolymerization and excretion.

In the present study, we found that total flavonoid content of *Chelidonium majus* extract is considerably higher in the flowering stage (Fig. 2). Considering the anticancer effect exhibited due to the flavonoid content, it can be suggested that extracts from the plant during flowering season could be effective in reducing cancer risks. It is, therefore, concluded that flavonoid fractions of *C. Majus* extract in the flowering stage could be considered as an effective anti cancer plant-derived medication [39].

## Total carotenoid content

It was found that the amount of total carotenoid content (mg/g dry) varied in extracts of leaves at various stages (Fig. 3). The data given in this figure indicate that leaves at vegetative stage contained the highest amount of total carotenoid (2.083 mg/g DW).

In this study, we confirmed that a considerable amount of carotenoids is present in leaf extracts of *Chelidonium majus*, especially during vegetative stage of the plant.

## Ferric-reducing antioxidant power (FRAP)

In this part of the research, fresh leaves were used instead of dry powder and the results were expressed as ferrous equivalent (Mm $Fe^{2+}$/gr FW) equivalent [23]. Figure 4

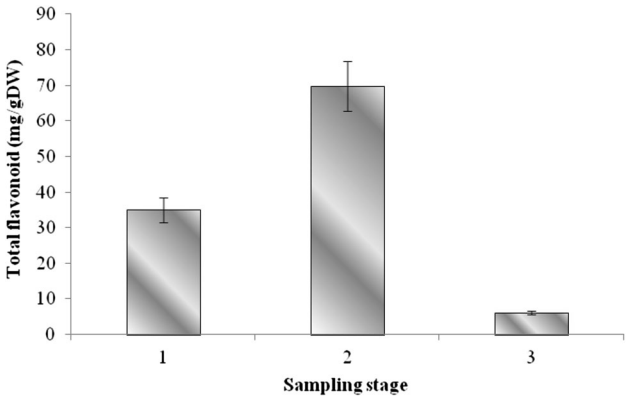


**Fig. 2** Total flavonoid content of different *Chelidonium majus* leaves extracts at *1* vegetative, *2* flowering and *3* fruiting stages

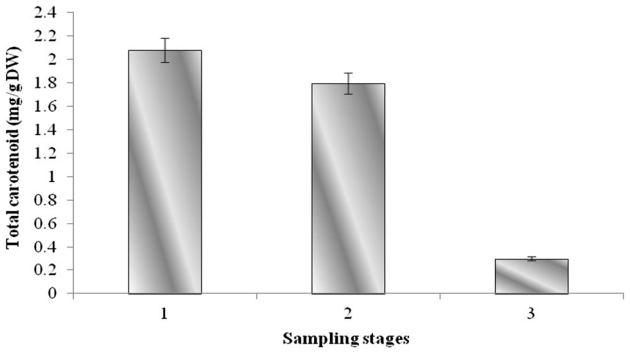


**Fig. 3** Total carotenoid content of different *Chelidonium majus* leaves extracts at *1* vegetative, *2* flowering and *3* fruiting stages

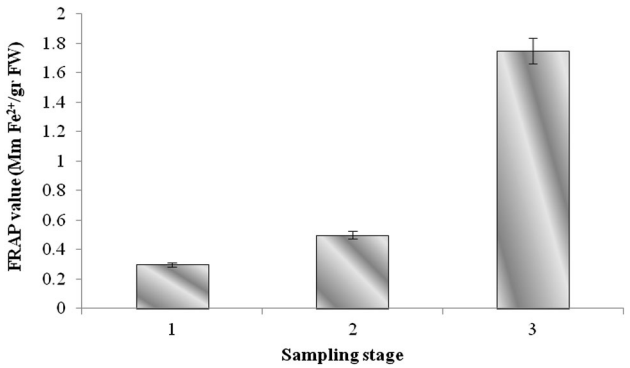


**Fig. 4** The results of FRAP test for different *Chelidonium majus* leaves extracts at *1* vegetative, *2* flowering and *3* fruiting stages

shows the plant extracts had highest ferric-reducing antioxidant power in the fruiting stage (1.75 ± 0.04 mg/g FW).

The antioxidant activity of *Chelidonium majus* during various stages of growth and development may depend on the extraction procedure and the type of plant part used for extraction. It has been reported that a number of various secondary metabolites, mostly antioxidant compounds are fluctuated in extract of *C. Majus* during various seasons [16]. The FRAP test results are also dependent on the extracts obtained from *C. Majus* plant in different parts of the world [27, 28].

## DPPH radical scavenging activity

The ability to scavenge DPPH radical was calculated from the following relationship [24].

$$\text{DPPH radical scavenging activity } (\%) = [(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})]/(\text{Abs}_{\text{control}})] \times 100$$

The $IC_{50}$ in mg/mL was calculated from the DPPH absorption curve at 517 nm using ascorbic acid as positive control. Figure 5 shows the ability of various extracts for scavenging DPPH radicals in terms of ascorbic acid

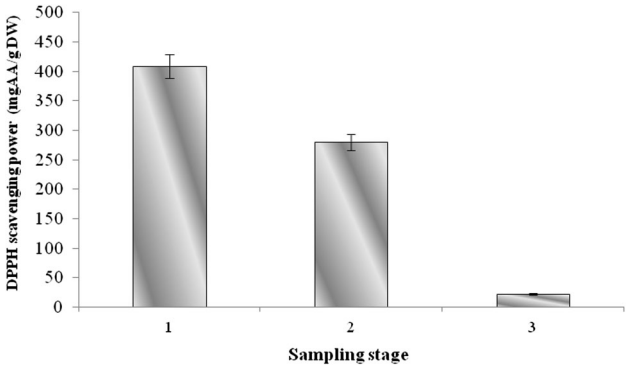


**Fig. 5** The results of DPPH test for different *Chelidonium majus* leaves extracts at *1* vegetative, *2* flowering and *3* fruiting stages. *AA* ascorbic acid

equivalent. According to the results, maximum DPPH radical scavenging activity was reached at growing stage (408/88 ± 24/83 g/g DW).

The results of present study indicate a high antioxidant power for *C. majus* extract as examined by DPPH. It is suggested that the potent antioxidant activity exhibited by the plant can provide a considerable protection against oxidative stress. Increased formation of ROS may lead to the development of malignancy, and the normal rates of ROS generation could help to reduce cancer development [31].

According to the literature, the strong antioxidant activity observed by *C. majus* extract does not depend on alkaloid and transition metal contents [12, 14]. Massive dose of *C. majus* (1.5–3 g/kg/day) administrated orally has caused slight but significant reduction in glutathione level and SOD activity in the liver of animal [10]. Therefore, high doses of *C. majus* extract might compromise hepatic antioxidant protection.

## Anthocyanins

A considerable concentration of anthocyanins (mg/g DW) was found in the extracts of *C. majus*, especially during growth and flowering stages (Fig. 6). This was supported by the results reported by other researchers for anthocyanin content of some food products [25]. Based on the results depicted in Fig. 6, the highest amount for anthocyanin (0.233 ± mg/g DW) was reached at the growing stage of the plant.

Anthocyanins are red, purple or blue vacuolar pigments with water solubility. Their wide range of color depends highly on the pH, and they are members of flavonoids.

In common with all flavonoids, the in vitro antioxidant properties of anthocyanins are not preserved in vivo [35]. It has been observed that consumption of foods rich in anthocyanins leads to their rapidly excretion [36]. However, the increase in antioxidant capacity of blood seen after these types of foods is the result of elevated uric acid, the final product of flavonoids' metabolism.

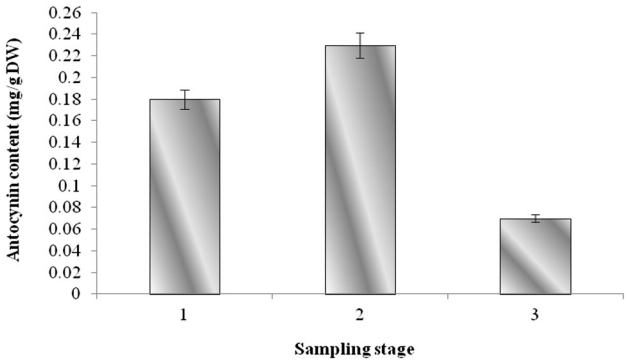


**Fig. 6** Concentration of anthocyanins in different extracts of *Chelidonium majus* leaves at *1* growth, *2* flowering and *3* fruiting stages

## Soluble sugar content

The value of soluble sugar content was expressed as mg/gr dry weight [30]. It was found that the content of sugar also varies during growth and development of the plant and it reaches its highest value during flowering stage (Fig. 7). A significant difference is observed between this value and soluble sugar during fruiting stage ($0.338 \pm 0.009$ mg/g DW). However, the soluble sugar during growth stage did not differ significantly from this highest amount.

It has been reported that the sugar content in leaf and stem of cassava contributes to the accumulation of starch in the tuberous root [30]. It is worth bearing in mind that the sugar content in this medicinal plant does not contribute to its medicinal properties. The soluble sugar content of *Chelidonium majus* has not been reported in the literature. However, a protein-bound polysaccharide has been isolated from water extracts of the plant capable of modulating the immune system [37]. This type of immunomodulatory activity is very important for the effect of extract on various cancers as the immune system is usually absent or very week in malignant cases [38–40].

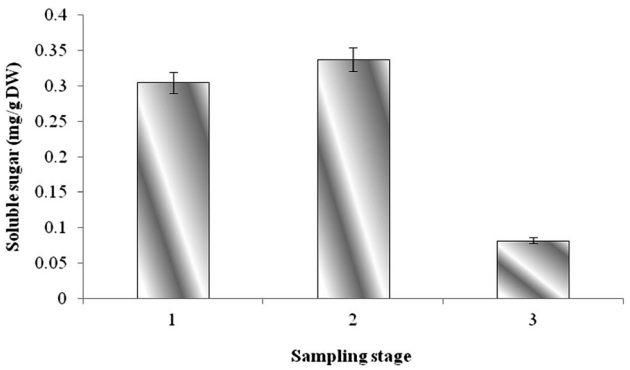


**Fig. 7** The amount of soluble sugar at different *Chelidonium majus* leaves extracts at *1* growth, *2* flowering and *3* fruiting stages

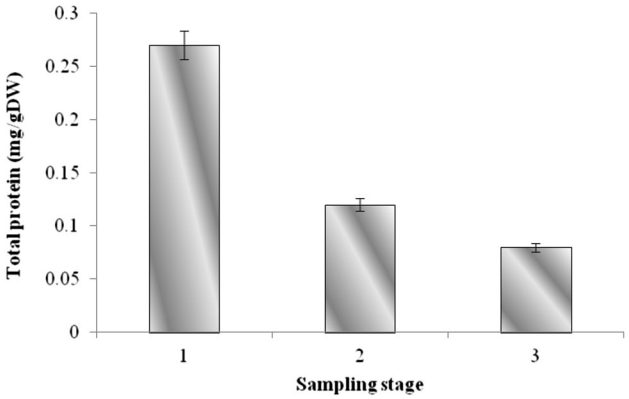


**Fig. 8** The amount of total protein at different *Chelidonium majus* leaves extracts at *1* growth, *2* flowering and *3* fruiting stages

## Total protein content

The total protein content in extracts was measured using a modified Bradford method. It was observed that maximum protein content is in the extracts from growing stage ($0.27 \pm 0.034$ mg/g DW). In Fig. 8, the total protein content during various stages of plant growth is compared.

Apparently, during the growth stage of the plant, proteins are produced faster in order to build up the main skeleton of the plant. The content of protein gradually decreases as the plant continues its growth and development.

In summary, the antioxidant activity of *Chelidonium majus* from Gilan province (North of Iran) depends on the phenological stages and the evaluation method. Despite the toxic properties of whole plant due to the presence of some alkaloids, it is a potent antioxidant in moderate doses. The methanolic extract of *Chelidonium majus* from North of Iran exhibited antioxidant properties during all stages of plant growth. The highest content of protein and sugar was found in growth and flowering stages. It is worth emphasizing that the total sugar content reported in this study had not been detected in extracts of *Chelidonium majus*.

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