J. Crop Prot. 2015, 4 (4): 487-496

Research Article

Herbicidal activity of constituents isolated from *Solanum elaeagnifolium* (Solanaceae)

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Abstracts: The bioactivity of chemical extracts from silverleaf nightshade *Solanum elaeagnifolium* Cav. seeds and leaves were tested for herbicidal activities, through water and ethanol extracts, against *Portulaca oleracea* L., *Corchorus olitorius* L., *Convolvulus arvensis* L. and *Echinochloa crus-galli* (L.) found in most *Zea mays* L. fields. Characterization of the isolated constituents from ethanol extract was conducted by various spectroscopic techniques. Purification of chloroform (100%) column fraction carried out by TLC plate using developing system; chloroform: ethanol: acetic acid (92:4:4) and hexane: chloroform: ethyl acetate (16:16:1) resulted chlorogenic acid, kaempferol and mangiferin. The second active column fraction eluted by chloroform: ethyl acetate was purified on TLC by chloroform: methanol: water (13:7:1) and butanol:water:formic acid (4:5:1) resulted in (coumaroyl glucoside, coumaroyl quince acid) and (kaempferol β-D-(6"-O-cis-cinnamoyl glucoside), dicaffeoyl quinic acids) respectively. The most active isolated component from *S. elaeagnifolium* seeds was chlorogenic acid which decreased *P.oleracea* total biomass fresh weight by (86.5%) followed by kaempferol β-D-(6"-O-cis-cinnamoyl glucoside) (84.4%), while a moderate effect was achieved from coumaroyl glucoside (79.37%), mangiferin (76.98), kaempferol (72.48%) and coumaroyl quince acid (66.47%); finally the lowest activity (63.6%) was achieved by dicaffeoyl quinic acids compared with the controls. Thus, the herbicidal activity of these constituents suggests their potential for development as natural herbicides.

Keywords: *Solanum elaeagnifolium,* allelochemicals, Polyphones, flavonoids, phytotoxic activity

Introduction

Some plant constituents cause several toxic effects against some weed species (Putnam 1988), giving novel sites and mechanisms of action for their newly obtained herbicides (Duke *et al.,* 2000). Silverleaf nightshade *Solanum elaeagnifolium* is a perennial shrub, widely distributed in Asia, Africa, Australia, and tropical and subtropical America (Chiale *et al*., 1991; Boyd *et al.,* 1984). It is listed as a noxious weed in its native region (Americas) and as an invasive alien plant in many other countries (Mekki, 2007). It was the dominant weed species in olive orchards at North Sinai, El Arish, Egypt (Balah, 2011). Highest mortalities of the red flour beetle *Tribolium castaneum* (Herbst) were recorded in larvae treated with methanol extract of *S. elaeagnifolium* seeds (Hamouda *et al.,* 2015). The morphological plasticity of *S. elaeagnifolium* could probably contribute to its adaptability and partly explain its establishment and continuing expansion in Austral (Zhu *et al.,* 2013).

Buck *et al*. (1960) isolated both the tropane

Handling Editor: Saeid Moharramipour

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Received: 06 October 2014, Accepted: 02 July 2015
Published online: 13 July 2015

alkaloid Solanine and steroidal alkaloid solanidine from *S. elaeagnifolium*. Glycoalkaloids as bioactive compounds were isolated from its seeds and leaves (Bekkouche *et al*., 2000). Chiale *et al*. (1991) identified kaempferol and kaempferol 3-glucoside as monoacylated flavonoid glucosides from its aerial parts. Cholesterol, campesterol, sitosterol, stigmasterol, As-avenasterol, A'-avenasterol, A'-stigmasterol and β-spinasterol were also identified from the seeds of 13 species of *Solanum*, including silverleaf nightshade (Keeler *et al*., 1990; Zygadlo, 1994). Amer *et al*. (2013) considered *S. elaeagnifolium* as a future source of antibacterial agent against the antibiotic resistant bacteria. Its foliage water-soluble extracts inhibited germination and root growth of cotton seeds and lettuce, respectively (Bothma, 2002). As a part of our continued work searching herbicidal substances against several important weeds as purslane *Portulaca oleracea*, Jute *Corchorus olitorius* L., bindweed *Convolvulus arvensis* and *barnyard* grass *Echinochloa crus-galli* in Egypt, this study aimed to evaluate the phyto-inhibitory effect of *S. elaeagnifolium* extracts and identify its active components through spectroscopic methods.

Materials and Methods

Silverleaf nightshade *Solanum elaeagnifolium* plant parts were collected from North Sinai, El Arish, Egypt and identified according to (Tackholm, 1974). Weed seeds of purslane *Portulaca oleracea,* barnyard grass *Echinochloa crus-galli* and bindweed *Convolvulus arvensis* were collected from maize field at El-Bahria oasis, and seeds of maize *Zea mays,* were obtained from the Agriculture Research Center, Cairo, Egypt.

Aqueous extraction

Ten grams of air-dried seeds and leaves were extracted by a rotary shaker for 5hr at 25 °C in 100 ml distilled water. The mixture was filtered through two layers of cheesecloth to remove debris, and subsequently through Whatman paper No # 4 and Millipore membrane. Then the aqueous extract was stored at -4 °C over night until used in bioassays.

Organic extraction and purification

Ground seeds (300 grams) were defatted at room temperature with 1000 ml of petroleum ether and extracted through Soxhlet using 95% aqueous ethanol. The ethanolic extract was filtered and the solvent was completely evaporated and fifty milligrams of the residue were dissolved in 10ml of ethanol for biological studies. The ethanolic extract was applied for chromatographic analysis on 100 gm silica gel for column chromatography (Merck, 60 mesh) in a glass column (2 × 100-cm). Silica gel was filled in methanol and eluted successively with 150 ml of petroleum ether, petroleum ether: chloroform (1: 1), chloroform, chloroform: ethyl acetate (1: 1), ethyl acetate, and methanol. Twelve fractions (75 ml of each) were collected from each solvent system, evaporated to dryness and weighed also; 5 mg aliquot was re-dissolved in 10 ml 70% aqueous ethanol for biological tests. Control was concurrently conducted. The most active fractions, which were eluted with 100% chloroform were purified using TLC by chloroform: ethanol: acetic acid mixture (92:4:4) and hexane: chloroform: ethyl acetate (16:16:1) developing solvent systems. While fractions eluted with chloroform: ethyl acetate were further purified using percolated TLC plate (20 × 20 cm) with chloroform: methanol: water (13:7:1) and (butanol: water: formic acid (4:5:1) developing system. The gotten spots were visualized by spraying with iodine vapor and UV lamp at 366 and 254 nm. The separated sample on the TLC sheets were collected and re-dissolved in methanol (Fig. 1). The solvent was completely removed and the obtained sample was exposed to mass spectrum using High Performance Liquid Chromatography coupled to a Thermo Finnigan Surveyor MSQ mass spectrometer detector. Positive ionization was performed using electrospray ionization with a nitrogen flow at 80 psi, a cone voltage of 70 V, a needle voltage of 3 kV, and a cone temperature of 600 °C. Mass data were collected over the range of the gradient program at a rate of one scan per two seconds. UV-VIS spectrophotometers THERMO (Nicolet evolution 300) were conducted at scan mode. (Mabry *et al.,* 1970).

Aqueous extract bioassay*:* The aqueous extract of silver-leaf nightshade (100mg ml^{-1}) was diluted

with distilled water to 0, 12.5, 25, 50 and 100mg ml^{-1} concentrations. Bioassay was set up in 9 cm Petri dishes. Ten seeds of each test plant were placed directly on the filter paper of each Petri dish following complete block design with four replicates. 5 ml of each extract concentration were added to each replicate and distilled water was used in control treatment. Petri-dishes were incubated in the dark at 27 ± 2 °C. Germination percentage (G %), root and shoot lengths of the seedlings was recorded after 7 days of incubation.

Ethanol extracts bioassay

Filter paper bioassay for ethanolic crude extracts at concentrations of 0, 500, 1000 and 2000 µg ml^{-1} using *P. oleracea* seeds was conducted as described by Wolf *et al.* (1984). Each concentration was added to a filter paper disc (Whatman No. 4) in 9 cm Petri dishes, moistened with 5 ml distilled water, and 10 seeds were sown onto it. The length of roots and hypocotyls of seedlings were determined 7 days post treatment. In the control treatment, the filter paper was impregnated with 2000 µl of ethanol after total evaporation of the solvents; it was moistened with 5 ml of distilled water.

Ethanol extracts bioassay using seedling

Seeds of *Z. mays*, *C. arvensis*, *E. crus-galli* and *P. oleracea* were surface sterilized using sodium hypochlorite (0.3% v/v) for 10-12 min and washed four times in sterile double-distilled water. Seeds were placed on static Murashige and Skoog (MS) basal media and allowed to germinate for seven days until roots and shoots emerged. Seven-day-old seedlings were transferred to tissue culture tube containing 5 mL of liquid MS media where the roots were submerged. Seedlings were treated with the concentrations of ethanolic seed extract (0, 250, 500, 1000 and 1500 µg ml^{-1}). In the control treatment 1500 µl of ethanol after total evaporation of the solvent, was moistened with 5 ml liquid MS medium. The purified fractions and compounds were bioassayed according to the above methods at 40 µg ml^{-1} in two ml of MS liquid media where one seedling was submerged. The pH distribution in most liquid media treatment with and without extracts ranged from pH 5.5-6.0. Plant cultures were maintained at a photo period of 16:8 h (L: D) at 30 ± 3 °C with daily an hour of shaking at 90 rpm on an orbital platform shaker. Total biomass of each seedling was recorded ten days after treatment.

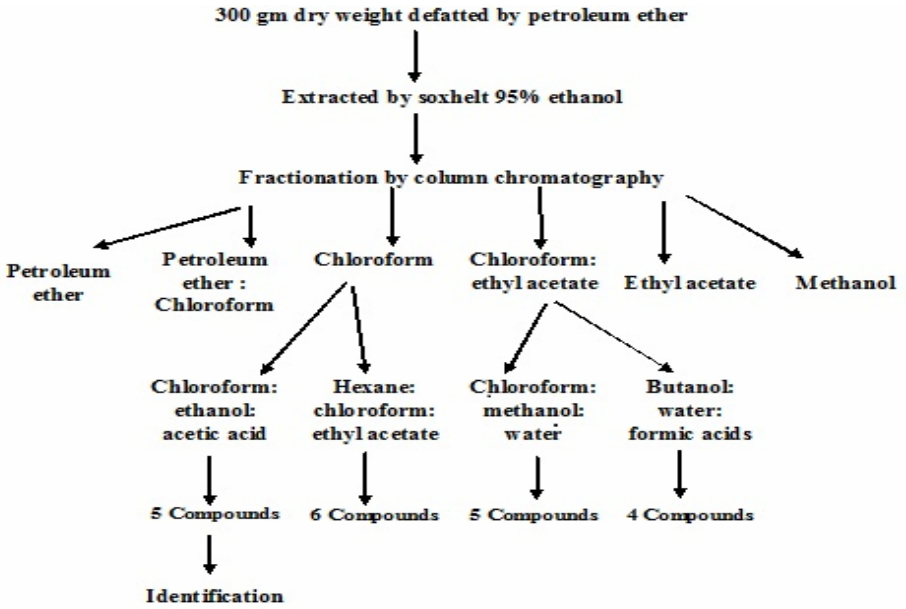

Figure 1 Diagram showing isolation and purification pattern of phytotoxic substances from seeds of *S. elaeagnifolium*.

Statistical analysis

Experiments were designed in a randomized complete block design with four replicates and data were statistically analyzed by ANOVA, according to Snedecor and Cochran (1990) and treatment means were compared by LSD at 5% level of probability. EC_{50} values for each growth parameter were calculated (Finney, 1971).

Results and Discussion

Pre-emergence activity of aqueous extracts

The obtained aqueous extract of *S. elaeagnifolium* seeds at a concentration of 100 mg ml^{-1} completely inhibited *P. oleracea* and *C. arvensis* and negatively affected germination and seedling growth. This extract at 100 mg ml^{-1} significantly inhibited *Z. mays*, and *E. crus-galli* germination processes up to 72.41% and 81.48%, their shoot system growth (71.23 and 80.12%) as well as their root system growth up to 75.08% and 84.36%, respectively compared with control (Table 1). The leaves aqueous extract at the same concentration had significant inhibitory effects on *Z. mays*, *P. oleracea, C. arvensis,* and *E. crus-galli* with inhibition percents of 62.07, 100, 65.22 and 66.67% on their germination; 76.7, 100.0, 67.7 and 59.3% on their shoot system growth and 68.31,100, 57.43, 66.67 on their root system growth, respectively in comparison to control. The obtained data indicate that the most susceptible plant to the tested extracts was *P. oleracea* with EC_{50} values 4.5, 0.51 and 9.39 mg ml^{-1}(seeds extract), 27.48, 31.86 and 33.50 mg ml^{-1} (leaf extracts) for germination, root length and shoot length. However *Z. mays* was less susceptible plant to *S. elaeagnifolium* leaf extracts (Table 1).

Filter paper test of ethanolic extracts on seeds of *P. oleracea*

Using the filter paper test, at concentrations of 0, 500, 1000 and 2000 µg ml^{-1} the *S. elaeagnifolium* seeds ethanol extract significantly inhibited the germination (40.9%) of *P. oleracea* only at 2000 µg ml^{-1} Seedling shoot length was significantly decreased at 500, 1000 and 2000 µg ml^{-1} by 74.6%, 89.85% and 89.18 %, however seedling shoot length was inhibited with 36.3%, 60.8% and 72.6 %, respectively in comparison to control (Fig. 2).

Table 1 Quantitative activity of *S. elaeagnifolium* aqueous extract on the treated plants growth parameters; shown as EC_{50} values.

Source of extract	Plant species	EC_{50} (mg ml^{-1})		
		Germination	Root Length	Shoot Length
Seeds	*Z. mays*	18.75	24.73	26.53
	P. oleracea	4.50	0.51	9.35
	Convolvulus sp.	21.75	17.92	23.45
	E. crus-galli	29.90	24.80	26.12
Leaves	*Z. mays*	50.01	46.36	43.95
	P. oleracea	27.48	31.86	33.50
	Convolvulus sp.	46.23	41.80	40.35
	E. crus-galli	49.80	38.60	39.50

Phytotoxicity assay against weed seedlings in liquid media

Crude ethanol extracts of *S. elaeagnifolium* seeds proved at 0, 250, 500, 1000 and 1500 µg ml^{-1} to be phytotoxic to *P. oleracea, C. arvensis* and *E. crus-galli* weeds total biomass fresh weight by achieved EC_{50} of 593.36, 1120.0 and 962.51 µg $ml^{-}1$, respectively (Table 2).

Purification and characterization of phytotoxic compounds

Further purification to the active column fractions (Table 3) eluted with 100% chloroform (F_3 and F_4)) conducted by per coated TLC plates (Table 4) using developing system; chloroform: ethanol: acetic acid (92:4:4) afforded five major spots with two active compounds (R_f 0.46 and 0.59). The primary active compound (R_f 0.46) had a UV absorbance λ_{max} (328) nm in methanol and a molecular mass of (354.15). This fraction was identified as chlorogenic acid ($C_{16}H_{18}O_9$),

whose spectral properties were identical with those of a commercial sample and retention time (Rt) 20.2 min at HPLC-UV profile and in agreement of Simirgiotis *et al.* (2013). The second herbicidal compound (R_f 0.59) was appeared on HPLC profile at RT; 37.05 min and had λ_{max} (336) nm in methanol and a molecular ions of (286). It was identified as kaempferol ($C_{15}H_{10}O_6$) by comparing its chromatographic and spectral properties with an authentic sample (Badawy *et al.* 2013). This column fraction was also developed by hexane: chloroform: ethyl acetate (16:16:1) and afforded six spots with one appearing at Rt; 39.34 min, R_f 0.63 that had a UV absorbance λ_{max} (345) nm in methanol and a molecular mass of (422). Its spectra were close to data published by (Elabbar *et al.*, 2014) and identified as mangiferin ($C_{19}H_{18}O_{11}$).

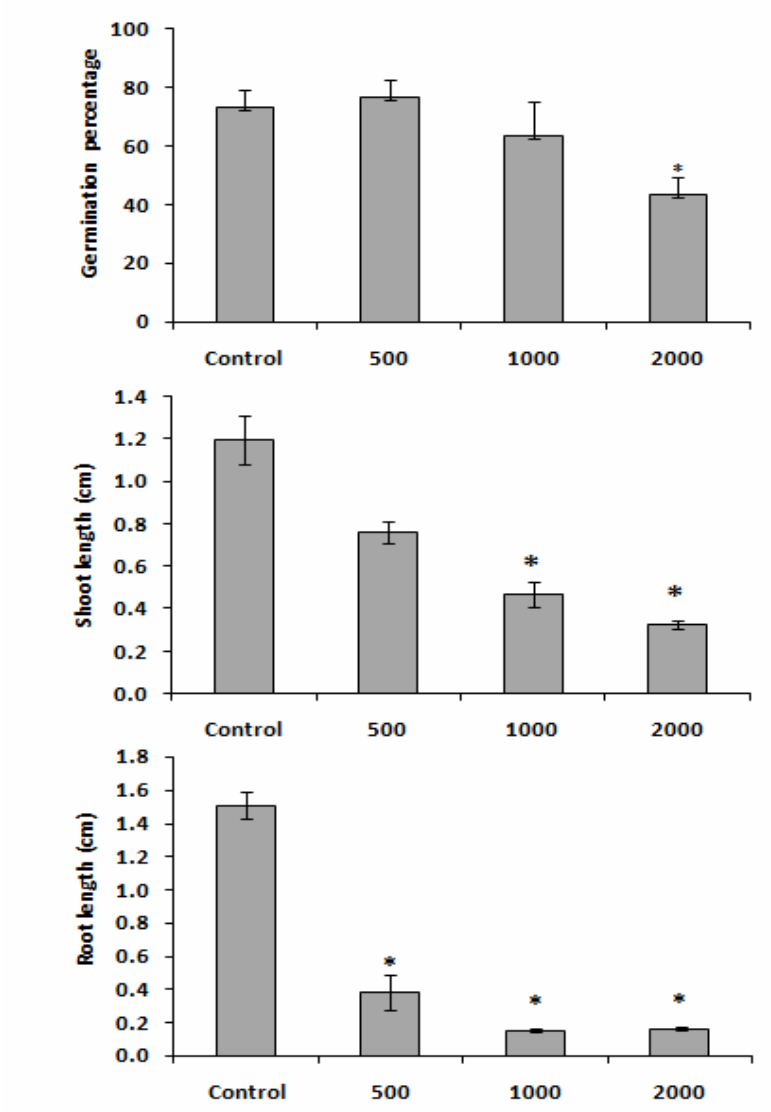

Figure 2 Filter paper test of ethanolic seeds extracts from *S. elaeagnifolium* on *P. oleracea* germination and growth parameters.

Table 2 Effect of *S. elaeagnifolium* seeds ethanol extract on weeds total biomass 10 days after treatments.

Concentration (µg/ml)	% of inhibition over the controls		
	P.oleracea	*C. arvensis*	*E. crus-galli*
250	30.15*	16.52	26.54
500	46.73*	31.30	37.04*
1000	63.82*	36.52*	49.38*
1500	77.39*	61.74*	58.64*

Table 3 Effect of active column chromatographic fractions of S. elaeagnifolium seeds extract on weeds total biomass. 10 days after treatments.

Fractions	% Inhibition over the control		
	P.oleracea	*C. arvensis*	*E. crus-galli*
F1	44.02	27.68	28.61
F2	76.75*	50.28	35.83
F3	97.94*	69.40*	97.59*
F4	98.49*	75.99*	86.36*
F5	97.52*	72.03*	83.69*
F6	96.56*	66.29*	39.04
F7	95.46*	66.67*	29.68
F8	94.22*	60.92	30.75
F9	90.37*	31.83	12.03
F10	67.81	50.66	11.50
F11	42.64	39.27	51.07
F12	45.12	52.45	42.25

*: Significant at $p < 0.05$.

Investigation of the chloroform: ethyl acetate (F_5 and F_6) column fractions developed on preparative TLC plate (20 x 20 cm) with chloroform: methanol: water (13:7:1) afforded five spots with two active compounds (R_f 0.47 and 0.53). The first one appeared on HPLC profile at Rt 4.55 min and had a UV absorbance λ_{max} (337) nm in methanol and a molecular mass of 326 resulting from ESI-MS *m/z* = 365 ($M + K^+$) and was identified as coumaroyl glucoside (R_f 0.47), the mass spectral of this compound is an agreement with that reported by Katsuragi *et al.* (2011). The second active compound appeared on HPLC profile at Rt 14.5 min and had λ_{max} (284.6) nm in methanol, the spectrum demonstrated major fragment at (337) and was identified as coumaroyl quince acid (R_f 0.53). Also this fraction developed on TLC by butanol/water/formic acid (4:5:1) resulted in four spots with two active compounds (R_f 0.63 and 0.73). The first one introduced at Rt 5.5 min and λ_{max} (234.5) nm and a molecular ions of (582) derived from the fragment ions m/z = 583, it identified as kaempferol 3/β-D-(6"-O-cis-cinnamoyl glucoside) (R_f 0.63), whose spectral properties were identical with those of previous published by (Chiale *et al.*, 1991). The second compounds(R_f 0.73) and λ_{max}(320) nm in methanol and a molecular mass Calc. by (515.1) within *m/z* = 515.1 which indicated the presence of dicaffeoyl quinic acid ($C_{25}H_{24}O_{12}$). This was further supported by commercial sample and its retention time on HPLC profile at the same Rt 4.33 min. The structure proposed by spectral data found in previous published data for the above allelopathic compounds (Hiradatea *et al.*, 2004) and confirmed by their HPLC retention time (Table 5).

Table 4 Activity of isolated purified compounds from *S. elaeagnifolium* seeds on *P. oleracea* total biomass fresh weight.

TLC developing system	CC. eluted by chloroform		CC. eluted by Chloroform: Ethyl acetate	
	Chloroform:Ethanol: Acetic acid 92:4:4	Hexane:Chloroform: Ethyl acetate 16:16:1	Butanol/Water/Formic acid 4:5:1	Chloroform:Methanol: Water 13:7:1
Spot1	29. 42	13.58	15.22	18.25
Spot2	86.50*	9.06	8.67	66.47*
Spot3	39.38	62.26*	84.45*	79.37*
Spot4	72.48*	3.40	55.32*	28.77
Spot5	20.80	76.98*	18.82	ND
Spot6	ND	3.77	ND	ND

CC. = column chromatography, ND = Not detected, *: Significant at $p < 0.05$.

Table 5 HPLC-MS characteristics of allelopathic compounds detected in extract of *Solanum elaeagnifolium* seeds.

Compounds	m/z	Fragments	HPLC Retention time (min)	λ_{max} (nm)
Chlorogenic acid	354 (M^+ + 1)	163.2,293.2,355.2	20.2	328
Kaempferol	286 (M^+ + 4)	130.1,143.4,156.5,194.9,264.2,290.5	37.05	320
Mangiferin	422 (M^+ + Na)	131,163.2,228.5,294.8,387.4,441.3	39.34	345
Coumaroyl glucoside	326 (M^+ + K)	127.1,203.1,311.2,365	4.55	326
Coumaroyl –quince acid	337 (M^+ + Na)	117,135,145,163,181,337.1	14.5	284.6
kaempferol β-D-(6"-O-cis-cinnamoyl glucoside	583 (M^+)	173.1,245,269.1,404.9,501.1,583	5.5	234.5
Dicaffeoyl quinic acids	516 (M^+ + 1)	187.1,245.1,269.1,351.1,433.2,515	4.33	224.5, 234.7

Chlorogenic acid was the most active isolate which decreased *P.oleracea* total biomass fresh weight by (86.5%) followed by kaempferol β-D-(6"-O-cis-cinnamoyl glucoside) (84.4%), coumaroyl glucoside (79.37%), mangiferin (76.98), kaempferol (72.48%), coumaroyl quince acid (66.47%) and dicaffeoyl quinic acids by (63.6%) (Fig. 3).

It could be conculded that the filter pepper bioassay and tested species were capable of detecting phytotoxicity of *S. elaeagnifolium* seeds and leaves extracts rapidly and economically although in limited quantities. Both extracts had a negative effect against the germination and seedling growth of tested plants. However, seed extracts demonstrated higher activity than leaf extracts based on their EC_{50}. Thereby seed extracts were chosen for extraction by aqueous ethanol and further purification.

According to the estimated $EC_{50,}$ the most susceptible plant was *P. oleracea* and exhibited highly positive response to both water and organic soluble compounds extracts than C. *arvensis*, and *E. crus-galli* as determined by their EC_{50}

Whereas, *C. arvensis* was more sensitive to *S. elaeagnifolium* water soluble compounds than *E. crus-galli* and vice versa. Root growth of the tested species was very sensitive to both water and organically soluble extracts which might be due to the direct contact with the phytotoxic compounds while seedling shoot length was more responsive than germination to extract allelochemicals. The use of a growth medium in tissue culture tubes in bioactivity test permits more accurate, reliable measurement of seedling length than does filter paper test in Petri dishes. According to the simple dose response dynamics, the increase in concentration increases the negative influence on tested plant parameters. The obtained data indicated that most allelochemicals were present in water extracts in much lower concentrations than in organic extracts.

Chlorogenic acid showed inhibition of seed germination and subsequent seedling growth in dark treated seeds more than light treated seeds of *Artemisia herba alba* in most concentrations (Al-Charchafchi and Al-Quadan, 2006). Coumarin, as main and typical component of *S. chamaejasme*, might act as a plant growth inhibitor which can severely inhibit cell growth at low concentrations (Abenavoli *et al.,* 2003). The fact that polyphenols are known natural products with potent herbicidal activity makes this topic more worthy and may require future investigation. Chlorogenic acid also has antioxidant activity comparable to that of L-ascorbic acid, which is a well known antioxidant (Kim *et al.,* 1997). The above mentioned results should greatly increase the opportunities available to others for further identification of *S. elaeagnifolium* purified phytochemicals. Based on the results of this study, *S. elaeagnifolium* has strong allelopathic potential and suppressive ability on growth parameters of *P. oleracea*, *C. olitorius, C. arvensis* and *E. crus-galli* weeds.

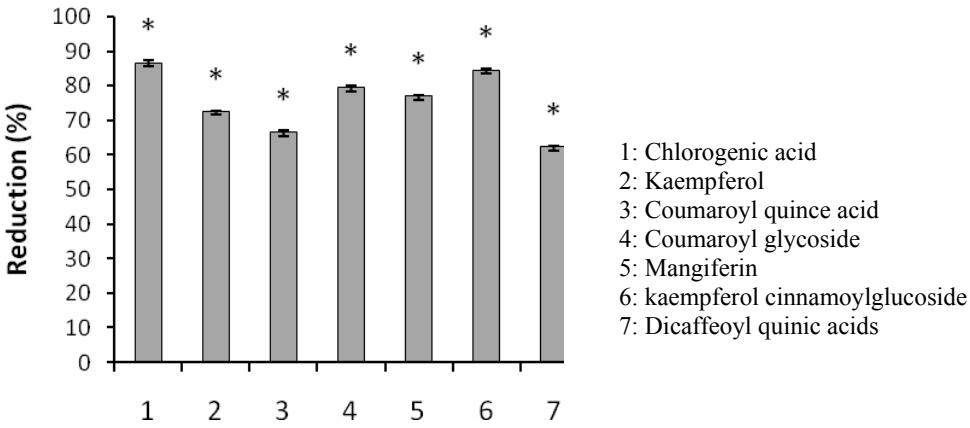

Figure 3 Phytotoxicity of allelopathic compounds extracted from seeds of *S. elaeagnifolium* on *P. oleracea* seedling total biomass fresh weight.

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خاصیت علف‌کشی ترکیبات استخراج شده از علف هرز تاج‌ریزی برگ نقره‌ای *Solanum elaeagnifolium* (Solanaceae)

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دریافت: ۱۴ مهر ۱۳۹۳؛ پذیرش: ۱۱ تیر ۱۳۹۴

چکیده: خاصیت علف‌کشی عصاره آبی و متانولی برگ و بذر علف هرز تاج‌ریزی برگ نقره‌ای *Solanum elaeagnifolium* Cav. روی علف‌های هرز مزارع ذرت شامل خرفه *Portulaca oleracea* L.، آزی وش *Corchorus olitorius* L.، پیچک صحرایی *Convolvulus arvensis* L. و سوروف *Echinochloa crus-galli* (L.) آزمایش شد. خصوصیات ترکیبات جدا شده از عصاره اتانولی به‌روش اسپکتروسکوپی مطالعه شد. خالص‌سازی فاز کلروفرم (خالص) به‌روش کروماتوگرافی لایه نازک (TLC) با استفاده از ترکیب کلروفرم:اتانول: استیک اسید (۹۲:۴:۴) و هگزان: کلروفرم:اتیل استات (۱۶:۱۶:۱) منجر به جدا شدن کلروژنیک اسید، کامپفرول و مانجیفرین شد. دومین فرکشن حاصل از کلروفرم:اتیل استات به‌وسیله TLC توسط ترکیبات کلروفرم:متانول: آب (۱۳:۷:۱) و بوتانول:آب: اسید فرمیک (۴:۵:۱) به‌ترتیب منجر به جدا شدن "کومارویل گلوکوزاید و کومارویل کوینیک اسید" و "کامپفرول بتا-دی و دیکافویل کوینیک" اسید شد. ترکیبب کلروژنیک اسید ۸۶/۵ درصد، کامپفرول بتا-دی ۸۴/۴ درصد، کومارویل گلوکوزاید ۷۹/۳۷ درصد، مانجیفرین ۷۶/۹۸ درصد، کامپفرول ۷۲/۴۸ درصد و کومارویل کوینیک اسید ۶۶/۴۷ درصد و درنهایت دیکافویل کوینیک ۶۳/۶ درصد از وزن تر علف هرز خرفه را در مقایسه با شاهد کاهش دادند. بنابراین ترکیبات جدا شده می‌توانند برای تولید علف‌کش‌های طبیعی مورد توجه قرار گیرند.

کلید واژگان: علف هرز تاج‌ریزی برگ نقره‌ای، آللوکمیکال‌ها، پلی‌فن‌ها، فلاونوئیدها، اثرات گیاه‌سوزی