Journal of Medicinally Active Plants

Volume 4
Issue 1 *Vol 4 Issues 1-2*

June 2015

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Manuscript received: November 5, 2014.

Keywords: *Apium graveolens*, Chinese celery, phenolics, rosmarinic acid, essential oil,

ABSTRACT

The essential oil, phenolic acids, and other constituents in three accessions of celery (*Apium graveolens* L) were investigated to determine phytochemical variability that lead to differences in yield and phytochemical profiles. The celery accessions were all grown in pots under the same environmental conditions. Analyses were done on two planting of three harvests each to determine and compare the levels of anthocyanin, carotenoids, chlorophyll, phenolic acids, rosmarinic acid, soluble sugars, tannins, and essential oil content. Except for carotenoids, significant variations in the phytochemical profiles among the three celery accessions were observed.

INTRODUCTION

Celery (*Apium graveolens* L. Apiaceae family), is a biennial plant grown in Egypt and in East Asian countries. The plant, which is native to the wetlands of Egypt, Sweden, and countries of southern Europe, is used as a food, medicine, and natural seasoning for flavoring and aroma. Noted for thinner petioles and a more dispersed physical form than American celery, the plants are valued for their flavor, nutritional elements, and medicinal applications.

The celery plant is recognized for the content of vitamins A, B_1, B_9, C, E, and K, in addition to the minerals Ca, Mn, Mg, P, Fe, and Zn (Shad et al., 2011; Domagaa-Swiatkiewicz and Gasto, 2012). The leaf blades with petioles are commonly used in salads, while the seeds are used to reduce the lipid level in the blood, helping to avoid cardiovascular diseases (Shehata and Soltan, 2012), muscle spasms (Saini et al., 2014), inflammation (Abdel-Moein et al., 2011), and other health problems.

An earlier study with these celery accessions examined the morphological and molecular properties of the plant material. The current study reports on phytochemical variability of the three celery accessions to determine the possibility of plant improvement for use of celery as a flavoring, nutritional, and medicinal source.

MATERIALS AND METHODS

Plant materials. A total of three celery (*Apium graveolens* L.) accessions (Egypt wild type, Balady, and Green Leaves) were evaluated for phyto-chemicals in this study (Helaly, et al., 2014). Wild type seeds were sourced from plants growing near Albosaily, Behira Governorate, Egypt. The Balady and Green Leaves seed were purchased, respectively, from Harraz Market in Cairo, Egypt and Reimer Seed Company, in Mt. Holly, North Carolina, U.S.A.

Seeds of the three celery accessions were sown in two plantings (Planting 1 in October and Planting 2 in January) using a commercial growing media (LC1 Mix, Sun Gro Horticulture, Vancouver, Canada), contained in separate 50 cm x 50 cm square plastic flats. The seeded flats were subsequently dampened with water and placed in a controlled environmental chamber (Model EF7, Conviron, Winnipeg, Canada) at the University of Massachusetts, Amherst, MA, for germination at 20^{o}C with a 14 h day/10 h night cycle using a mixture of fluorescent and incandescent bulbs (PAR = 135 μmol $m^{-2}s^{-1}$ and R-FR ratio = 1.92) (Helaly, 2014).

At four weeks after seeding, three replicates of 10 plants from each celery accession were randomly selected and transplanted into 3.8 L plastic pots filled with the commercial growing media BX (Pro-Mix Inc., Quakertown, PA). After transplanting, the containers with seedling were placed in a glasshouse (minimum temperature 22^{o}C, natural sunlight) at the University of Massachusetts for growth and development.

Beginning at 44 days after transplanting, the leaves (leaf blades and petioles) of the plants were harvested at 30 (planting 1) and 31 (planting 2) day intervals by cutting the petioles at approximately 2 cm above the media surface. The freshly harvested leaves from 10 separate plants per replicate within each accession were immediately weighed using an electronic balance to determine fresh weights. After weighing, each replicate sample was cut into sections that were 3 to 5 cm long, sealed in plastic bags, and frozen at -80^{o}C until used in all subsequent analyses.

Chlorophyll and carotenoids. Chlorophyll and carotenoid levels of leaf tissue were determined using the spectrophotometric methods described by Al-Amier and Craker (2007); Hipkins and Baker (1986); and Yu (2011). From each of the replicates in each accession, 50 mg samples of the leaves were randomly chosen from among the sections of leaf tissue and placed in separate 5 mL vials containing 3 mL of 100% methanol. The vials were covered with aluminum foil to prevent light initiated loss of the chlorophyll and stored at 23^{o}C. After 2 h, each sample was thoroughly mixed. The methanol extract was decanted into clean vials and the extract was stored at 4 ^{o}C until the absorbance was measured in a spectrophotometer (Hitachi model U-2000) at 650 nm and 665 nm to determine the chlorophyll content and at 470 nm to determine carotenoid content.

Chlorophyll concentration in the methanol extract was determined using the formula: chlorophyll concentration = 25.8 x A_{650} + 4.0 x A_{665}, where A_{650} and A_{665} are the absorbance at 650 and 665 nm, respectively. The concentration of carotenoids was determined using the formula: carotenoid concentration = (1000 x A_{470} – 1.91Chl a – 95.15 Chl b) /272). Chlorophyll and carotenoid concentrations were subsequently converted to mg/g of tissue.

Anthocyanin. The anthocyanin content of the celery leaves was determined according to procedures outlined by Manchinelli (1990). Randomly chosen, 10 replicate leaf samples (50 mg each) of the frozen tissue collected from each celery accession were placed in 5 mL vials containing 3 mL of acidified 100% methanol (1% HCl, w/v). The vials were covered with aluminum foil and placed on a constantly moving mechanical shaker for two days at 3-5^{o}C for anthocyanin extraction. The methanol extracts were subsequently clarified by filtration through Whatman No. 1 filter paper. Each extract absorbance was measured with a spectro-photometer (Hitachi model U-2000) at 530 nm for anthocyanin and at 657 nm to measure contaminating chlorophyll in the acidic extraction solution (Manchinelli, 1990).

Anthocyanin concentrations in the extracts were determined using the formula: Anthocyanin concentration = $A_{530} - 0.25A_{657,}$ where A_{530} and A_{657} are the absorbance at 530 and 657 nm, respectively, with 25% of the A_{657} reading subtracted to account for chlorophyll breakdown products. The concentration of anthocyanin was subsequently converted to mg anthocyanin/g of tissue.

Soluble sugars. Total soluble sugars (g/100 g DW) in each accession were determined colorimetrically according to the method outlined by Duois et al. (1956).

Phenolics. Phenolic levels in the leaf tissue were determined using a modification of the method of Chandler and Dodds (1983). A 50 mg (fresh weight) sample from each of 10 replicate samples of each accession were submerged in 2.5 mL of 95% ethanol at 0^{o} C for 48 h. Afterwards, the samples were homogenized and centrifuged at 13,000 x g for 10 min to separate the tissue from the extract. A 1 mL aliquot of the supernatant was transferred to a 16 mm x 100 mm test tube and thoroughly mixed with 1 mL of 95% ethanol and 5 mL of distilled water. Subsequently, 0.5 mL of 50% Folin-Ciocalteu reagent was added to each test tube, allowed to react for 5 min before 1 mL of 5% Na_2CO_3 was added with thorough mixing to stop the chemical reaction. After 60 min, the absorbance of the solution was measured spectrophotometrically at 725 nm using an Agilent model 8453 spectrophotometer with 95% ethanol as

a blank and solutions of gallic acid as standards. The level of phenolic acids in each sample was determined in gallic acid equivalents by comparison to the standard solutions. Phenolic acid levels equal gallic acid equivalents/g fresh tissue weight.

Tannins. Tannin content of the samples was determined as described by Price et al. (1978), using a modified vanillin-HCl methanol method. The vanillin-HCl reagent was prepared just prior to use by mixing equal volumes of 8% concentrated HCl in methanol and 1% vanillin in methanol. A ground leaf tissue sample (200 mg dry wt.) was placed in a 25 mL Erlenmeyer flask and 10 mL of 1% concentrated HCl in methanol was added to the flask. The flask was capped and the contents thoroughly mixed by placing on a mechanical shaker for 20 min after which the contents were transferred to centrifuge tubes and centrifuged at 510 x g for 5 min. A one mL sample of the supernatant was pipetted into a test tube containing 5 mL of vanillin-HCl reagent, thoroughly mixed, and incubated at 30°C for 20 min. The absorbance of the mixture was measured at 450 nm with an Agilent 8453 spectrophotometer and compared with prepared standards (mg/mL) of catechin, which provided a color intensity equivalent to tannin. Tannin level of the accessions was determined as: Percent tannin = [Catechin equivalents (mg/L) x 10 mL/200 mg] x 100.

Rosmarinic acid. The level of rosmarinic acid in each of the accessions was determined using the modified UV assay of López-Arnaldos et al. (1995). A total of 50 mg of leaf tissue were collected from nodes six through ten below the tip of the main stem from each accession. The samples were submerged in 3 mL of a 50:50 mixture of methanol and distilled, deionized water and incubated at 55°C for 2 h to extract the rosmarinic acid. A one mL sample of the extract was diluted with 5 mL of methanol, thoroughly mixed, and the absorbance measured at 333 nm. The concentration of rosmarinic acid in each sample was determined using the formula: Absorbance (A_{333}) = ϵbc, where ϵ is the extension coefficient = 19,000 L/mole*cm, b is the width of the quartz cuvettes = 1 cm, and c is the concentration rosmarinic acid.

Essential oil. The essential oils were extracted from the leaves of three plants per replicate and from the seeds of each of the celery accessions, using steam distillation for 3 h in a modified Clevenger-type apparatus. The extracted oils were dried over Na_2SO_4 to remove traces of water, and the oil yield was measured and expressed as the total units of oil per unit fresh weight (µL/plant).

The essential oil constituents were separated by gas chromatography using a Shimadzu GC-2014A equipped with a FID and a 30 m x 0.25 i.d. Supelcowax capillary column. The injector temperature was 220 °C and the detector temperature was 250 °C. The carrier gas was He at a flow rate of 39 mL/min using a 40:1 split. The temperature was programmed for 60°C isothermal for 1 min and then at an increasing rate of 5 °C/min to 240 °C, and held for 1 min. A total of 42 Individual oil constituents were identified by comparison of retention times and co-injections with authentic standards. For comparison purposes among the accessions, only major oil constituents (those representing a minimum of one percent of the essential oil) are reported as percentage of the total oil.

Statistical analyses. Data were analyzed using ANOVA following the procedures outlined in Snedecor and Cochran (1980). Means were separated using the least significant differences ($LSD_{0.05}$) when an initial F-test indicated differences among averaged values.

RESULTS

Chlorophyll levels in celery leaves varied among the three accessions (Table 1). The highest chlorophyll content was in the Balady accession in both growing seasons, whereas the lowest value of chlorophyll content was obtained from Egyptian wild type. Anthocyanin content was relatively high in the Egyptian wild type with a concentration more than in the other accessions. The Egyptian wild type had the lowest level of chlorophyll, but had the highest amount of anthocyanin. Balady accession, however, had the highest level of chlorophyll and lowest level of anthocyanin. The carotenoid content was highest in the Balady accession, but was not significantly different from the other two accessions.

The Wild type accession was significantly higher in phenolic acids than the other two accessions in the first growing season, but not in the second growing season. No significant differences in phenolic acid production were noted among the three accessions in the second season. Only minimum differences were observed among the three accessions for rosmarinic acid levels, except for a lower level in the wild type in the first harvest. Tannins contents of all three accessions were higher in the first harvest as compared with the second harvest.

Table 1. Constituent levels in leaves of celery accessions.

Constituents	Harvest year	Accessions			LSD @ 0.05
		Wild type	Baldy	Green leaves	
		------- mg/g FW ---------			
Chlorophyll (mg/g FWt)	1	1.20	2.15	1.86	0.16
	2	1.24	2.20	1.82	0.16
Carotenoids (mg/g FWt)	1	1.08	1.66	1.44	ns
	2	0.69	1.12	0.95	ns
Anthocyanins (mg/g FWt)	1	4.74	0.31	0.76	0.35
	2	5.60	0.36	0.76	0.71
Phenolic acids (mg/g FWt)	1	37.20	31.50	32.20	2.22
	2	30.90	31.20	32.80	1.95
Rosmarinic acid (mg/g FWt)	1	1.54	1.74	1.78	0.15
	2	1.70	1.75	1.77	0.05
Tannins (mg/g FWt)	1	2.75	2.07	2.79	0.36
	2	2.35	1.79	2.19	0.43
Soluble sugars					
Glucose	1	7.00	9.13	7.61	0.42
Fructose	1	7.31	9.53	7.94	0.31
Sucrose	1	5.61	7.19	6.61	0.36
Glucose	2	9.53	9.47	11.50	0.44
Fructose	2	9.13	9.08	11.02	0.36
Sucrose	2	7.58	8.92	8.92	0.39

Variation in soluble sugars (sucrose, glucose, and fructose) was observed in the celery accessions. The measured soluble sugar content was higher in the second harvest than in the first harvest. Fructose was the most abundant sugar detected in the accessions during the first harvest and glucose was the most abundant in the second year.

Essential oil yields differed among the three celery accessions with the Wild type accession producing more oil than the Balady and the Green leaves accessions, in all harvests of both the first and second planting and in the oil extracted from the seeds (Table 2). Of the three accessions, the Wild type also had the highest oil content in the second planting.

The essential oil analysis of celery leaves and seeds detected a total of 42 compounds of which 11 compounds [(Z)-3-hexenol, α-pinene, myrcene, limonene, β-caryophyllene, α-humulene, β-selinene, α-selinene, kessane, 3-butylphthalide, sedanenolide] were predominant, representing 91.1 to 92.6% of the total leaf oil and 91.0 to 91.3% of the total seed oil (Table 3).

Table 2. Essential oil in celery leaves and seeds.

Accession	First season (% oil)			Seed oil (%)
	1st harvest	2nd harvest	3rd harvest	
Wild type	2.28	2.10	2.44	4.40
Balady	1.61	1.39	1.67	3.75
Green leaves	0.61	1.44	1.50	3.10
LSD @ 0.05	1.20	0.57	0.55	1.20
	Second season (% oil)			
	1st harvest	2nd harvest	3rd harvest	
Wild type	2.22	2.11	2.28	-
Balady	1.67	1.50	1.61	-
Green leaves	0.56	1.28	1.44	-
LSD @ 0.05	1.50	0.59	0.75	-

Limonene was the main constituent in the celery essential oil, varying from 53.6% to 63.5% of the total oil with the higher percentages associated with the harvest 2 and harvest 3. The limonene level in the essential oil extracted from the seeds was highest in the Green leaves accession. Among the tested accessions, the level of sedanenolide varied from 2.80% in the Wild type to 0.32% in Balady and 0.21% in Green leaves.

DISCUSSION

The measured variability in the chlorophyll, carotenoid, anthocyanin, sugar, and essential oil content reflect the genetic heterogeneity among the tested celery accessions and the plant responses to the growth environment. An earlier study documented differences in the DNA profiles among the three accessions used in the current work (Helaly, et al, 2014). Such heterogeneity is common among various plant accessions (Pirbalouti, et al., 2013; Shojaiefar, et al., 2015).

Heterogeneity enables plant species to adapt to different environmental conditions, but in cultivated

crops can lead to phenotypes with reduced vigor and unpredictable yields (Al-Amier, et al., 2005; Whitham and Slobodchikoff, 1981). The diversity and heterogeneity broadens the genetic base and enables plant breeders to develop breeding programs towards the improvement of existing crop plants and production of environmentally tolerant genotypes with high yields.

Table 3. Essential composition in leaves of celery accessions and seeds.

Accessions	Wild type				Balady				Green leaves			
	Harvest			Seed oil	Harvest			Seed oil	Harvest			Seed oil
Oil constituents	1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd	
	(% of oil)											
z-3-Hexen1-ol	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.09	0.10	0.10	0.10	0.10
α–Pinene	1.20	1.20	1.20	1.01	1.30	1.40	1.40	1.20	1.20	1.40	1.30	1.12
Myrcene	1.30	1.26	1.27	1.20	1.40	1.40	1.37	1.20	1.20	1.23	1.23	1.20
Limonene	53.60	55.40	58.50	58.60	57.40	58.50	59.00	59.00	61.50	62.30	63.10	63.50
β-Caryophyllene	6.23	6.55	6.55	5.36	5.45	5.52	5.65	4.50	6.45	6.44	6.66	5.50
α-Humulene	1.90	1.80	1.70	1.80	1.50	1.60	1.50	1.40	1.33	1.24	1.44	1.50
β-selinene	10.30	10.50	10.50	11.50	9.24	9.25	9.27	10.12	10.12	10.25	10.50	10.60
α-Selinene	1.25	1.24	1.50	1.50	2.10	2.11	2.13	2.40	1.40	1.34	1.35	1.30
Kessane	3.40	3.30	3.30	3.50	2.50	2.60	2.60	2.70	2.50	2.50	2.60	2.50
3-Butylphthalide	2.30	3.40	3.50	3.50	2.22	2.30	2.50	3.10	1.50	1.40	1.50	1.40
Sedanenolide	2.50	2.70	2.80	2.80	2.80	0.32	0.33	0.35	0.21	0.25	0.27	2.40

Except for the carotenoid content, significant differences in the phytochemicals and sugars were observed among all the accessions. In the Balady and Green leaves accessions, an inverse relationship between total chlorophyll and anthocyanin content existed. The Wild type accession had a relatively high anthocyanin content. Higher levels of anthocyanin may protect these plants against abiotic stresses (Chalker-Scott, 1999; Lev-Yadun and Gould, 2009). Our results for the soluble sugar contents are in agreement with those of Ruprez and Toledano (2003).

Differences among the essential oil yields were most likely due to differences in the genetic composition of the genotypes since all genotypes were grown at the same location. The observed differences may be due to different genetic factors and different chemotypes that can influence the oil compositions. The relatively high content of kessane detected in the seeds of the Wild type accession (2.5-3.7%) is similar to the results by Philippe et al. (2002), and may suggest a relationship between Indian celery and the Egyptian Wild type.

The essential oil constituents in the studied celery accessions are in common agreement with other studies on essential oil in celery plants (Van Wassenhove et al., 1990; Mišić, et al., 2008; Sellami et al., 2012).

ACKNOWLEGEMENTS

This material is based on work supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, the Massachusetts Agricultural Experiment Station, and the Stockbridge School of Agriculture. Project No. MAS000729.

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