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RESEARCH ARTICLE

Caffeoyl phenols and alkamides of cultivated *Echinacea purpurea* and *Echinacea atrorubens* var. *paradoxa*

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Abstract

Echinacea purpurea (L.) Moench was recently introduced into Taiwan. In the present study, the biomass, the contents of caffeoyl phenols, and the content of dodeca-2*E*,4*E*,8*Z*,10*E*-tetraenoic acid isobutylamide plus dodeca-2*E*,4*E*,8*Z*,10*Z*-tetraenoic acid isobutylamide (alkamides **8** and **9** respectively,) of locally selected line CLS-P2 and two introduced cultivars Magnus and White Swan of *E. purpurea* and an introduced *E. atrorubens* var. *paradoxa* were compared. The results indicated that both biomass and phytoactive constituents varied considerably among the introduced cultivars and selected line. Line CLS-P2 grew better and produced more aerial and ground parts than introduced cultivars Magnus and White Swan. It also produced more caffeoyl phenols, particularly cichoric acid and caftaric acid in its leaves than Magnus and White Swan. All the *E. purpurea* cultivars and line produced same amounts of alkamides **8** and **9** in their flower heads and leaves. But White Swan produced more alkamides **8** and **9** in its roots than CLS-P2 and Magnus. Line CLS-P2 was less homogenous in genetic background as compared to the introduced cultivars. *E. atrorubens* var. *paradoxa* also grew well in Taiwan, but it produced less aerial and ground dry mass than *E. purpurea*. *E. atrorubens* var. *paradoxa* produced more echinacoside in its flower heads, leaves, and root parts, while *E. purpurea* plants had more cichoric acid and caftaric acid in their flower heads and leaves. *E. atrorubens* var. *paradoxa* also produced more alkamides **8** and **9** in flower heads and leaves, while *E. purpurea* produced more alkamides **8** and **9** in roots.

Keywords: *Alkamides; biomass; caffeic acid derivatives;* Echinacea atrorubens*;* Echinacea purpurea*; purple coneflower; yield*

Introduction

Echinacea is an herbaceous perennial of *Asteraceae* family that grows throughout the eastern and central United States and southern Canada. Three species of *Echinacea* are valued as medicinal herb: *E. purpurea* (L.) Moench*, E. pallida* (Nutt.) Nutt., and *E. angustifolia* DC (Mistríková & Vaverková, 2007). All three species show pharmacological activity, which appears to result from the combined effects of caffeoyl phenols, alkamides, and polysaccharides (Binns et al., 2002; Randolph et al., 2003; Raduner et al., 2006; Pillai et al., 2007). However, *E. purpurea* is the most cultivated and widely used of the three species, due to ease of cultivation and total use of the whole plant (Wills & Stuart, 1999; Seidler-Lozykowska & Dabrowska, 2003; Kreft, 2005). Extracts and compounds are commercially produced from the various parts of *E. purpurea* for the prevention of common cold, flu, respiratory infections and inflammations, and the stimulation of immunomodulation (Mahady et al., 2001; Goel et al., 2005; Vimalanthan et al., 2005; Hinz et al., 2007).

The seeds of *Echinacea purpurea* cv. Magnus and White Swan were purchased from Park Seed, Greenwood, SC. The seeds of *E. atrorubens* var. *paradoxa* were obtained from Seeds of Change, Santa Fe, NM. The seed population CLS-P2 of *Echinacea purpurea* selected from a consecutive mass selection program was obtained from National Chung Hsing University.

The phytochemical traits of medicinal plants, depending on growing sites, climate conditions, and

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genetic modifications, vary considerably among cultivated populations (Millauskas et al., 2004). *E. purpurea* was recently introduced and appeared to grow well in Taiwan (Chen et al., 2007). However, introduction of this species into large scale cultivation requires homogenous plant materials, and it is known that selection of morphologically superior *E. purpurea* lines would result in elevated phytochemical content in each of the major constituents when compared to non-selected lines (Binns et al., 2002). The present study compared the biomass productions of two introduced *E. purpurea* cultivars and a selected population through a breeding program. The contents of total phenolic, several caffeoyl derivatives, and alkamides were also determined and compared among the tested *E. purpurea* cultivars and selected populations. Various alkamides have been isolated and identified from the roots and flowers of *E. purpurea* (Binns et al., 2002), with dodeca-2*E*,4*E*,8*Z*,10*E*-tetraenoic acid isobutylamide (alkamide **8**) and dodeca-2*E*,4*E*,8*Z*,10*Z*-tetraenoic acid isobutylamide (alkamide **9**) being predominant (Kim et al., 2000). Therefore, only alkamide **8** and alkamide **9** were determined in the present study. Additionally, *E. atrorubens* var. *paradoxa*, which was reported to have caffeoyl derivatives and alkamides in its root (Bauer & Foster, 1991), was also included for phytochemical comparison.

Materials and methods

In June 2003, all the seeds were soaked in running water for 8 h, and then planted in 104-plugs filled with a mixture of peat moss and vermiculite (3:1) at depth of 1.5 cm, and watered as necessary. The indoor-raised seedlings with 4 to 5 leaves were transplanted to the experiment farm of the Department of Agronomy, National Chung Hsing University in July 2003. The seedlings were planted on raised 2-row bed plots (1 m wide and 6 m long with 30 cm bed spacing) covered with silver-black polyethylene sheets for weed control. The plant spacing was 30 × 30 cm. Pre-plant fertilizers were applied at the rates of 100 kg N ha^{-1}, 60 kg P_2O_5 ha^{-1} and 100 kg K_2O ha^{-1}.

For biomass determinations, plant samples composed of two rows 3 m long were taken at the full flower stage. The number of flower heads produced per plant were counted and recorded, and then the whole plants were ploughed up and were separated into leaves, stems, flower heads, and root. All the sampled plant materials were dried in a forced hot air dryer at 43°C to a moisture content of 10 after drying for 4 to 7 days, and weighed for biomass determinations.

The total phenol content was estimated by a colorimetric assay based on procedures described by Taga et al. (1984). Dried ground tissue (50 mg) was extracted by using 3 mL of 60% (v/v) methanol containing 0.3% (v/v) HCl for 60 min, and then centrifuging at 18,000 *g* for 15 min. A 10 μL aliquot of tissue extract was dissolved in 200 μL of 2% (v/v) Na_2CO_3 and 10 μL of Folin and Ciocalteu's phenol reagent (50%, v/v) was added. The mixture was left to stand at room temperature for 30 min. Absorbance measurement was taken at 725 nm using a spectrophotometer, and caffeic acid was used in the construction of the standard curve.

For caffeic acid derivative determinations, the tissue extract used for total phenol determination (20 μL) was filtered through a 0.2 μm syringe filter (Minisart RC 15, Sartorius) and then analyzed using a HPLC (Hitachi, Tokyo, Japan) consisting of pump (L-7100), column oven (655A-52) (35°C), UV-VIS detector (L-4200) (330 nm) and auto sampler (L-7200) (Hu & Kitts, 2000). The column used was Mightysil RP-18 GP 5 μm 150 × 4.6 mm (Kanto, Tokyo, Japan). Two different eluents were used: A) acetonitrile/water 10:90, B) acetonitrile/water 25:75. Various levels of caftaric acid, chlorogenic acid, cynarin, echinacoside and cichoric acid were used in the construction of standard curves.

For quantification of alkamide **8** and alkamide **9**, dried ground tissue (50 mg) was extracted with 2.5 mL acetonitrile for 5 min and centrifuged at 18,000 *g* for 15 min (Perry et al., 1997). The supernant (20 μL) was filtered through a 0.2 μm syringe filter (Minisart RC 15, Sartorius) and then analyzed using a HPLC (Hitachi, Japan) consisting of pump (L-7100), column oven (655A-52) (35°C), UV-VIS detector (L-7420) (254 nm) and auto sampler (L-2200). The column used was Mightysil RP-18 GP 5 μm 250 × 4.6 mm (Kanto, Tokyo, Japan). Two different eluents were used: A acetonitrile 100% and B: water. Various levels of alkamide **8** plus alkamide **9** (alkamide **8** and **9**) were used in the construction of standard curves.

The experimental design was a randomized complete block design with four replicates. All data were subjected to an analysis of variance and when a significant ($P < 0.05$) F ratio occurred for treatment effects, a least significant difference (LSD) was calculated.

Results and discussion

Both *Echinacea* species grew vigorously under natural conditions. However, the morphological traits in harvested *Echinacea* plants were highly variable, as indicated by the relatively greater standard deviations in comparison with means, of the examined samples (Table 1). Kreft (2005) indicated that only a small portion of the morphological variability could be explained by environmental and cultural conditions, with the inter-individual differences being the main source of variability. *Echinacea* plant is an obligate crosser and is

self-incompatible (Li, 1998; Van Gaal et al., 1998). These results might explain in part why *Echinacea* plants have greater morphological variability.

Cultivar differences had no statistically significant effect on the days to floral formation in both introduced and locally selected *E. purpurea* plants (Table 1). Nevertheless, selected line CLS-P2 grew taller and produced more stems, and subsequently produced more flower heads than introduced cultivars Magnus and White Swan (Table 1). As a result, CLS-P2 produced more biomass, both in aerial and ground portions, than Magnus or White Swan (Table 2). The greater aerial biomass production of CLS-P2 was mainly attributable to its greater leaf production (Table 2). CLS-P2, Magnus and White Swan produced dry leaf tissues of 44.50, 11.92 and 14.81 g plant^{-1}, respectively. CLS-P2 also out yielded in stems and flower heads than introduced cultivars, but the differences in biomass were not significant at 5% statistical level (Table 2). However, the calculated coefficients of variation (the ratio of standard deviation and mean × 100%), which are the indirect indicators of heterogeneity for the tested morphological and agronomic traits (data not shown), reveal that the locally selected CLS-P2 (59% on average across all 11 morphological and agronomic traits) is less homogenous than introduced Magnus (31%) and White Swan (42%). Thus, a continuous mass selection program is a must for further improving the homogeneity in morphological and agronomic traits of line CLS-P2.

E. atrorubens var. *paradoxa* required a longer time to reach flora formation than *E. purpurea* line CLS-P2 (Table 1). It produced less aerial parts, resulting from a combination of lower flower head, stem, and leaf yields, than CLS-P2 (Table 2). Additionally, it produced fewer rhizomes than CLS-P2. Nevertheless, *E. atrorubens* var. *paradoxa* produced more root dry mass than CLS-P2 (Table 2).

Phenolic substances extracted from leaves and flower heads of *E. purpurea* plants have been used for the treatment of various types of illness (Thygesen et al., 2007). As with the morphological and agronomic traits, the contents of total phenolics in flower head, leaf and root tissues also oscillated greatly (Tables 3, 4, and 5). The flower heads had more total phenolics than leaves (Tables 3 and 4). Significant phenolic content variations in flower heads also existed among the tested *E. purpurea* cultivars and line, with White Swan containing more total phenolics in flower heads than CLS-P2 and Magnus (Table 3). On the other hand, highest leaf content of total phenolics was found in CLS-P2, followed by Magnus and White Swan (Table 4). The root content of total phenolics was relatively low in comparison with that of flower heads and leaves (Table 5). The locally selected CLS-P2 had greater root content of total phenolics than newly introduced Magnus and White Swan.

The content of total caffeoyl derivatives such as cichoric acid, caffeic acid and chlorogenic acid represents the greatest portion of phenolic substances in

Table 1. Morphological traits of *Echinacea* species, cultivars and line.

Species	Days to floral formation	Plant height (cm)	Stems (No. plant^{-1})	Flower heads (No. plant^{-1})
E. purpurea line CLS-P2	75.1 ± 17.8^{a}	46.21 ± 13.41^{a}	4.79 ± 2.01^{a}	19.40 ± 13.71^{a}
E. purpurea cv. Magnus	81.8 ± 14.3^{a}	38.50 ± 7.71^{ab}	2.00 ± 0.82^{b}	11.93 ± 4.89^{ab}
E. purpurea cv. White Swan	89.8 ± 17.6^{a}	36.13 ± 5.23^{b}	2.38 ± 0.86^{b}	15.12 ± 6.08^{a}
E. atrorubens var. *paradoxa*	97.9 ± 19.7^{a}	30.25 ± 12.80^{b}	2.60 ± 1.71^{b}	2.70 ± 2.36^{b}
	Pr > F	Pr > F	Pr > F	Pr > F
	0.1332	0.0001	< 0.0001	0.0001

Data are present as mean and standard deviation.
Means within columns followed by the same letter are not significantly different at $P_{0.05}$ probability level.

Table 2. Agronomic traits (g dry weight plant^{-1}) of *Echinacea* species, cultivars and line.

Species/cultivars	Aerial parts	Ground parts	Flower heads	Stems	Leaves	Rhizomes	Roots
E. purpurea line CLS-P2	92.93 ± 48.83^{a}	31.48 ± 22.52^{a}	31.60 ± 22.41^{a}	16.83 ± 14.33^{a}	44.50 ± 19.41^{a}	14.71 ± 12.53^{a}	16.77 ± 11.41^{b}
E. purpurea cv. Magnus	50.80 ± 14.82^{b}	15.22 ± 4.61^{b}	26.08 ± 6.85^{ab}	12.80 ± 5.00^{ab}	11.92 ± 4.79^{b}	6.33 ± 2.74^{b}	8.89 ± 3.30^{b}
E. purpurea cv. White Swan	59.06 ± 22.80^{b}	15.93 ± 8.03^{b}	30.71 ± 15.57^{a}	13.54 ± 5.25^{ab}	14.81 ± 7.05^{b}	5.21 ± 3.71^{b}	10.72 ± 5.34^{b}
E. atrorubens Var. *paradoxa*	37.51 ± 19.54^{b}	27.21 ± 6.88^{ab}	12.40 ± 6.77^{b}	4.67 ± 3.55^{b}	20.44 ± 13.01^{b}	3.82 ± 2.09^{b}	23.39 ± 5.86^{a}
	Pr > F	Pr > F	Pr > F	Pr > F	Pr > F	Pr > F	Pr > F
	< 0.0001	0.0004	0.0466	0.0228	< 0.0001	< 0.0001	0.0106

Data are present as mean and standard deviation.
Means within columns followed by the same letter are not significantly different at $P_{0.05}$ probability level.

Table 3. The contents (on dry weight base) of total phenolics, caffeoyl derivatives and alkamides 8 and 9 in the flower heads of *Echinacea* species, cultivars and line.

Species	Total phenolics mg g^{-1}	Total caffeoyl derivatives mg g^{-1}	Cichoric acid mg g^{-1}	Caftaric acid mg g^{-1}	Chlorogenic acid mg g^{-1}	Cynarin Mg g^{-1}	Echinacoside mg g^{-1}	Alkamides **8** and **9** μg g^{-1}
E. purpurea line CLS-P2	191.3± 33.0^{b}	125.3 ± 25.8^{b}	94.50 ± 21.71^{b}	23.82 ± 5.05^{a}	5.96 ± 3.76^{a}	0.30 ± 0.21^{a}	1.12 ± 0.55^{b}	197.2 ± 45.8^{b}
E. purpurea cv. Magnus	188.3 ± 31.4^{b}	116.7 ± 25.1^{b}	90.01 ± 21.42^{b}	21.53 ± 4.47^{ab}	3.87 ± 1.14^{a}	0.32 ± 0.05^{a}	1.41 ± 0.22^{b}	240.5 ± 30.6^{b}
E. purpurea cv. White Swan	232.6 ± 22.4^{a}	145.5 ± 27.0^{a}	115.13 ± 24.91^{a}	22.40 ± 4.12^{b}	6.50 ± 2.74^{a}	0.17 ± 0.04^{a}	1.67 ± 0.39^{b}	182.2 ± 89.2^{b}
E. atrorubens var. *paradoxa*	165.4 ± 23.5^{b}	147.0 ± 34.0^{a}	4.13 ± 0.97^{c}	7.54 ± 1.59^{c}	8.76 ± 10.61^{a}	0.26 ± 0.08^{a}	126.6 ± 25.10^{a}	991.9 ± 412.7^{a}
	Pr > F	Pr > F	Pr > F	Pr > F	Pr > F	Pr > F	Pr > F	Pr > F
	< 0.0014	< 0.0299	< 0.0001	< 0.0001	0.2893	0.2862	< 0.0001	< 0.0001

Data are present as mean and standard deviation.
Means within columns followed by the same letter are not significantly different at $P_{0.05}$ probability level.

Table 4. The contents (on dry weight base) of total phenolics, caffeoyl derivatives and alkamides 8 and 9 in the leaves of *Echinacea* species, cultivars and line.

Species	Total phenolics mg g^{-1}	Total caffeoyl derivatives mg g^{-1}	Cichoric acid mg g^{-1}	Caftaric acid mg g^{-1}	Chlorogenic acid mg g^{-1}	Cynarin mg g^{-1}	Echinacoside mg g^{-1}	Alkamides **8** and **9** μg g^{-1}
E. purpurea line CLS-P2	83.91 ± 19.01^{a}	61.73 ± 21.52^{a}	40.34 ± 17.73^{a}	20.62 ± 6.27^{a}	0.15 ± 0.19^{b}	0.51 ± 0.46^{a}	0.79 ± 0.61^{b}	97.74 ± 9.29^{b}
E. purpurea cv. Magnus	70.42 ± 23.11^{b}	34.23 ± 7.78^{b}	24.23 ± 6.83^{b}	9.24 ± 3.00^{b}	0.23 ± 0.25^{a}	0.32 ± 0.09^{ab}	0.59 ± 0.15^{b}	102.21 ± 12.50^{b}
E. purpurea cv. White Swan	69.83 ± 26.50^{b}	31.75 ± 11.83^{b}	20.03 ± 8.28^{b}	11.19 ± 3.99^{b}	0.14 ± 0.10^{b}	0.27 ± 0.17^{b}	0.42 ± 0.26^{b}	94.13 ± 3.99^{b}
E. atrorubens var. *paradoxa*	39.13 ± 14.80^{c}	20.02 ± 7.78^{b}	4.13 ± 0.97^{c}	Trace	0.43 ± 0.35^{a}	0.16 ± 0.08^{ab}	15.43 ± 7.30^{a}	156.12 ± 26.71^{a}
	Pr > F	Pr > F	Pr > F	Pr > F	Pr > F	Pr > F	Pr > F	Pr > F
	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0289	< 0.0001	< 0.0001

Data are present as mean and standard deviation.
Means within columns followed by the same letter are not significantly different at $P_{0.05}$ probability level.

Table 5. The contents (on dry weight base) of total phenolics, caffeoyl derivatives and alkamides 8 and 9 in the roots of *Echinacea* species, cultivars and line.

Species	Total phenolics mg g^{-1}	Total caffeoyl derivatives mg g^{-1}	Cichoric acid mg g^{-1}	Caftaric acid mg g^{-1}	Chlorogenic acid mg g^{-1}	Cynarin mg g^{-1}	Echinacoside mg g^{-1}	Alkamides **8** and **9** μg g^{-1}
E. purpurea line CLS-P2	56.01 ± 11.90^{a}	16.53 ± 5.83^{c}	9.73 ± 3.76^{b}	6.33 ± 2.22^{b}	0.10 ± 0.05^{b}	3.59 ± 1.10^{a}	0.31 ± 0.12^{b}	517.5 ± 235.7^{b}
E. purpurea cv. Magnus	38.92 ± 9.35^{b}	19.71 ± 5.39^{c}	11.72 ± 3.85^{b}	7.27 ± 1.91^{b}	0.08 ± 0.01^{b}	2.16 ± 0.84^{b}	0.72 ± 0.28^{b}	411.8 ± 30.6^{b}
E. purpurea cv. White Swan	43.01 ± 12.32^{b}	27.40 ± 9.26^{b}	13.59 ± 5.49^{a}	13.32 ± 4.04^{a}	Trace	2.10 ± 2.17^{b}	0.47 ± 0.19^{b}	1285.7 ± 967.2^{a}
E. atrorubens var. *paradoxa*	39.01 ± 12.63^{b}	62.93 ± 23.34^{a}	0.17 ± 0.13^{c}	0.07 ± 0.01^{c}	0.88 ± 1.05^{a}	0.20 ± 0.06^{c}	61.80 ± 23.72^{a}	147.6 ± 43.3^{b}
	Pr > F	Pr > F	Pr > F	Pr > F	Pr > F	Pr > F	Pr > F	Pr > F
	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0002	< 0.0001	< 0.0001	< 0.0001

Data are present as mean and standard deviation.
Means within columns followed by the same letter are not significantly different at $P_{0.05}$ probability level.

E. purpurea plants (Thygesen et al., 2007). In the present study, greater variations were also found in the content of total caffeoyl derivatives in the flower heads, leaves, and roots (Tables 3, 4 and 5). Statistically significant differences in the flower head contents of caffeic acid derivatives existed among the tested *E. purpurea* cultivars (Table 3). Total caffeoyl derivatives were 125.3, 116.7, and 145.5 mg g^{-1} dry weight for CLS-P2, Magnus and White Swan, respectively. In all three cultivars and line, harvested dry flower heads contained highest

cichoric acid content and followed by caftaric acid and chlorogenic acid (Table 3). The contents of cynarin and echinacoside in flower heads were relatively low as compared to cichoric acid, caftaric acid or chlorogenic acid (Table 3).

The contents of caffeoyl derivatives in leaves also differed by *E. purpurea* cultivar (Table 4). The caffeoyl derivatives were 61.73, 31.75 and 20. 02 mg g^{-1} dry weight for line CLS-P2, cultivar Magnus and cultivar White Swan, respectively. Among the five caffeoyl derivatives examined in the present study, CLS-P2 leaves had greater cichoric acid, caftaric acid, cynarin and echinacoside levels than Magnus and White Swan (Table 4). In all three cultivars and line, harvested dry leaves contained highest cichoric acid content and followed by caftaric acid and echinacoside (Table 4). The contents of chlorogenic acid and cynarin in leaves were relatively low as compared to cichoric acid, caftaric acid and chlorogenic acid (Table 4).

The contents of caffeoyl derivatives in dry roots also varied considerably among three *E. purpurea* cultivars and selected line (Table 5). The caffeoyl derivatives were 16.53, 19.71, and 27. 40 mg g^{-1} dry weight for CLS-P2, Magnus and White Swan, respectively. In all three cultivars and line, harvested dry roots contained highest cichoric acid content and then followed by caftaric acid, cynarin, echinacoside, and chlorogenic acid (Table 4). Only a trace amount of chlorogenic acid was detectable in the roots of White Swan (Table 5).

Cichoric acid is one of the most important markers affecting the market quality of *E. purpurea* (Thygesen et al., 2007). Qu et al. (2005) reported that the level of cichoric acid varied considerably between flower heads and roots of *E. purpurea.* The *E. purpurea* grown in Slovenia had cichoric acid of 10.76 and 15. 82 mg g^{-1} dry weight in flower head and leaf tissues of the plant (Kreft, 2005). The contents of cichoric acid in German grown *E. purpurea* were 7. 6 mg g^{-1} dry weight in roots with the flowers and leaves containing 13 mg g^{-1} dry weight (Wills & Stuart, 1999). The Australian grown *E. purpurea* flowers, roots and leaves contained ranges of 29.5-38.3, 10.4-23.8, and 4.1-15. 3 mg g^{-1} dry weight respectively (Wills & Stuart, 1999). The levels of cichoric acid presented in our study were much higher than their findings.

Many alkamides have been isolated and identified from the roots and flowers of *E. purpurea* (Kim et al., 2000; Stuart & Wills, 2000; Binns et al., 2002; Clifford et al., 2002), with dodeca-2*E*,4*E*,8*Z*,10*E*-tetraenoic acid isobutylamide (alkamide **8**) and dodeca-2*E*,4*E*,8*Z*,10*Z*-tetraenoic acid isobutylamide (alkamide **9**) being predominant (Kim et al., 2000), particularly in the young tops (Qu et al., 2005). In the present study, alkamides **8** and **9** were detectable in flower heads, leaves and root portions of *E. purpurea* plants (Tables 3, 4, and 5). However, the alkamides **8** and **9** content differed significantly in each of the tissues assayed, with roots containing highest levels of alkamides **8** and **9**. These results are in agreement with other reports (Wills & Stuart, 1999; Qu et al., 2005; Thygesen et al., 2007). The alkamides **8** and **9** contents were also affected by cultivar. Line CLS-P2 had the highest alkamides **8** and **9** content in dry leaves among the tested cultivars and line (Table 4), while White Swan accumulated considerably higher alkamides **8** and **9** content in dry roots than Magnum and line CLS-P2 (Table 5). However, no statistically significant differences in flower heads alkamides **8** and **9** content were obtainable among the tested *E. purpurea* cultivars and line (Table 3).

E. atrorubens variety *paradoxa* was reported to have caffeoyl derivatives and alkamides in its root (Bauer & Foster, 1991; Binns et al., 2002). Our data re-confirm their findings. Both caffeoyl derivatives and alkamides **8** and **9** were present in the flower heads, leaves and roots of *E. atrorubens* variety *paradoxa* (Tables 3, 4, and 5). In fact, *E. atrorubens* variety *paradoxa* contained more total caffeoyl derivatives in dry flower heads and roots than *E. purpurea* (Tables 3 and 5). However, the profiles of total caffeoyl derivatives differed between *E. atrorubens* variety *paradoxa* and *E. purpurea*. Instead of cichoric acid and caftaric acid, it was echinacoside that accumulated in the highest levels in flower heads, leaves, and roots of *E. atrorubens* variety *paradoxa* (Tables 3, 4, and 5). Moreover, *E. atrorubens* variety *paradoxa* accumulated more alkamides **8** and **9** in the flower heads and leaves of the plants (Tables 3 and 4), whereas it had relatively lower alkamides **8** and **9** content in the roots (Table 5).

In conclusion, the present results indicate that the biomass production of cultivated *E. purpurea* plants varies considerably among the introduced cultivars Magnus and White Swan and locally selected line CLS-P2. The selected line CLS-P2 appears to grow better and produce more aerial and ground parts than introduced cultivars Magnus and White Swan. But CLS-P2 is less homogenous in genetic background than Magnus and White Swan. The contents of total caffeoyl phenols and alkamides **8** and **9** within the various parts of plant tissues of line CLS-P2 also oscillated greatly, but with less magnitude of variation in comparison with that of morphological and agronomic traits. Line CLS-P2 produced more caffeoyl phenols, particularly cichoric acid and caftaric acid in their leaves than cultivars Magnus and White Swan. The production of caffeory phenols and alkamides **8** and **9** also differed by species. *E. purpurea* plants generally produce more caffeoyl phenols, particularly cichoric acid and caftaric acid, in its flower heads and leaves. On the other hand, *E. atrorubens* variety *paradoxa* accumulated more echinacoside in its flower heads, leaves, and roots parts. *E. atrorubens* variety *paradoxa* also accumulated more alkamides **8** and **9** in flower heads and leaves, while *E. purpurea* accumulated more alkamides **8** and **9**

in roots. It is known that the introduction of *E. purpurea* into commercial scale cultivation requires more homogenous plant population. Thus, a mass selection program should be continued to further improve the homogeneity in morphological, agronomic, and phytochemical traits for *E. purpurea* line CLS-P2.

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