

Growth, Phenolic Compounds and Antioxidant Activity of Some Medicinal Plants Grown under Organic Farming Condition

¹M.Y. Khalil, ²A.A. Moustafa and ¹N.Y. Naguib

¹Department of Cultivation and Production of Medicinal and Aromatic Plants, N.R.C., Giza, Egypt

²Department of Biochemistry, Faculty of Agriculture, Cairo University, Giza, Egypt

Abstract: Eight medicinal plants were cultivated in the Experimental Farm Station of the National Research Centre at Shalakan Kalubia Governorate, Egypt, during the two consecutive seasons 2003/2004. Plants were grown under organic farming conditions, as the soil was treated with organic compost without using mineral or chemical fertilization. The herbs of the plants were harvested and subjected to the estimation of phenolic compounds and antioxidative activities in their extracts. The results showed that plant growth parameters varied greatly. Marigold plants were significantly the highest, while sage plants resulted significantly in the heaviest fresh and dry herb weight. Salicylic acid was the highest phenolic compound, compared to the other fourteen phenolics. Salicylic was present in high contents in all studied plants except sage which contained the largest quantity of pyrogallol. Catechol, protocatechic and cinnamic acid were the phenolics present in the lowest quantity. Different plants greatly varied in the contents of the phenolic compounds. Antioxidant activity increased as ethanol extract raised from 100 to 150 and 200µl extract. Sage showed very strong antioxidant capacity (91.34% inhibition of peroxidation at 200 µl extract), followed by dragonhead and plantago. Other plants showed moderate antioxidant activities. Further work on the effect of organic farming compared to chemical one on phenolics and antioxidant activity as well as on different medicinal plants are suggested.

Key words: Phenolic compounds • antioxidative activities • marigold (*Calendula officinalis*) • dragonhead (*Dracocephalum moldavica* L.) • fennel (*Foeniculum vulgare*) • plantago (*Plantago afra* L.) • clary (*Salvia verbenaca*) • sage (*Salvia viridis*) • sideritis (*Sideritis montana*) • milk thistle (*Silybum marianum*)

INTRODUCTION

Approximately 80% of the world population depend exclusively on plants for their health and healing. Whereas in the developed world, reliance on surgery and pharmaceutical medicine is more usual but in the recent years, more and more people are complementing their treatment with natural supplements [1]. Furthermore, motivation of people towards herbs are increasing due to their concern about the side effect of drugs, those are prepared from synthetic materials. The people want to concern their own health rather than merely submitting themselves to impersonal health care system. Many botanical and some common dietary supplements are good sources of antioxidants and anti-inflammatory compounds [2, 3].

Plant materials containing phenolic constituents are increasingly of interest as they retard oxidative degradation of lipids and thereby improving quality and nutritional value of food [4, 5]. The importance of the antioxidant constituents of plant material is the maintained of health and protection from coronary heart disease and cancer [6]. They are vital substances that possess the ability to protect the body from damage caused by free radical induced oxidative stress [7, 8]. Several researches on the phenolic constituents and antioxidant activities in various plants have been conducted [9-13]. Meanwhile, Galvez *et al.* [12] pointed out that there was a correlation between antioxidant capacity and phenolics content. However, Kahkonen *et al.* [10] stated that antioxidants activity does not necessarily correlate with high amounts of phenolics.

Organic farming was recommended by United Nations Organizations [14, 15] as it ensures safety products for human health as well as environments [16]. Organic manuring have beneficial impacts on soil properties and produce safe plants with good, neat sources of better availability of nutrients [17, 18]. Many investigators obtained best results by using organic compost for several medicinal and aromatic plants, as on some aromatic plants [19]; on fennel [20, 21]; on rosmary (*Rosemarinus officinalis*) [22]; on three *Mentha* species [23]; on *Hyosyamus muticus* [24] and on *Sidritis montana* [25].

Medicinal and aromatic plants a gift of nature are being used against various infections and diseases in the world since past history. *Calendula officinalis* (marigold) was cultivated by Egyptians, Greeks and Arab [26]. It is well known as cleansing and detoxifying herb [27] and antiviral, antitumor, have mutagenic and cytotoxic properties [28]. *Dracocephalum moldavica* L. (dragonhead) a member of Family Lamiaceae, is a hardy annual plant with aromatic, balm scented, green foliage [29]. It is a pain killer [30] and antitumor and anti-rheumatic [31]. Fennel (*Foeniculum vulgare* Mill.), Family Apiaceae is a short-lived aromatic and medicinal herb, indigenous to Europe and cultivated in India, China and Egypt [32]. Choi and Hwang [33] stated that fennel had anti-inflammatory, analgesic and antioxidant activities. Plantago (*Plantago afra* L.), Family Plantaginaceae, is native to Mediterranean, an annual, erect, glandular-hairy caulescent herb [29]. It had a long history of popular traditional uses as medicine in many countries for treating various diseases varying from cold to cancer and viral hepatitis [27]. Galvez *et al.* [12] observed that *Plantago* spp. showed also good antioxidant activity. *Salvia verbenaca* (Clary) Family Lamiaceae, from the latin 'Salvere', meaning to heal, save or to be safe and unharmed indicating the medical value of the plants of salvia. Its medicinal virtues are rather more powerful for disordered of states of digestion, violent cases of hysteria [34]. Herb drunk has been recommended as a very helpful in all women's diseases and ailments [35]. Sage (*Salvia viridis*) is antioxidant in cheeses, pickles, vegetables and beverages [34]. Erdemoglu *et al.* [13] pointed out that sage have a potential source of antioxidants of natural organ as its extract possess a significant scavenger activity against DPPH free radical and an inhibitory effect on H₂O₂. Sideritis (*Sideritis montana* L.), Family Labiatae, has an important pharmaceutical utilization to treat a

variety of disorders [36]. Its extract has an oxidative activity attributed to the presence of flavonoids and phenylpropanoid glycosides [35]. *Silybum marianum* Gaertn. (milk thistle, mary thistle) Family Asteraceae, is famous in curing a whole range of liver and gall bladder. Its leaves has an oxidant capacity more than vitamins C and E. the leaves of plant have white veins that look as if milk was spilled upon them-according to legend, the milk of the Virgin Mary. It can help reverse the damage done from eating poisonous mushrooms.

This study aimed to estimate the vegetative growth characters, phenolic compounds and oxidative activities of eight aromatic and medicinal plants grown under organic farming conditions.

MATERIAL AND METHODS

Eight medicinal plants were cultivated under organic farming conditions at two successive seasons of 2003/2004 and 2004/2005, in the Experimental Farm Station of National Research Centre, at Shalakan Kalubia Governorate, Egypt. Seeds of marigold (*Calendula officinalis*), dragonhead (*Dracocephalum moldavica* L.), fennel (*Foeniculum vulgare*), plantago (*Plantago afra* L.), clary (*Salvia verbenaca*), sage (*Salvia viridis*), sideritis (*Sideritis montana*) and milk thistle (*Silybum marianum*) were sown at 15th October of both seasons, in plots of 3.06m² (1.75 X 1.75m) on rows 35cm, in between and the distances between plants were 35cm. Every plot contained 25 plants. The experimental soil was clay loamy having the following properties: 5.3% coarse sand, 30.6% fine sand, 38.7% silt and 25.4% clay, 0.56 dsm⁻¹, E.C., 8.1 pH, 3.2% CaCO₃, 512ppm total soluble salts, 0.11% total nitrogen, 41.1, 17.0 and 401ppm N, P(P₂O₅) and K(K₂O) respectively and 22.2, 3.9, 2.1 and 2.6 mg⁻¹ DTPA extractable Fe, Mn, Zn and Cu consecutively. The soil was thoroughly plows and mixed with organic compost, a product of Green Valley for Organic Products Co, SAE, Egypt, at the rate 18m³/feddan. The properties of the compost were: 35.4% moisture content, 7.0 pH, 1.2 mmhos/cm² EC, 263.0 mg/kg ammonium nitrate, 45.6% organic matter, 18.89% organic carbon, 39.56% ash, 16:1 C/N ratio and 1.4, 0.466 and 1.21% total N, P and K, respectively. The treatments were replicated thrice. Agriculture routine practices as irrigation, weeding... etc, were carried out whenever required. The herbs were harvested at 15th February 2004 and 2005 and the following data was assessed:

Vegetative growth: A sample of five plants in each replicate were randomly taken and the following characters were recorded: plant height (cm) from soil surface to the summit of the plant and fresh and dry weights (g/plant).

Chemical analysis:

1-Sample preparation: Fresh herb of the eight kinds of plants were collected, cleaned and immediately stored at 20°C until lyophilized (Delta, condenser temperature -45°C, pressure 0.01 m bar). After lyophilization, the freeze-dried tissues were ground to pass a 0.5mm sieve and allowed to equilibrate in open air.

2-Extraction and hydrolysis: The phenolic compounds were extracted according to Hertog *et al.* [37]. Extracts were prepared as follows: 40 ml of 62.5% aqueous methanol was added to 0.5 g of freeze-dried sample material. 10ml of 6 M HCl was added to this extract with careful mixing. The extraction solution thus obtained consisted of 1.2 M HCl in 50% aqueous methanol (v/v). After refluxing at 90°C for 2h with regular swirling, the extract was allowed to cool and was subsequently made up to 100ml with methanol and sonicated for 5 min. approximately 2ml was filtered through a 0.45-µm filter for organic solvents prior to injection.

3-High Performance Liquid Chromatography (HPLC)

analysis: Identification of individual phenolic compounds of the plant extracts was performed on a Hewlett-Packard HPLC (Model 1100). Using a hypersil C₁₈ reversed-phase column (250 x 4.6 mm) with 5 µm particle size. Injection by means of a Rheodyne injection valve (Model 7125). A constant flow rate of 1 ml min⁻¹ was used with two mobile phases: (A) 0.5% acetic acid in distilled water at pH 2.65; and solvent (B) 0.5% acetic acid in 99.5% acetonitrile. The elution gradient was linear starting with (A) and ending with (B) over 35 min., using an UV detector set at wavelength 254 nm [38]. Phenolic compounds of each sample were identified by comparing their relative retention times with those of the standards mixture chromatogram. The concentration of an individual compound was calculated on the basis of peak area measurements and then converted to mg phenolic/100g dry weight. All chemical and solvents used were HPLC spectral grade. Standard phenolic compounds were obtained from Sigma (St. Louis, USA) and Rom Merck-Sheuchardt (Munich, Germany Chemical Companies).

4-Antioxidant activity in a linoleic acid system:

Antioxidant activity assay was carried out by using the linoleic acid system. Linoleic acid emulsion (0.02 M) was prepared with linoleic acid (0.2804 mg) and Tween 20 (0.2804 g) in phosphate buffer (50 ml, 0.05 M, pH 7.4). A reaction solution, containing extracts (50-200 µl). Linoleic acid emulsion (2.5 ml) and phosphate buffer (2.3 ml, 0.2 m, pH 7.0) were mixed with a homogenizer. The reaction mixture was incubated at 37°C in the dark and the degree of oxidation was measured according to the thiocyanate method (0.1 ml, 30%) and sample solution (0.1 ml). After the mixture was stirred for 3 min, the peroxide value was determined by reading the absorbance at 500 nm and the inhibition percentage of linoleic acid peroxidation was calculated as (%) inhibition = $[1 - (\text{absorbance of sample at 500 nm}) / (\text{absorbance of control at 500 nm})] \times 100$. All tests were run in duplicate and analysis of all samples was done in triplicate.

- Blank was carried out in similar procedure and its value was subtracted from the obtained values of the treatments.
- The layout of the investigation was designed to provide complete randomized (CRD).
- Data obtained (mean of the two seasons), were subjected to standard analysis of variance procedure and the values of L.S.D. were obtained when the calculated 'F' values were significant at 5% level [39].

RESULTS AND DISCUSSION

Growth parameters: Data presented in Table 1 indicated that the eight medicinal plants varied greatly in their growth parameters, with significant differences among most of them. The marigold was significantly the highest plant (90.0 cm), followed by fennel (75.6 cm). Whereas plantain plants were significantly the shortest ones with mean height of 28.4 cm. Other plants varied between the highest and lowest plants with significant differences among them, except between clary and siderites.

Dry weight of the herbs attained similar trend to their fresh weights. Sage produced significantly the heaviest fresh and dry weights of the herb, which were 266.4 and 76.5g, respectively, compared to most plants. The siderites herb which came in the second rank, as weighed 147 and 38.8g fresh and dry weight, consecutively. The fresh and dry weights of the eight plants herb were in a

Table 1: Growth parameters for the medicinal plants grown under organic farming condition

Plants	Growth character		
	Plant height (cm)	Fresh weight (g/plant)	Dry weight (g/plant)
<i>Calendula officinalis</i> (marigold)	90.0	133.7	36.5
<i>Dracocephalum moldavica</i> (dragonhead)	61.3	86.5	26.8
<i>Foeniculum vulgare</i> (fennel)	75.6	98.8	27.3
<i>Plantago afra</i> (plantago)	28.4	60.2	18.1
<i>Salvia verbenaca</i> (clary)	51.0	94.0	27.6
<i>Salvia viridis</i> (sage)	70.1	266.4	76.5
<i>Sideritis montana</i> (sideritis)	48.6	147.9	38.8
<i>Silybum marianum</i> (milk thistle)	44.8	181.3	50.4
LSD at 0.05	5.4	12.3	2.9

descending order as the following: sage (*Salvia viridis*) < sideritis (*Sideritis montana*) < marigold (*Calendula officinalis*) < fennel (*Foeniculum vulgare*) < clary (*Salvia verbenaca*) < dragonhead (*Dracocephalum moldavica* L.) < plantain (*Plantago afra* L.) < milk thistle (*Silybum marianum*) with significant differences in most cases.

In this concern Bailey [29] and Hartnoll [40] reported that different plants vary greatly in their heights and vegetative growth characters as well as their flowering characteristics.

Phenolic compounds: Fifteen phenolic compounds were identified in plant herbs extracts (Table 2). These constituents were catechol, cinnamic acid, chlorogenic, coumarin, ferulic acid, hydroquinone, hydroxybenzoic, o-coumaric acid, p-coumaric acid, phenol, protocatechuic, pyrogalllic acid, resorcinol, salicylic acid and vanillin.

The eight plants under study showed great variations in their content of the different phenolic compounds.

In general, salicylic acid was the major phenolic compound in the herb of all the studied plants, except in sage which pyrogalllic acid was the major phenolic compound. Salicylic acid ranged between 449.54mg in fennel and 83.55mg in milk thistle herb. The least phenolic constituent was catechol which was detected in two plants only, sideritis (0.67 mg) and plantain (0.40 mg); whereas proto-catechuic, was absent in these two plants only, but its content in the other herbs was very low as it ranged between 0.13 and 1.16mg/100mg dry weight, in clary and marigold, consecutively. Hydroquinone was detected in four plants only: marigold (63.55 mg), milk thistle (23.72 mg), fennel (6.94 mg) and sideritis (2.19 mg).

Regarding different plants, the largest phenolic compounds after salicylic acid were pyrogalllic acid and p-coumaric acid in clary (77.46 mg each); ferulic acid (53.45 and 26.47 mg) in dragonhead and fennel, respectively; resorcinol (55.48 mg) in marigold, coumarin (75.69 mg) in milk thistle, pyrogalllic acid (34.58 mg) in plantain and

Table 2: The concentrations of phenolic compounds in eight medicinal herbs at their vegetative stage (mg/100g D.W.)

Phenolic compounds	Plants							
	1	2	3	4	5	6	7	8
Pyrogalllic acid	77.46	26.39	13.04	11.88	7.29	34.58	259.98	17.10
Hydroquinone	--	--	6.94	63.55	23.72	--	--	2.19
Resorcinol	16.94	16.64	2.86	55.48	18.34	19.18	7.29	97.52
Protocatechuic	0.13	0.63	0.41	1.16	1.08	--	0.52	--
Catechol	--	--	--	--	--	0.40	--	0.67
Hydroxyl benzoic	1.17	1.48	0.49	5.01	1.18	1.00	1.22	73.68
Chlorogenic acid	17.89	5.84	5.98	6.25	12.06	6.76	12.12	4.72
Phenol	34.00	31.03	61.17	34.01	44.67	18.46	65.44	33.42
Vanillin	30.64	7.76	8.53	--	10.52	8.75	29.11	12.73
p-coumaric acid	77.46	16.39	13.04	11.88	7.29	34.58	26.00	7.56
Ferulic acid	11.86	53.45	26.47	10.58	8.26	28.71	98.00	6.89
Salicylic acid	89.41	111.89	449.54	181.43	83.55	220.15	139.55	362.20
o-coumaric acid	34.33	23.12	14.94	38.55	32.02	--	4.92	12.05
Coumarin	27.73	5.93	4.63	3.53	75.69	14.47	14.15	1.88
Cinnamic acid	8.30	3.09	2.77	2.00	0.56	5.02	--	0.45

1 = Clary, 2 = Dragonhead, 3 = Fennel, 4 = Marigold, 5 = Milk thistle, 6 = Plantain, 7 = Sage, 8 = Sideritis

Table 3: Antioxidant activity of ethanol extracted for eight medicinal herbs at their vegetative stage (mg/100 g D.W.)

Plants	Inhibition of peroxidation (%)		
	100 µl extract	150 µl extract	200 µl extract
<i>Calendula officinalis</i> (marigold)	44.86±2.01	49.74±2.21	63.11±2.37
<i>Dracocephalum moldavica</i> (dragonhead)	69.74±3.11	80.14±3.47	88.74±4.11
<i>Foeniculum vulgare</i> (fennel)	61.72±3.01	68.71±3.51	76.14±3.48
<i>Plantago afra</i> (plantain)	68.14±3.01	79.63±3.84	87.61±4.11
<i>Salvia verbenaca</i> (clary)	64.31±2.86	71.81±3.41	77.11±3.71
<i>Salvia viridis</i> (sage)	71.78±3.69	83.74±3.99	91.34±4.98
<i>Sideritis montana</i> (siderites)	53.64±2.43	64.27±2.81	73.21±3.11
<i>Silybum marianum</i> (milk thistle)	46.18±2.11	53.71±2.35	68.69±2.84
LSD at 0.05	2.94	3.90	3.99

-Each value is expressed as mean±SE (n = 3)

resorcinol (97.52mg) in siderites. While the lowest compounds content were protochatechonic at the rates of 0.13, 0.41, 0.52, 0.63 and 1.16 mg/100 g dry weight in clary, fennel, sage, dragonhead and marigold herb, respectively. The lowest phenolic compound content in plantain was catechol (0.40 mg), but in siderites and milk thistle it was the cinnamic acid at the rates of 0.45 and 0.56 mg/100 g dry weight.

The results agrees with Nakatani [2] that phenolic compounds ranged between plants according to their genus species, varieties and cultivars.

Antioxidant activity: Antioxidant activities at three different concentrations of ethanol (100, 150 and 200 µl extract) was evaluated against linoleic acid peroxidation in the reaction mixture of the eight medicinal plant herbs are presented in Table 3.

The results revealed that the inhibition of peroxidation was progressively increased by raising ethanol concentration. The material extracts had overall good antioxidant activity. Most of the examined herbs showed moderate antioxidant capacity. Sage, dragonhead and plantain herb showed considerably strong antioxidant response (over 87% inhibition) on 200 µ ethanol extract.

The inhibition of Me Lo hydroperoxides of plant extracts decreased in the following order: sage < dragonhead < plantain < clary < fennel < sideritis < milk thistle < marigold, with significant differences in most cases. Sage herb extract was characterized with the highest antioxidant activity (inhibition %) which was about 91.34±4.98% followed by the dragonhead extract (88.74±4.11%) at 200 µl extract whereas at 100 µl ethanol extract inhibition % was 71.78±3.69 and 69.74±3.11 for the two aforementioned plants, successively; without significant differences between them. The lowest

inhibition % was in the marigold extract with a percentage of 44.86, 49.74 and 63.11% at 100, 150 and 200 µl ethanol extract, respectively.

Antioxidants are vital substances which possess the ability to protect body from damage by free radical-induced oxidative stress [8]. Velioglu *et al.* [41] and Kim *et al.* [42] stated that natural antioxidants are usually phenolic and polyphenolic compounds. Such results coincide with those of Bol'shakova *et al.* [9] mentioned that plantain plant had more antioxidant property than *Salvia officinalis*. Trojakova *et al.* [11] reported that sage leaves extracts showed reasonable antioxidant activity. Kahkonen *et al.* [10] concluded that 90 different plants extracts varied widely in their antioxidant activities.

CONCLUSION

It can be noted that the studied plants grown under organic farming conditions varied greatly in their growth characters. Marigold was the highest plants, but sage plant produced the heaviest herb weight.

The phenolic compound showed marked variation in herb of the studied plants. Salicylic acid was the major phenolic in all plants herb, except the sage which had the largest content of pyrogalllic acid. Any other phenolic compounds mounted to half the content of salicylic. Antioxidant activity expressed as percentage of inhibition of peroxidation in herb extracts attained the following order: sage (*Salvia viridis*) < dragonhead (*Dracocephalum moldavica*) < plantain (*Plantago afra*) < clary (*Salvia verbenaca*) < fennel (*Foeniculum vulgare*) < sideritis (*Sideritis montana*) < milk thistle (*Silybum marianum*) < marigold (*Calendula officinalis*). More work on the benefits of organic farming compared to mineral

manuring and their sharing in phenolics and antioxidant activity is required. Furthermore, more studies on other medicinal plants would be a must.

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