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RESEARCH ARTICLE

Phytochemical composition and antifeedant activity of five *Vincetoxicum* taxa against *Spodoptera littoralis* and *Leptinotarsa decemlineata*

Sevda GÜZEL, Roman PAVELA, Ahmet İLÇİM, Gamze KÖKDİL

ABSTRACT

Five *Vincetoxicum* taxa (*V. canescens* subsp. *canescens*, *V. canescens* subsp. *pedunculata, V. fuscatum* subsp. *fuscatum, V. fuscatum* subsp. *boissieri* and *V. parviflorum*) were investigated for insect antifeedant activity against *Spodoptera littoralis* and *Leptinotarsa decemlineata*. At a dose of 500 μg/cm^2, 12 extracts were 100 % feeding deterrent, while 6 extracts showed 86.3-99.3 % antifeedant activity. The effective doses (ED_{50}) of the total ethanol extract from *V. canescens* subsp. *pedunculata* and of the methanol: dichloromethane (1:1) extract from *V. parviflorum* were 12, and 18 μg/cm^2, respectively, against *S. littoralis*, and that of the total ethanol extract from *V. fuscatum* subsp. *fuscatum* was 25 μg/cm^2 against *L. decemlineata*. The dichloromethane extracts of *V. parviflorum* and *V. canescens* subsp. *pedunculata* inhibited the growth of *S. littoralis* with ED_{50} values of 0.08 and 0.09 mg/g, respectively, and their LD_{50} values for larval mortality were 1.07 and 1.03 mg/g, respectively. Phytochemical analysis revealed the presence of cardiotonic glycosides, sugars, flavonoids and alkaloids in all investigated taxa. In the dichloromethane and methanol:dichloromethane (1:1) extracts, phenanthroindolizidine alkaloids were detected by LC/MS/MS.

Keywords: *Vincetoxicum*; antifeedant activity; phytochemical composition; *Spodoptera littoralis*; *Leptinotarsa decemlineata*

1. Introduction

The genus *Vincetoxicum* N.M. Wolf, (Apocynaceae; subfamily Asclepiadoideae) [1, 2] is represented by approximately 100 species which are distributed throughout Asia, Japan, Europe [3] and North America [4]. Some species of the genus (i.e. *V. nigrum* (L.) Moench , *V. hirundinaria* Medicus and *V. stocksii* Ali & Khatoon) have been used in European and Chinese traditional medicine for the treatment of neurosis, malaria, scrofula, rupture, injuries, fever, wounds, scabies [1, 5] and as expectorant, diuretic, emetic [6,7], laxative and diaphoretic agents [1]. There are some reports on biological activities of *V. rossicum* (Kleo.) Barb., *V. stocksii* and *V. hirundinaria* species including antibacterial and antifungal [8], antidiarrheal, antispazmodic [9], antileishmanial, antimalarial [5], cytotoxic [10], antifeedant and growth inhibition [7] effects in the literature. Phytochemical investigations revealed the presence of phenanthroindolizidine alkaloids [8, 10-13], steroidal glycosides [7, 14, 15], triterpenoids [15, 16], flavonoids, saponins, phenolics [7, 9], steroids [6, 15], volatile compounds, acetophenone [6] and alkanols [15] in *Vincetoxicum* species. In these phytochemical constituents, phenanthroindolizidine alkaloids are characteristic secondary metabolites of some Asclepiadaceae and Moraceae

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genera including *Cynanchum* L., *Vincetoxicum*, *Tylophora* R. Br., *Pergularia* L. and *Ficus* L. [10, 17]. These alkaloids exhibit interesting biological activities such as significant cytotoxic [18] and antitumor activities as well as anti-inflammatory [17], antimicrobial [8], antiviral [19], antiasthmatic [20], insecticidal and insect antifeedant [7, 8, 21] activities.

Vincetoxicum is one of the largest genera of the subfamily Asclepiadoideae in Anatolia and is represented by 10 taxa [22], which have not yet been the subject of any study of their phytoconstitutents and biological activities. As part of our studies on *Vincetoxicum* species, we report here on the phytochemical profiles, insect antifeedant activity, larval mortality and growth inhibitory effects of five *Vincetoxicum* taxa (*V. canescens* (Willd.) Decne. subsp. *canescens*, *V. canescens* subsp. *pedunculata* Browicz*, V. fuscatum* subsp. *fuscatum* (Hornem) Reichb.*, V. fuscatum* subsp. *boissieri* (Kusn) Browicz and *V. parviflorum* Decne.) against the larvae of *Spodoptera littoralis* Bois. (Boisduval) (Lepidoptera: Noctuidae) and *Leptinotarsa decemlineata* Say. (Coleoptera: Chrysomelidae).

2. Materials and Methods

2.1. Plant materials

Plants were collected from their natural habitats in different regions of Turkey during the summer of 2009, identified by one of the authors (S. Güzel) and confirmed by Dr. Ahmet İlçim, Department of Biology, Faculty of Arts and Science, Mustafa Kemal University (Antakya, Turkey). The dried voucher specimens were deposited in the Mustafa Kemal University Herbarium (Further details are provided in Table 1).

2.2. Chemicals and reagents

Dichloromethane and diethyl ether were purchased from Sigma-Aldrich (St. Louis, MO, USA) and all other chemicals and reagents of analytical grade were purchased from Merck (Darmstadt, Germany).

2.3. Phytochemical screening

Standard test procedures were used in phytochemical screening [23-26]. Alkaloids were detected by using Mayer's and Dragendorff's tests and Borntrager's test was used for anthraquinones. Coumarins were vizualized by their fluorescence in ammoniacal solutions. The presence of sugars was tested with Fehling's solution, and Molisch's and Selivanoff's tests [26]. Tannins were detected by their reactions with gelatin, $FeCl_3$ [26], bromine water and Stiasny reagent [24]. Cardiac glycosides and steroids were screened by using Keller-Kiliani, Baljet and Liebermann-Burchard tests. Cyanogenic glycosides were detected with picric acid/ sodium carbonate. Foam value was used to show the precence of saponins [25]. Flavonoids were detected with cyanidin test [25] and dilute NH_3, $Pb(Ac)_2$ and $FeCl_3$ [23]. Anthocyanins were tested by using some solutions including dilute H_2SO_4, $Pb(Ac)_2$, NaOH and amyl alcohol [23].

2.4. Extraction procedure

Aerial parts of plant materials were powdered, sequentially macerated three times with CH_2Cl_2, MeOH:CH_2Cl_2 (1:1, v/v) and MeOH (600 ml of solvent per 100 g of plant material) at room temperature to give a series of crude extracts (extracts A, B and C, respectively) [10]. Additionally, total ethanol

Table 1. List of plant materials, their origins and voucher references.

Taxon	Locality [b]	Voucher references
V. canescens subsp. *canescens*	C6: Kahramanmaraş, Engizek Mountain, Fallow fields, 1.000 m.	MKUH 1283
V. cancescens subsp. *pedunculata* *	B3: Afyon, Dinar; Kumalar Mountain, 1,500-1.600 m.	MKUH 1284
V. fuscatum subsp. *fuscatum*	B6: Kayseri, Pınarbası, Hınzır Mountain, 1.800 m.	MKUH 1315
V. fuscatum subsp. *boissieri* *	A5: Amasya, Ferhat Mountain, 460 m.	MKUH 1316
V. parviflorum *	A7: Trabzon, 1.200 m.	MKUH 1334

[b] Locality information is based on the Flora of Turkey grid system.

*Endemic taxon

extracts were prepared. Plant materials were extracted twice with 96 % ethanol (720 ml of EtOH per 100 g of plant material), sonicated for 30 min. and left overnight at room temperature with shaking [8]. After filtration through Whatman No.1 filter paper, solvents were evaporated under reduced pressure using vacuum evaporator (Heidolph-Rotar TLR 1000) at 35-40 °C, and the dry residues kept in the dark at 4 °C until use.

2.5. Preparation of alkaloid fractions

The crude extracts (A, B and C) were subjected to TLC [silica gel 60 F_{254} plates, Merck; Benzene:Acetone (5:1)] and alkaloids vizualized by using Dragendorff's reagent. The alkaloid-containing CH_2Cl_2 and MeOH: CH_2Cl_2 extracts were fractionated according to Staerk *et al*. (10). Briefly, crude extracts were dissolved in HCl (0.016 M, 600 ml), the solutions extracted five times with 200 ml of petroleum ether, the pH of the aqueous fractions adjusted to 9-10 with aqueous NH_3, and these fractions were re-extracted four times with 200 ml of diethyl ether [10]. The fractions were stored in the dark at 4 °C until use and analysis by LC/MS/MS.

2.6. LC/MS/MS analysis

Fractions were analysed in an Agilent 1200 (6460 Triple Quad LC/MS/MS system) equipped with binary pump, vacuum degasser, QQQ (MS/MS) detector. YMC Pro C18 (150x3.0 mm, 5 µm particle size) column (YMC, Japan) and operated in the positive ion mode. Phenanthroindolizidine alkaloids were identified online according to Cui *et al.* [27] using methanol / 0.02 M ammonium acetate (52:48, v/v) as the isocratic mobile phase at a flow rate of 0.3 ml/min.

2.7. Insects

S. littoralis: A laboratory colony of *S. littoralis* (Crop Research Institute, Prague, Czech Republic) was reared on a semisynthetic insect diet provided by Stonefly Industries (Bryan, TX, USA). Experiments were performed with pre-weighed, newly-molted 3rd instar larvae at 25±1 °C and a 16:8 h light: dark photoperiod.

L. decemlineata: Adults were collected annually from a potato field and a colony was maintained on potato plants cv. Agnia in an environmental chamber under conditions as above. 3rd instar larvae were used for the experiments.

2.8. Antifeedant activity

The plant extracts were tested for antifeedant activity in a no-choice test. Larvae of either insect, starved for 3h, were placed in 9 cm Petri dishes containing moist filter paper and four 1.5 cm disks of tomato (for *S. littoralis*) or potato (for *L. decemlineata*) leaves, respectively. Extracts were dissolved in acetone in a series of concentrations from 0.1 to 5 %. Subsequently, they were applied to the leaf discs at 10 µl cm^{-2}, to provide doses of roughly 500, 400, 300, 200, 150, 100, 50, 25 and 10µg cm^{-2}, respectively. For the control, acetone only was used. The sovent was allowed to evaporate, and after evaporation 2 starved larvae of either *S. littoralis* or *L. decemlineata* were placed into the centre of each dish. An experiment was terminated when the control group had consumed nearly 90 % of the leaf disks (about 7h), and the remaining areas of all leaf disks were assessed by a screener software program [7].

The feeding deterrence index (FDI) was calculated according to the equation

(FDI) = 100 X ((C-T)/(C + T))

C: area of the control leaf consumed by larvae

T: area of treated leaf consumed by larvae.

2.9. Effect on larval growth

A diet was prepared by mixing a defined amount of extract dissolved in water with the dry semisynthetic diet. Twenty newly emerged 3rd instar larvae of known weight were transferred into a 6 cm Petri dish and for 5 days supplied under standard rearing conditions (see Insects) *ad libitum* with the respective contaminated diet. Then the larvae were weighed and the growth index (GI) determined according to the equation (28):

The formula: GI (%) = 100−((T/C) X 100)

C: increase of larval weight in the control

T: increase of larval weight on contaminated diet

Each individual assay was repeated four times.

2.10. Larval mortality

Larval mortality of tested plant extracts on early 3rd instar larvae of *S. littoralis* were studied by evaluating mortality after 5 days applications and the application carried out orally. The diet for mortality test was prepared identically as described above and were administered to *S. littoralis* larvae. Control larvae were reared with a diet including only water.

The prepared diet was given *ad libitum* to new larvae of *S. littoralis*. 5 days later the larval mortality was evaluated. For each dose 4 replications of 20 larvae were studied. Whole larvae of each replicate were carried into plastic boxes (10 cm × 10 cm × 7 cm). The boxes were put in to a growth chamber (L16:D8, 25 ˚C) for 5 days. Death was noted by the time the larvae didn't react prodding with forceps [28].

2.11. Statistical analysis

The ED_{50} values causing 50 % feeding or growth inhibition and LD_{50} values causing 50 % larval mortality with CI_{95} (95 % confidence limit) values were assessed by probit analysis [29]. Before analysis, the percentages were transformed by arcsin $\sqrt{(x/100)}$ (7). For calculation EPA Probit Analysis Program (Version 1.5) was used.

3. Results

3.1. Phytochemical screening

The results of the qualitative screening of phytoconstituents are shown in Table 2. All plant materials showed positive results for cardioactive glycosides, sugars, flavonoids and alkaloids. Tannins was observed in *V. fuscatum* subsp. *fuscatum*, *V. fuscatum* subsp. *boissieri* and *V. parviflorum*. The other constituents were not detected in any of the tested plant materials.

Table 2. Phytochemical constituents of studied *Vincetoxicum* taxa.

Constituents	Taxon				
	V. canescens subsp. *canescens*	*V. canescens* subsp. *pedunculata*	*V. fuscatum* subsp. *fuscatum*	*V. fuscatum* subsp. *boissieri*	*V. parviflorum*
Alkaloid	+	+	+	+	+
Flavonoid	+	+	+	+	+
Anthocyanin	-	-	-	-	-
Coumarin	-	-	-	-	-
Saponin	-	-	-	-	-
Sugar	+	+	+	+	+
Tannin	-	-	+	+	+
Cyanogenic Glycoside	-	-	-	-	-
Anthracene Glycoside	-	-	-	-	-
Cardiactive Glycoside	+	+	+	+	+

+: presence of constituent
-: absence of constituent

Table 3. Percentage yielded of extracts from studied materials.

Taxon	Yield (%)					
	Extract A (CH_2Cl_2)	Crude alkaloid extract yield from Extract A (CH_2Cl_2)	Extract B (MeOH:CH_2Cl_2 (1:1))	Crude alkaloid extract yield from Extract B (MeOH:CH_2Cl_2 (1:1))	Extract C (MeOH)	Total EtOH extract
V. canescens subsp. *canescens*	4.03	0.62	10.30	0.53	8.79	13.78
V. cancescens subsp. *pedunculata* *	2.29	1.25	9.61	0.66	13.72	11.47
V. fuscatum subsp. *fuscatum*	4.34	0.65	12.28	0.98	9.9	15.51
V. fuscatum subsp. *boissieri* *	3.47	3.10	11.64	1.38	8.93	13.56
V. parviflorum *	3.32	5.40	12.91	0.58	8.43	11.28

*Endemic taxon

3.2. LC/MS/MS analysis of alkaloid containing fractions

The yields of the extracts are given in Tables 3. The extracts were subjected to LC/MS/MS for qualitative phenanthroindolizidine alkaloid analysis. The alkaloids 1, 2 and 3 (Figure 1, 2) were identified as secoantofine, secoantofine *N*-oxide and antofine *N*-oxide, respectively, by comparing their mass spectroscopic data with those in the literature [10, 11, 18] (Table 4). Compound 1 was not detected in *V. fuscatum* subsp. *fuscatum*

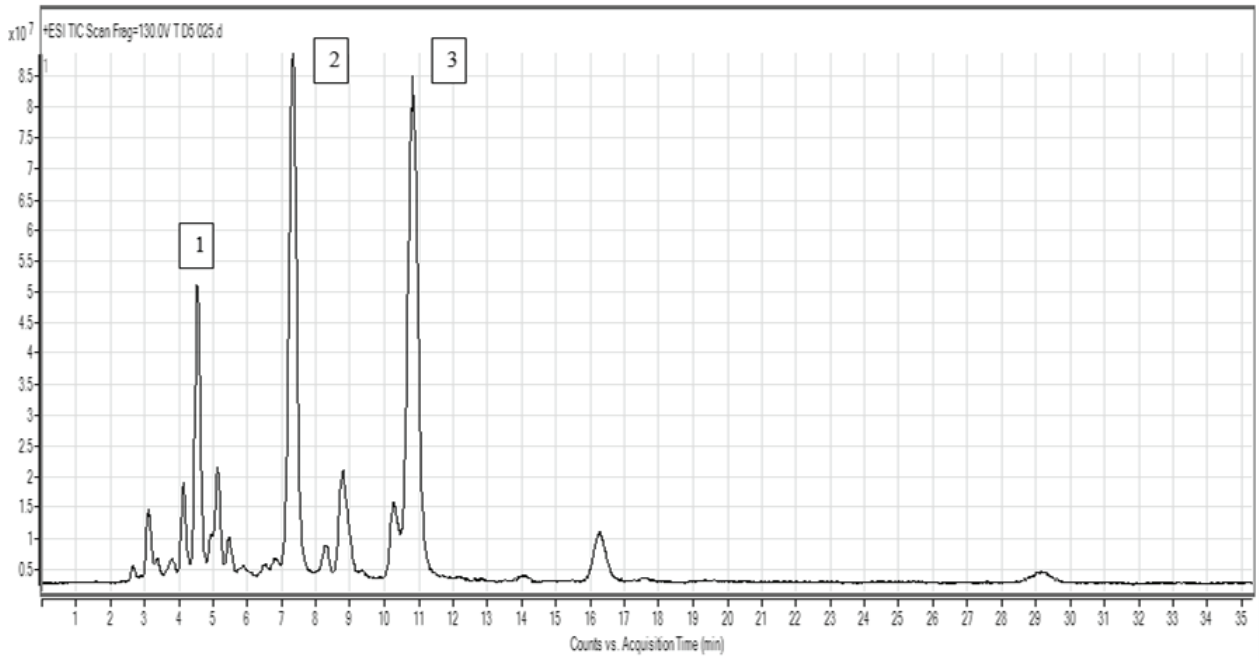

Figure 1. Total ion chromatogram of LC/MS/MS analysis of alkaloids fractions of CH_2Cl_2 extract of *V. parviflorum* in positive ESI mod. (1: Compound 1; 2: Compound 2; 3: Compound 3)

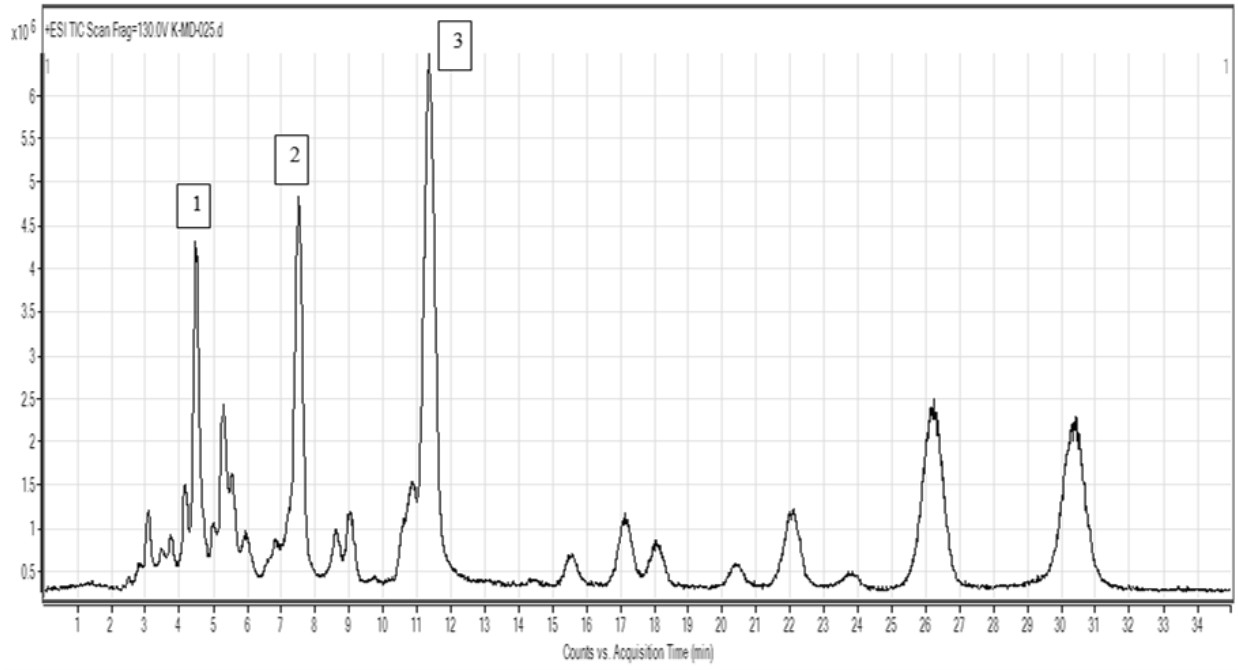

Figure 2. Total ion chromatogram of LC/MS/MS analysis of alkaloid fractions of CH_2Cl_2:MeOH (1:1) extract of *V. canescens* subsp. *canescens* in positive ESI mod. (1: Compound 1; 2: Compound 2; 3: Compound 3)

3.3. Antifeedant activity

The antifeedant activity of the four extracts of increasing polarity obtained from each of the five *Vincetoxicum* taxa was tested against larvae of the polyphagous pest *S. littoralis* and the oligophagous pest *L. decemlineata* by the leaf disc no-choice method. Antifeedant activities were initially determined at 500 μg/cm^2. Generally, almost all extracts exhibited antifeedant activity at this concentration, the larvae of *S. littoralis* being less sensitive to the extracts than those of *L. decemlineata* (Table 5). To obtain a quantitative estimate of the effectiveness of the extracts, concentrations causing a 50 % reduction of feeding activity (ED_{50}) were determined for those extracts that had caused 50% or higher inhibition of feeding at 500 μg/cm^2.

Against *L. decemlineata* larvae, only the total ethanolic extracts of the five taxa exhibited appreciable antifeeding activity, that from *V. fuscatum* subsp. *fuscatum* having an ED_{50} value of 25 μg/cm^2, while the ED_{50} values for the other taxa were in the range from 42 to 66 μg/cm^2 (Table 5). Against *S. littoralis* larvae, the total EtOH extract of *V. canescens* subsp. *pedunculata* and the MeOH:CH_2Cl_2 (1:1) extract of *V. parviflorum* was most effective (ED_{50} values of 12 μg/cm^2 and 18 μg/cm^2, respectively).

3.4. Growth inhibition and larval mortality

The plant extracts inhibited the growth of the *S. littoralis* larvae and caused mortality (Table 6). The CH_2Cl_2 extracts of *V. parviflorum* and *V. canescens* subsp. *pedunculata* were particularly inhibitory with ED_{50} values of 0.08 and 0.09 mg/g, respectively, and also caused highest larval mortality with LD_{50} doses of 1.07 mg/g and 1.03 mg/g, respectively.

Table 4. MS-MS data of compound 1, 2 and 3 in studied plants.

Compound	Rt (min)	MS^1(*m/z*)	MS^2(*m/z*) (relative abundances, %)
1	4.45	362 ((M-2H_2)+H^+)	346.30 (100), 318.30 (15)
2	7.47	382.30 $(M+H)^+$	297.3 (100), 265.2 (32), 202.1 (18), 159.10 (18)
3	11.2	380.30 $(M+H)^+$	362.3 (15), 295.4 (50), 294.4 (100)

Table 5. Antifeedant activity and effective doses of tested extracts against larvae of *Leptinotarsa decemlineata* and *Spodoptera littoralis*.

Taxon	Extract	Feeding deterrence index (%)[a] after exposure maximal dose		Effective doses			
		L. decemlineata	*S. littoralis*	*L. decemlineata*		*S. littoralis*	
		500 μg/cm^2	500 μg/cm^2	$ED_{50}(CI_{95})$[b]	Chi[c]	$ED_{50}(CI_{95})$[b]	Chi[c]
V. canescens subsp. *canescens*	CH_2Cl_2	43.6±5.1	100.0±0.0	ND	-	45(41-49)	3.111
	MeOH:CH_2Cl_2 (1:1)	99.3±2.7	44.2±5.7	335(316-356)	0.509	ND	-
	MeOH	3.9±1.8	41.3±2.7	ND	-	ND	-
	Total EtOH	100.0±0.0	72.1±1.6	66 (53-77)	1.833	320(311-337)	0.298
V. canescens subsp. *pedunculata*	CH_2Cl_2	24.7±5.2	74.7±5.2	ND	-	172(169-188)	2.123
	MeOH:CH_2Cl_2 (1:1)	2.9±3.3	72.9±2.3	ND	-	129(111-138)	2.115
	MeOH	77.7±3.2	48.3±7.5	434(421-475)	2.125	ND	-
	Total EtOH	93.9±2.8	100.0±0.0	55(47-64)	1.787	12(11-15)	1.222
V. fuscatum subsp. *fuscatum*	CH_2Cl_2	92.7±3.3	62.7±2.3	158(133-181)	0.181	398(389-412)	0.678
	MeOH:CH_2Cl_2 (1:1)	100.0±0.0	59.3±7.1	185(163-197)	2.895	431(356-492)	1.236
	MeOH	100.0±0.0	73.7±3.5	195(167-203)	3.181	132(125-148)	2.115
	Total EtOH	100.0±0.0	73.3±2.2	25(20-29)	4.112	110(103-115)	3.315
V. fuscatum subsp. *boissieri*	CH_2Cl_2	89.5±5.3	79.2±2.3	141(107-170)	0.161	323(292-353)	1.575
	MeOH:CH_2Cl_2 (1:1)	59.2±2.5	43.7±2.5	489(427-516)	3.128	ND	-
	MeOH	40.1±6.5	42.1±5.5	ND	-	ND	-
	Total EtOH	100.0±0.0	100.0±0.0	42(38-48)	3.125	33(25-38)	2.333
V. parviflorum	CH_2Cl_2	100.0±0.0	62.3±3.1	43(36-49)	2.867	489(422-512)	3.122
	MeOH:CH_2Cl_2 (1:1)	86.3±2.8	100.0±0.0	302(276-327)	3.232	18(13-25)	2.485
	MeOH	57.4±2.8	32.3±2.5	495(487-512)	3.821	ND	-
	Total EtOH	100.0±0.0	88.2±3.1	65(53-76)	0.936	218(201-225)	0.335

[a] Mean FDI (±S.E.), numbers present the deterrent effect. FDI = ((C×T)/(C + T))×100, where C and T are the control and treated leaf consumed by the insect.
[b]Effective doses (in μg/cm^2) causing 50% (ED_{50}) feeding deterrence of *L. decemlineata* or *S. littoralis* larvae relative to the control. CI_{95}: 95% confidence intervals were given in parenthesis.
[c]Chi-square value, significant at $P < 0.05$ level. ND: Not determined

Table 6. The effect of crude extract of *Vincetoxicum spp.* plants incorporated into larval diet on growth inhibition and larval mortality of *S. littoralis* larvae. The extracts were in the diet for 5 d.

Taxon	Extract	Growth inhibition		Larval mortality	
		ED_{50} (CI_{95})[a] mg/g	Chi[c]	LD_{50} (CI_{95})[b] mg/g	Chi[c]
V. canescens subsp. *canescens*	CH_2Cl_2	0.24 (0.17-0.31)	1.253	3.82 (2.31-4.28)	0.568
	MeOH:CH_2Cl_2 (1:1)	0.82 (0.72-0.95)	0.235	3.63 (2.59-4.24)	1.215
	MeOH	6.41 (5.13-7.47)	1.023.	17.44 (16.97-20.05)	1.124
	Total EtOH	0.45 (0.38-0.54)	0.338	3.42 (2.36-5.96)	1.325
V. canescens subsp. *pedunculata*	CH_2Cl_2	0.09 (0.04-0.11)	1.225	1.03 (0.74-1.88)	0.552
	MeOH:CH_2Cl_2 (1:1)	0.69 (0.60-0.78)	0.005	2.78 (2.09-3.34)	0.521
	MeOH	1.19 (1.05-1.35)	0.462	4.93 (3.82-5.07)	0.123
	Total EtOH	0.17 (0.09-0.23)	0.445	3.33 (2.94-4.83)	0.448
V. fuscatum subsp. *fuscatum*	CH_2Cl_2	0.75 (0.66-0.84)	1.258	2.65 (2.15-3.64)	0.998
	MeOH:CH_2Cl_2 (1:1)	1.31 (1.19-1.44)	0.256	3.53 (2.97-4.49)	0.569
	MeOH	ND	-	ND	-
	Total EtOH	0.72 (0.59-0.91)	1.338	8.17 (7.61-9.46)	0.589
V. fuscatum subsp. *boissieri*	CH_2Cl_2	0.23 (0.18-0.28)	1.586	1.59 (1.18-2.48)	0.788
	MeOH:CH_2Cl_2 (1:1)	1.84 (1.73-1.95)	0.567	3.81 (3.42-4.47)	0.988
	MeOH	1.77 (1.59-1.93)	0.324	7.21 (5.79-7.89)	1.568
	Total EtOH	0.23 (0.16-0.29)	0.457	2.77 (1.78-3.12)	1.528
V. parviflorum	CH_2Cl_2	0.08 (0.03-0.11)	0.328	1.07 (0.76-2.01)	1.859
	MeOH:CH_2Cl_2 (1:1)	0.63 (0.52-0.67)	0.355	2.06 (1.64-2.31)	1.028
	MeOH	2.83 (2.48-3.26)	0.695	13.24 (12.87-15.07)	0.987
	Total EtOH	0.29 (0.23-0.33)	0.332	1.93 (1.49-3.06)	1.331

[a]Effective doses causing 50% (ED_{50}) growth inhibition of *S. littoralis* larvae relative to the control.
[b]The lethal dose (LD_{50}) causing 50% mortality of larvae compared with the control.
CI_{95}: The corresponding 95% confidence intervals were given in parenthesis. ND: Not determined

4. **Discussion**

In the present study phytochemical constituents, antifeedant, growth-inhibitory and toxic activities of extracts of increasing polarity obtained from aerial parts of five *Vincetoxicum* taxa were evaluated. Phytochemical screening tests showed that cardioactive glycosides, sugars, flavonoids and alkaloids were main secondary metabolites of all studied taxa. Additionally, both subspecies of *V. fuscatum* and *V. parviflorum* contained tannin. The presence of alkaloids were detected in all CH_2Cl_2, MeOH:CH_2Cl_2 (1:1) and MeOH extracts by TLC. The LC/MS/MS analysis results showed that alkaloid fractions contained three compounds. The MS/MS spectrums of the compounds were compared with literature data. The analysis revealed that compound 1, 2 and 3 were secoantofine, secoantofine *N*-oxide and antofine *N*-oxide, respectively. These results confirm previous research with *Vincetoxicum* species regarding the precence of phenanthroindolizidine alkaloids in *Vincetoxicum* taxa. Further isolation and structure elucidation studies of pure compounds are needed for the confirmation of the structures of these compounds.

Lavault *et al.* [11] isolated two isomers of antofine *N*-oxide from *V. hirundinaria*. Besides these alkaloids Staerk *et al.* [10 and 18] reported that antofine *N*-oxide, secoantofine *N*-oxide, antofine and secoantofine were phenanthroindolizidine alkaloids of *Cynanchum vincetoxicum* (L.) Pers. (syn. *Vincetoxicum hirundinaria*). Staerk *et al.* [12] also showed that *V. pumilum* contained antofine and antofine *N*-oxide. Furthermore, antofine was found in *V. nigrum* and *V. rossicum* in different studies by some authors [8, 13, 30].

Polyphagous pest *S. littoralis* and oligophagous pest *L. decemlineata* are leaf-eating pests that cause destructive damage on many economically important crops [7]. *S. littoralis* which distributed among Africa and Mediterranean Europae can feed on several plants [31] such as cotton, flax, groundnuts, rice, tea, tobacco and many vegetables belong to 40 families [7]. *L. decemlineata* known as "Colorado potato beetle" developed on Solanaceae family plants. During all development stages this species were characterized by high and impressive feeding rates, and higly fecundity [7] moreover it has the ability to develop field resistance to approximately every insecticide used against it and nowadays they developed resistance to over than 40 chemical insecticides [32]. So pest management became important problem of agriculture, public health and environment

[28]. Different methods such as using synthetic pesticides are prefered for pest management, but fast acting synthetic pesticides causes several ecological problems [33]. Recently, biological pesticides have been originated from plants and plant-derived products [34] such as extracts take attention, because they are safer, more friendly and more efficient alternatives to synthetic pesticides [35].

Antifeedant activity of plant extracts and plant products have been reported in various researches [7, 8, 28, 36]. Various secondary metabolites including alkaloids [7, 36], tannins [37], terpenes [38], essential oils [39] and phenolics [36] were reported as potent insect antifeedants.

Anti-insect activity of *V. rossicum* had been reported against *Allantus cinctus* (L.), *Drepana arcuata* Wlk. and *Ostrinia nubilalis* Hbn. by Mogg *et al*. [8]. Pavela [36] indicated larvacidal activity of methanol extract from *V. hirundinaria* against *Culex quinguefasciatus* Say larvae at maximal tested dose of 1.000 ppm. Pavela [7] also showed that the methanolic extract of *V. hirundinaria* had significant antifeedant activity against *S. littoralis* (99.2 ±1.5 feeding deterrence index) and *L. decemlineata* (72.1±6.5 feeding deterrence index) larvae at maximal tested dose of 500 μg/cm^2. Also he found that *V. hirundinaria* estimated the highest effectiveness with effective doses (ED_{50}) 11 μg/cm^2 and (ED_{90}) 99 μg/cm^2 against *S. littoralis* larvae. *Ge et al*. [40] reported that antofine *N*-oxide and antofine isolated from *Cynanchum mongolicum* were toxic compounds against *S.litura* but these compounds were less active than total alkaloids.

In this study, the antifeedant activity bioassay clearly indicated that five *Vincetoxicum* taxa were effective against both tested pest species and our results are agreement with above mentioned literature data. The phytochemical and antifeedant activity studies on these *Vincetoxicum* taxa were the first time. However, further isolation, structure elucidation and antifeedant activity studies on especially *V. parviflorum* and *V. canescens* subsp. *pedunculata* are needed to determine bioactive constituents that would be responsible from activity and to prepare standardized extracts or compounds as commercial bio-pesticide.

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