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Effects of gibberellic acid (GA_3) on phenolic compounds and antiradical activity of marigold (*Calendula officinalis*)

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Abstract

Calendula sp. is an important medicinal and industrial plant with various bioactivities. The field study was conducted in Jiroft region of Kerman, Iran, to observe the effects of gibberellic acid (GA_3) on phenolic compounds and antiradical activity of Marigold (*Calendula officinalis*) extract. The levels of GA_3 were 0, 50, 150 and 250 mg L^{-1} in three spraying stages (first, second and third). The phenolic content was measured by the Folin–Ciocalteau method and the antioxidant activity by DPPH (2, 2-diphenyl-1-picrylhydrazyl) method. Results of this study showed that application of GA3 at first stage and second stage (first and second) significantly increased the content of phenolic compounds in *C. officinalis* (61.96 and 62,74 mg gallic acid/100 g dry weight respectively) while at third stage decreased the phenolic compounds. In all spraying stages, by increasing of GA_3 concentration, phenolic compounds of *C. officinalis* extracts were increased. Antiradical activity of *C. officinalis* extracts showed effects similar to the phenolic compounds. *C. officinalis* had phenolic compounds and scavenged free radicals in-vitro and using of different GA_3 levels could increase its phenolic compounds and antiradical activity.

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Introduction

Calendula officinalis (pot marigold, common marigold, garden marigold, English marigold, or Scottish marigold) is a plant in the genus *Calendula* of the family *Asteraceae*. It is probably native to southern Europe, though its long history of cultivation makes its precise origin unknown, and it may possibly be of garden origin (Gazim *et al.,* 2008). This plant is an annual plant with yellow to orange flowers and includes a high number of carotenoids such as flavoxanthin, lutein, rubixanthin, b-carotene, g-carotene, and lycopene (Pintea *et al.,* 2003). These carotenoids have been found to have antioxidant, antimicrobial and antiproliferative properties. Research suggests that it can be very protective against prostate cancer (Dahan, *et al.,* 2008).

Plant growth regulators (PGRs), either produced naturally by the plant or synthetically by a chemist, are small organic molecules that act inside the plant cells and alter the growth and development of plants. PGRs can be broadly divided into two groups: plant growth promoters (auxins, gibberellins and cytokinins) and bio inhibitors (ABA, methyljasmonate). GA_3, increases stem length, the number of flower per plant and induces fruit setting (Azuma *et al.,* 1997). Recent research has shown that GA_3 treatment leads to higher polyphenol and anthocyanin content (Teszl´ak *et.al,* 2005).

Polyphenols are a group of secondary plant compounds, which not only plays an important role in the plant itself as protective agents against fungus attack and UV irradiation, but also has a positive effect on human health (Bravo, 1998). Therefore, recent interest in food phenolics has increased greatly because of their antioxidant, antimutagen and free radical-scavenging abilities and their potential effects on human health in the prevention of many of the major contemporary chronic diseases. There are reported data describing *C. officinalis* extracts as rich in polyphenols (Efstratiou *et al.,* 2012; Ercetin *et al.,* 2012). Alteration of poly phenolic and anthocyanin content, evoked by GA_3 treatment of grapevines would thus have a direct impact on antioxidative potential (Teszl´ak *et al.,* 2005).

Since no data exists on the impact of GA_3 Marigold treatment on polyphenol content, and antiradical activity, present study was conducted in order evaluation of the potential antioxidant activity and also estimation of the phenolic content of *C. officinalis* at three spraying stages and different GA_3 levels.

Materials and methods

Material chemicals

Phenolic acid standard (gallic acid), Folin–Ciocalteu reagent, sodium carbonate, methanol and DPPH were purchased from Merck (Darmstadt, Germany). Gibberellic acid (GA_3), Tween 20 were from Fluka (Buchs, Switzerland), all chemicals were of reagent grade.

GA_3 treatment of grapevine flowers

Airial parts of marigold were sprayed at three stages (first, second and third) with an aqueous GA_3 solution (containing 0.2% Tween 20). GA_3 was dissolved in deionised water directly on site before use to ensure constant application doses of 0, 50, 150 and 250 mg L^{-1}.

Preparation of the extracts

The leaf and steam parts of *C. officinalis* were separated by hand, air-dried at room temperature, and powdered in a mechanical grinder. Parts of plant were weighed accurately in a digital balance (Shimadzu AW320), the extraction solvent was methanol–water (4:1 v/v), and extraction carried out at ambient temperature (20°C) for 24 h using a laboratory shaker. The ratio of methanol and water which result the highest yield of phenolic compounds and flavonoids during preliminary trials selected as best ratio. Similar ratio of methanol to water was used by biglari *et al.* (2008). Each extract was filtered with whatman No. 1 filter paper. The obtained filtrate evaporated to dryness at 40°C in a rotary evaporator (Buchi Laborator). Then all the extracts were dried by a freeze dryer and dried sample constituents stored at 4°C until use (Arabshahi-Delouee and Urooj, 2006).

Estimation of total phenolic compounds

Total phenolic content of each extract was determined by the Folin–Ciocalteu micro method (Slinkard and Singleton 1977). Briefly, 20 µl of extract solution were mixed with 300 µl of Na2CO3 solution (20%), then 1.16 ml of distilled water and 100 µl of Folin–Ciocalteu reagent added to mixture after 1 min and 8 min respectively. Subsequently, the mixture was incubated in a shaking incubator at 40¬°C for 30 min and its absorbance was measured at 760 nm. Gallic acid was used as a standard for calibration curve. The phenolic content was expressed as gallic acid equivalents by using the following linear equation were obtained from calibration curve:

$$A = 0.98\ C + 9.321 \times 0.001 \quad (1)\quad R2 = 0.9965$$

Where A is the absorbance and C is concentration as gallic acid equivalents (µg/ml).

DPPH radical scavenging activity

The ability of extracts to scavenge DPPH radicals was determined according to the Bios (1958) method. Briefly, 1 ml of a 1 mM methanolic solution of DPPH was mixed with 3 ml of extract solution in methanol (containing 50–400 µg of dried extract). The mixture was then homogenized vigorously and left for 30 min in the dark place (at room temperature). Its absorbance was measured at 517 nm and activity was expressed as percentage of DPPH scavenging relative to control using the following equation:

$$\text{DPPH scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100 \quad (2)$$

Statistical analysis

All these experiments were replicated three times, and the average values are reported. The Effects of different GA_3 levels (*Calendula officinalis*) at three spraying stages on phenolic compounds and antioxidant activity of Marigold were determined using the analysis of variance (ANOVA) method, and significant differences of means were compared using Duncan's test at P<0.05 significant level using the SAS software (2001) program.

Result and discussion

Phenolic compounds

In order to determine how GA_3 application might affect phenolics content of *C. officinalis*, both leaves and the steams were harvested to compare the level of phenolics in the plants.

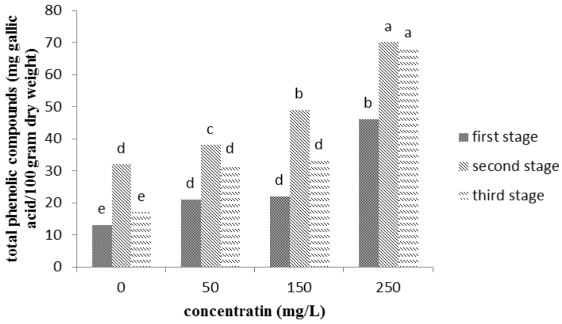

Fig. 1. Effects of: GA_3 on phenolic compounds of *C. officinalis*. Each observation is a mean±SD of 3 replications. In each figures means with same superscripts had no significant difference with each other (P>0.05).

As can be seen from figure 1, concentration and spraying stages of GA_3 had significant effect on (P<0.05) total phenolic content. By increasing in concentration, total phenolic compound increased. the highest total phenolic content was obtained from the *C. officinalis* plants treated with GA_3 at second stage, and was significantly greater than the first and third stage. The reduction in phenolics content between third stage and other stages may be due to the developmental stage of the plants within each group, timing of GA_3 application and a complex metabolic process occurring during the shift from vegetative to reproductive growth (Sharaf-Eldin *et al.,* 2007).

Sharaf-Eldin *et al.,* (2007) showed that although GA_3 increased phenolic compounds in leaves, it decreased it in the flower receptacles of globe artichoke. Our results are in agreement with the finding of Anastasia *et al.,* (2012) who reported GA_3, IAA and kinetin significantly increased specific phenolic compounds (gallic acid and rutin) in lentil plants.

DPPH radical scavenging activity

The results of DPPH radical scavenging activity of *C. officinalis* extracts affected by different GA_3 levels and spraying stages were shown in figure 2. Free radicals which are involved in the process of lipid per-oxidation are considered to play a major role in numerous chronic pathologies, such as cancer and cardiovascular diseases among others (Dorman *et al.,* 2003). The DPPH radical has been widely used to evaluate the free radicals scavenging ability of various natural products and has been accepted as a model compound for free radicals originating in lipids (Porto *et al.,* 2000).

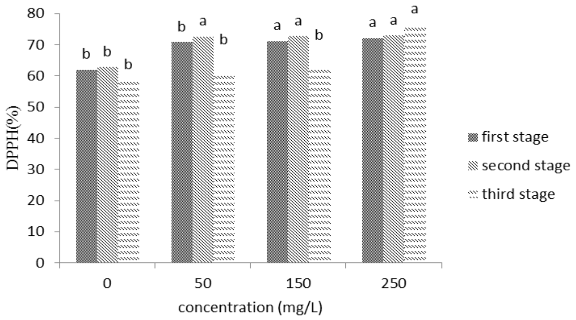

Fig. 2. Effects of: GA_3 on DPPH radical scavenging activity of *C. officinalis*. Each observation is a mean±SD of 3 replications. In each figures means with same superscripts had no significant difference with each other (P>0.05).

As it can be seen from figure 2, second stage that contained the highest amount of total phenolics, was found to be the most active radical scavenger followed by first stage and third stage. A high correlation between free radical scavenging and the phenolic contents has been reported for fruits (Gao *et al.,* 2000; Arabshahi Delouee and Urooj, 2006). Figure 2 also showed that DPPH radical scavenging increased by increasing GA_3 concentration and no significant differences ($P< 0.05$) were found between DPPH radical scavenging of most concentrations and spraying stages.

Conclusions

The presence of phenolic compound in high concentration in the *C. officinalis* could be of interest as a primary source of naturally occurring bioactive substance. Total phenolic content (TPC) and antiradical activity of *C. officinalis* were varied with different GA_3 levels and spraying stages. TPC and antiradical activity increased by increase of concentration from 0 to 250 mg L^{-1} at all spraying stages. The results obtained in this study provide the first documentation on the phenolic profile and antioxidant properties of *C. officinalis* as affected by GA_3. Thus, the significant occurrence of bioactive phenolic compounds along with the interesting antioxidant activity of *C. officinalis* treated with PGRs make them useful for daily inclusion in the human diet.

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