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Physiological effect of some antioxidant polyphenols on sweet marjoram (*Majorana hortensis*) plants

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Abstract. *El-Moursi A, Talaat IM, Balbaa LK. 2012. Physiological effect of some antioxidant polyphenols on sweet marjoram (*Majorana hortensis*) plants. Nusantara Bioscience 4: 11-15.* Two pot experiments were conducted in the screen of the National Research Centre, Dokki, Cairo, Egypt to study the physiological effect of foliar application of some antioxidant polyphenols on growth and chemical constituents of sweet marjoram plants (*Majorana hortensis* L.). Plants were treated with curcuminoids, cinnamic acid and salicylic acid, each at 5 and 10 mg/L except the control plants. The results indicate that foliar application of curcuminoids increased growth parameters under study. Total sugars were also increased as a result of foliar application of curcuminoids. On the other hand, oil %, oil yield, and nitrogen % were decreased as a result of curcuminoids treatments. Cinnamic acid at 5 mg/L resulted in the tallest plants in most cases. Application of cinnamic acid at 10 mg/L significantly increased oil % and total oil yield/plant. Sugar content followed the same trend. Treatment of sweet marjoram plants with salicylic acid significantly increased oil % and oil yield, especially in plants treated with 10 mg/L SA. Total sugars % and total nitrogen % followed the same trend. The main constituents of the plant essential oil were also markedly affected.

Keywords: sweet marjoram, antioxidant polyphenols, curcuminoids.

Abstract. *El-Moursi A, Talaat IM, Balbaa LK. 2012. Pengaruh fisiologis beberapa polifenol antioksidan terhadap tanaman marjoram manis (*Majorana hortensis*). Nusantara Bioscience 4: 11-15.* Dua percobaan pot telah dilakukan di rumah kaca Pusat Penelitian Nasional, Dokki, Kairo, Mesir untuk mempelajari pengaruh fisiologis aplikasi foliar beberapa polifenol antioksidan pada pertumbuhan dan kandungan kimia tanaman marjoram manis (*Majorana hortensis* L.). Tanaman diperlakukan dengan kurkuminoid, asam sinamat dan asam salisilat, masing-masing sebanyak 5 dan 10 mg/L, kecuali tanaman kontrol. Hasil yang diperoleh menunjukkan bahwa aplikasi foliar dari kurkuminoid meningkat parameter pertumbuhan tanaman yang diteliti. Total gula juga meningkat akibat aplikasi foliar kurkuminoid. Di sisi lain, persentase minyak, hasil minyak dan persentase nitrogen menurun akibat perlakuan kurkuminoid. Perlakuan asam sinamat pada 5 mg/L menghasilkan tanaman tertinggi dalam keseluruhan percobaan. Perlakuan asam sinamat pada 10 mg/L secara signifikan meningkat persentase minyak dan kandungan minyak total/tanaman. Kadar gula menunjukkan kecenderungan yang sama. Perlakuan tanaman marjoram manis dengan asam salisilat secara signifikan meningkatkan persentase minyak dan kandungan minyak yang dihasilkan, terutama pada tanaman yang diperlakukan dengan asam salisilat sebanyak 10 mg/L. Total persentase gula dan total persentase nitrogen menunjukkan kecenderungan yang sama. Konstituen utama dari minyak atsiri tanaman juga sangat terpengaruh.

Key words: marjoram manis, polifenol antioksidan, kurkuminoid.

INTRODUCTION

Marjoram (*Majorana hortensis* L) is an annual, sometimes biennial herb or sub-shrub, with an erect, square, slightly hairy stem. The grayish leaves are opposite, oval and short-stalked. The small, white or purplish two-lipped flowers are arranged in roundish clusters (‘knots') in the leaf axil. The fruit consists of four smooth nutlets, which ripen only in warm regions (Figure 1). All parts of the plant are pleasantly aromatic.

The flowering stems are the medicinal parts. Their constituents include 1-2% of essential oil with a spicy fragrance containing terpinines and terpinol, plus tannins, bitter compounds, carotenes and vitamin C. These substances give sweet marjoram stomachic, carminative, choleretic, antispasmodic and weak sedative properties. In herbalism, it is used mainly for various gastrointestinal disorders and to aid digestion. It is also an ingredient of ointments and bath preparations used to alleviate rheumatism (Stodola and Volàk 1992).

Curcuminoids are antioxidant polyphenols and what is considered as curcumin or a derivative of curcumin with different chemical groups that have been formed to increase solubility of curcumins and make them suitable for drug formulation. These compounds are polyphenols and produce a pronounced yellow color. Many curcumin characters are unsuitable for use as drugs by themselves. They have poor solubility in water at acidic and physiological pH, and also hydrolyze rapidly in alkaline solutions (Péret-Almeida et al. 2005). Therefore, curcumin derivatives are synthesized to increase their solubility and hence bioavailability (Tomren 2007). Curcuminoids are

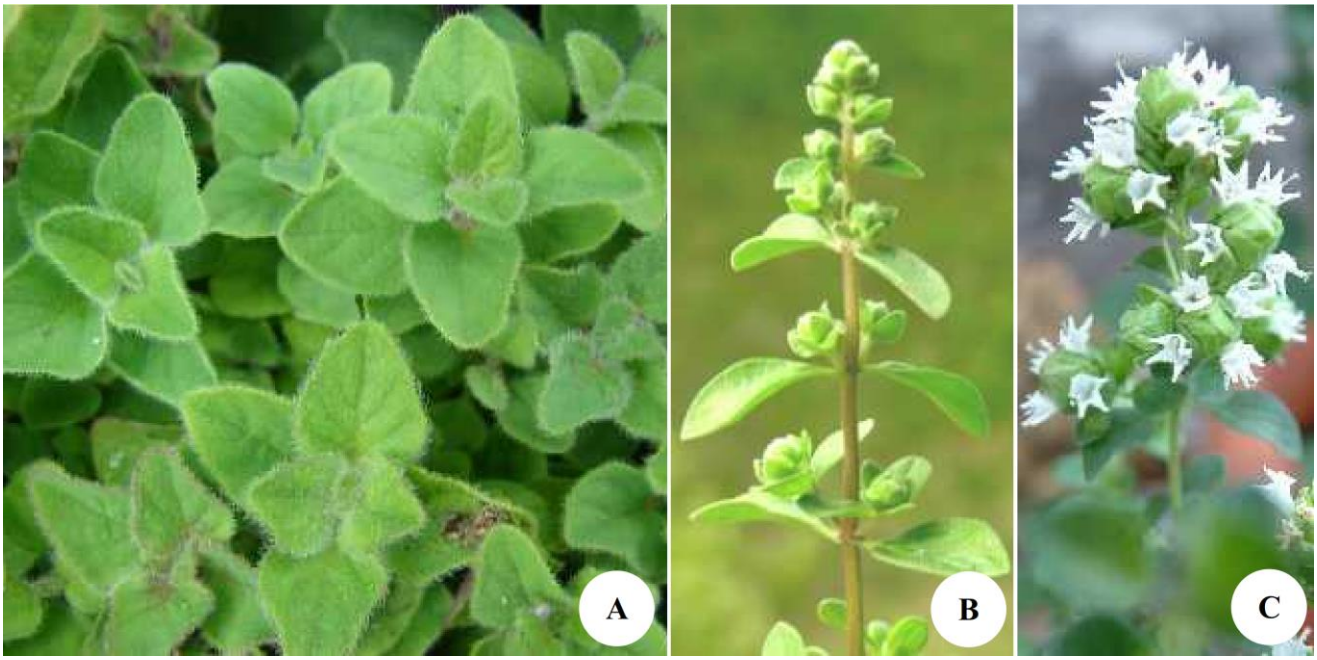

Figure 1. Sweet marjoram plant. A. general appearance, B. Spike, C. Flower (photos from several sources).

soluble in dimethyl sulfoxide (DMSO), acetone and ethanol (Tiyaboonchai 2007), but are poorly soluble in lipids. It is possible to increase curcuminoid solubility in aqueous phase with surfactants or co-surfactants (Jayaprakasha et al. 2006). Curcumin derivatives have been synthesized that could possibly be more potent than curcumin itself. Most common derivatives have different substituents on the phenyl groups (Tiyaboonchai 2007). There is currently an increasing demand for demethoxycurcumin and (curcuminoids) because of their recently discovered biological activity (Tønnesen et al. 2002).

Many investigators studied the role of trans-cinnamic acid in stimulating growth and activating plants. It was reported that plants synthesize large amounts of phenylpropanoid acids, mainly hydroxycinnamic acids, which are often found in conjugated forms, such as glycosides or Glc esters. These conjugates have been identified in numerous plants (Molgaard and Ravn 1988 and Herrmann 1989). Glucosides may be bioactive by themselves as defense compounds or they may be storage forms (Dixon 2001). On the other hand, 1-O-acyl Glc esters may serve as activated intermediates analogous to CoA thioesters in plant secondary metabolism (Villegas, and Kojima 1986; Lehfeldt et al. 2000). Glycosylation of hydroxycinnamic acids to form both glycosides and Glc esters is catalyzed by a group of enzymes called glucosyltransferases (GTs), which transfer the Glc residue from mostly UDP-activated Glc (Mock and Strack 1993). Related GTs are known that glycosylate other compounds, such as flavonoids (Cheng et al. 1994), alkaloids (Moehs et al. 1997; Kita et al. 2000), terpenoids (Jones et al. 1999), cyanohydrins (Reed et al. 1993, thiohydroxymates, and plant hormones, Jackson et al. 2001). Many glycosyltransferases are able to glycosylate more than one aglycon, and they appear to recognize only the part of the molecule where glycosylation takes place (Hoesel 1981). Glycosylation normally takes place in the cytosol, but Glc conjugates are found in the vacuole (Vogt and Jones 2000). Salicylic acid (SA) was reported to play a role of natural inductor of thermogenesis in *Arum* lily, induces flowering in a range of plants, controls ion uptake by roots and stomatal conductivity (Raskin 1992).

The aim of the present study was to investigate the effect of some antioxidant phenolic compounds (curcuminoids, cinnamic acid, and salicylic acid) on the growth and chemical constituents of sweet marjoram plant.

MATERIAL AND METHODS

Growth of sweet marjoram plant

Two pot experiments were carried out during two successive seasons of (2007/2008- 2008/2009) at the screen of National Research Centre (NRC), Dokki, Cairo, Egypt. Seeds of sweet marjoram were secured from Horticulture Research Institute, Agricultural Research Centre, Ministry of Agriculture, Egypt. The seeds were sown in the nursery on 21st February 2007 and 2008, respectively. 45 days later, the seedlings were transferred into clay pots 30 cm in diameter, each pot contained 8 kg loamy clay soil. Fifteen days after sowing, the seedlings were thinned leaving two uniform plants. Each pot received equal and adequate amounts of water and fertilizers. Phosphorous as calcium

superphosphate was mixed with the soil before sowing at the rate of 4.0 g/pot. Three grams of nitrogen as ammonium sulfate in three applications (one g for each) with two weeks intervals started 30 days after sowing. Also, two grams of potassium sulfate was added as soil application. Other agricultural processes were performed according to normal practice. 30 days after planting, transplants were sprayed with different concentrations of curcuminoids, cinnamic acid or salicylic acid, each at (5, 10 mg/L) in addition to control plants which were sprayed with distilled water.

Chemical constituents of sweet marjoram plant

Curcuminoids were extracted from ginger plants and were secured from Department of Natural Products, NRC, Dokki, Cairo, Egypt. Treatments were distributed in a completely randomized block design with three replications, each replicate comprising three pots. The plant herbage was harvested, by cutting 5 cm above the soil surface, and plant growth characters in terms of plant height, number of branches, and herbage fresh and dry weights were recorded. Total sugars percent were determined according to Dubois et al. (1956). Total nitrogen was determined using the modified Micro-Kjeldahl method according to Jackson (1973).

Chemical composition of essential oil

Samples from the fresh herbage of each treatment were separately subjected to hydro-distillation in order to determine the percentage of essential oil according to the Egyptian Pharmacopoeia (1984). Qualitative and quantitative determination of the different main constituents of marjoram oil, obtained from the first cut from each treatment had been carried out in parallel with authentic samples of different oil components by GLC technique. The qualitative identification of the main oil fractions was carried out by comparing the relative retention time of different peaks with those of the pure authentic samples. The quantitative determination was achieved by the peak area percentage, which was measured for each fraction; to study the changes in the constituents of marjoram oil as a result of the effect of different treatments used.

For this purpose, gas-liquid chromatographic apparatus (VARIAN-3700), equipped with FID, Hp 4270 Integrator, was used for the separation of marjoram oil fractions of the samples. The analysis conditions were as follows: The chromatography was fitted with (2m x 1/8``) columns, peaked with Diatomic G.Hb, (100-120) mesh, and coated with 10% DEGS. 12 Ft. S.S. The columns were operated, using a temperature program, a linear increase with rate of 4°C/min, from (70°C to 190°C); with nitrogen at 30 mL/min, as a carrier gas. The flow rates for hydrogen and air were 30 and 300 mL/min, respectively. Detector temperature was 280°C. Chart speed was 0.5 cm/min range: 32; sample size was about 2 mL. Sensitivity of the apparatus was 18-8 x32. The standard material was injected with the samples of marjoram oil under the same conditions.

Data analysis

Data obtained were subjected to standard analysis of variance procedure. The values of LSD were obtained whenever F values were significant at 5% level as described by Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Growth of sweet marjoram plant

Data presented in Table 1 show that curcuminoids treatment at 5 mg/L significantly promoted plant height, number of branches, fresh and dry weights of herb in both cuttings. Fresh and dry weights of herb followed the same trend. Application of curcuminoids at 10 mg/L resulted in a marked decrease in the number of branches in the second cut. Cinnamic acid at 5 mg/L resulted in the tallest plants in most cases, number of branches, fresh and dry weights of herb followed the same trend. Salicylic acid treatments significantly increased plant height, number of branches, fresh and dry weights of herb, especially in plants treated with 5 mg/L SA (Table 1).

In this concern, Raskin (1992) reported that salicylic acid (SA) is an endogenous growth regulator of phenolic nature, which participates in the regulation of physiological processes in plants. SA, for example, plays a role as natural inductor of thermogenesis in *Arum* lily, induces flowering in a range of plants, controls ion uptake by roots and stomatal conductivity.

Talaat (2005) reported that foliar application of salicylic acid (50 or 100 µM) enhanced the vegetative growth of geranium plants, especially at 100 µM concentration. Talaat and Balbaa (2010) also reported that exogenous application of trans-cinnamic acid on basil plants considerably increased plant growth at both the two cuttings. It was also recognized that the most promising results of vegetative growth criteria (i.e., plant height, number of branches, fresh and dry weights of herb) were obtained from plants treated with trans-cinnamic acid (250 mg/L).

Chemical constituents of sweet marjoram plant

Data presented in Table 2 show that oil % and total oil yield/plant were significantly decreased as a result of foliar spray of curcuminoids at 5 mg/L and 10 mg/L. These results hold true for essential oil % and total oil yield/plant in both cuttings. Total nitrogen % followed the same trend. On the other hand, total sugars % was pronouncedly increased as a result of curcuminoids treatments.

Data also indicate that application of cinnamic acid, especially at 10 mg/L significantly increased oil % and total oil yield/plant. Sugar content followed the same trend. On the other hand, total nitrogen % was markedly decreased as a result of cinnamic acid treatments.

Meanwhile, treatment of sweet marjoram plants with salicylic acid significantly increased oil % and oil yield, especially in plants treated with 10 mg/L SA. Total sugars % and total nitrogen % followed the same trend.

In this respect, Talaat and Balbaa (2010) reported that chemical analysis of the leaves of sweet basil at both the

first and second cuts indicated that the contents of total essential oil % and oil yield in basil herb were significantly increased as a result of foliar application of trans-cinnamic acid. Similar results were obtained for total carbohydrates and total soluble sugars, Total nitrogen, total phosphorus, and total potassium contents. Iron and zinc contents followed the same trend.

These findings were in agreement with those obtained by Tari et al. (2002) who reported that SA application resulted in a significant increase in total soluble sugar content in leaves of Camellia cuttings thus maintaining the carbohydrates pool in the chloroplasts at a high level. This increase may be implicated in osmotic adjustment as it has been described in tomato SA-treated plants (Tari et al. 2002). Talaat (2005) also reported that foliar application of salicylic acid (50 or 100 µM) increased total sugars %, total protein (µg/g FW), essential oil %, and essential oil yield. Kaveh et al. (2004) reported that very low dose (50 µM) of SA considerably enhanced the growth and carbohydrate metabolism of tea cuttings. Addition of 50 µM SA produced the most remarkable effects. There was a 2 fold significant increase in leaf area, leaf fresh weight, and leaf dry weight. Leaf TSS was also doubled by this treatment. Invertase activity in SA treated cuttings was higher than in control with a significant increase for 50 µM SA.

Table 1. Effect of some antioxidant polyphenols on vegetative growth of sweet marjoram plants

Treatments (mg/L)	Plant Height		Number of branches		Fresh wt. of herb		Dry wt. of herb	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Curcuminoids 5	46.67	28.00	21.67	23.00	75.10	48.78	31.20	17.08
Curcuminoids 10	43.67	26.00	17.67	17.67	75.01	39.71	30.53	13.61
Cinnamic acid 5	49.00	27.00	19.33	22.00	79.63	55.47	27.47	28.11
Cinnamic acid 10	51.00	33.67	23.33	28.33	93.18	76.82	32.78	30.27
Salicylic acid 5	46.33	30.33	18.00	27.00	77.10	74.86	25.98	22.15
Salicylic acid 10	48.00	32.00	20.67	28.00	88.16	75.15	32.87	29.83
Control	42.33	25.67	14.00	20.00	75.30	41.72	24.27	13.73
LSD (5%)	3.30	1.19	2.34	2.18	4.07	4.15	4.32	3.60

Table 2. Effect of some antioxidant polyphenols on chemical constituents of sweet marjoram plants

Treatments (mg/L)	Oil %		Oil yield		Total sugars %	Total nitrogen %
	1st cut	2nd cut	1st cut	2nd cut	1st cut	1st cut
Curcuminoids 5	0.34	0.29	0.26	0.14	16.4	9.2
Curcuminoids 10	0.25	0.26	0.19	0.10	16.95	8.25
Cinnamic acid 5	0.38	0.46	0.30	0.25	15.15	9.69
Cinnamic acid 10	0.41	0.48	0.38	0.37	16.85	7.06
Salicylic acid 5	0.39	0.42	0.30	0.32	16.1	9.69
Salicylic acid 10	0.42	0.44	0.37	0.33	16.35	11.88
Control	0.36	0.38	0.27	0.16	14.15	9.69
LSD (5%)	0.06	0.07	0.05	0.05	N.S.	N.S.

Chemical composition of essential oil

To study the effect of different treatments on essential oil composition of sweet marjoram plants the oil of each liquid chromatography and the main compounds and their relative percentages are shown in (Table 3). Linalool ranged from 10.62% in plants treated with 5 mg/L salicylic to 32.36% in plants treated with 10 mg/L curcuminoids. The highest content of α-terpineol (38.11%) was observed in plants received 10 mg/L curcuminoids.

In this respect, Talaat (2005) reported that foliar treatment of pelargonium plants with salicylic acid at the rate of 50 µM/L resulted in the highest content of citronellol. Gamal El-Din and Reda (2006) also reported that treatment of chamomile plants with salicylic acid, especially at 60 µM/L resulted in quantitative increases of some essential oil constituents.

Table 3. Effect of curcuminoids on essential oil constituents of sweet marjoram plants.

Treatments (mg/L)	Curcuminoids 5	Curcuminoids 10	Cinnamic acid 5	Cinnamic acid 10	Salicylic acid 5	Salicylic acid 10	Control
α-pinene	1.55	0.76	1.16	1.40	0.12	1.03	1.06
β-pinene	7.09	0.72	0.38	2.51	6.51	7.02	7.03
camphene	7.35	1.49	0.80	8.85	6.90	6.68	7.90
d-limonene	17.28	5.01	7.88	7.95	13.84	14.16	17.11
Cymene	7.63	8.74	6.71	23.30	8.12	7.06	6.49
Linalool	11.20	32.36	15.81	10.62	14.76	14.13	16.83
α-terpineol	29.05	38.11	35.40	31.68	30.89	33.21	28.56
Geraniol	3.09	-	1.94	5.10	5.88	5.98	1.72
Carvone	2.73	-	4.33	0.81	1.21	0.97	3.49
Eugenol	1.59	-	0.81	0.11	0.19	0.19	0.86
Citronellol	-	-	0.26	1.88	0.12	0.13	1.72
Ethyl cinnamate	-	0.22	0.50	2.14	1.65	0.49	-
Carvacrol	1.88	3.87	1.73	0.17	0.17	0.19	0.16
Thymol	0.55	1.82	0.19	0.27	0.34	0.21	0.28
Known	90.99	92.88	77.62	95.15	91.19	92.61	93.7
Unknown	9.01	7.12	22.38	4.85	8.81	7.39	6.3

CONCLUSION

From the above-mentioned data, it could be concluded that the antioxidant polyphenols (curcuminoids, cinnamic acid, and salicylic acid) might play a role in plant phytochemical mechanisms through affecting the metabolism of terpenes, essential oil, carbohydrates and proteins, but further studies are needed to learn more about these mechanisms.

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