IAAOJ, Health Science, 2015, 3(2), 01-09

EVALUATION OF ANTIFUNGAL EFFECT OF DIFFERENT POLARITY EXTRACTS FROM FIVE *VINCETOXICUM* TAXA AGAINST *ASPERGILLUS FUMIGATUS*

Sevda GUZEL[1], Roman PAVELA[2], Gamze KOKDIL[1]

[1]Faculty of Pharmacy, Department of Pharmacognosy, Yenisehir Campus, Mersin University, 33169 Mersin, Turkey

[2]Crop Research Institute, Ruzyne 16106, Prague, Czech Republic

e-mail: sevdacanguzel@yahoo.com

ABSTRACT

Plants, plant extracts and natural compounds are important source of new drugs. *Vincetoxicum* species are traditionally used for the treatment of many disease in European and Chinese folk medicine. *V. canescens* subsp. *canescens* has been used for treatment of fungal infections in traditional medicine in east part of Turkey. Many biological activities including antibacterial, antifungal, antidiarrheal, antispasmodic, antileishmanial, antimalarial, cytotoxic, antifeedant and growth inhibition have been shown for several *Vincetoxicum* species (e.g *V.rossicum, V.stocksii, V.nigrum, V.hirundinaria*) in scientific reports.

In this study five *Vincetoxicum* taxa (*V. canescens* subsp. *canescens*, *V. canescens* subsp. *pedunculata, V. fuscatum* subsp. *fuscatum, V. fuscatum* subsp. *boissieri* and *V. parviflorum*) were collected from their natural habitats in different parts of Turkey and thirty different polarity extracts (dichloromethane, methanol:dichloromethane (1:1), methanol) and ten total ethanol extracts were prepared from their roots and aerial parts. These extracts were evaluated for their possible antifungal activity against widespread filamentous fungus *Aspergillus fumigatus* by using the agar dilution method.

In tested forty extracts, the highest antifungal effect was detected for dichloromethane extracts obtained from roots and aerial parts of *V. parviflorum* and roots of *V. canescens* subsp. *canescens* against *A. fumigatus (*45.86 % inhibition value). This is the first study on antifungal effect of *Vincetoxicum* species against *A. fumigatus*.

Keywords: Antifungal activity, *Aspergillus fumigatus*, Agar dilution method, *Vincetoxicum*

**The Ministry of Agriculture of the Czech Republic supported this work with Project No. RO0415.

BEŞ *VINCETOXICUM* TAXONUNUN FARKLI POLARİTEDEKİ EKSTRELERİNİN *ASPERGILLUS FUMIGATUS*'A KARŞI ANTİFUNGAL ETKİSİNİN DEĞERLENDİRİLMESİ

ÖZET

Bitkiler, bitki ekstreleri ve doğal bileşikler yeni ilaçların önemli kaynağıdır. *Vincetoxicum* türleri Avrupa ve Çin halk tıbbında bir çok hastalığın tedavisinde geleneksel olarak kullanılmaktadır. *V. canescens* subsp. *canescens* Türkiye'nin doğusunda geleneksel tıpda fungal enfeksiyonların tedavisinde kullanılmaktadır. Çeşitli *Vincetoxicum* türleri (örneğin *V.rossicum, V.stocksii, V.nigrum, V.hirundinaria*) için antibakteriyal, antifungal, antidiyareik, antispazmodik, antileishmaniyal, antimalaryal, sitotoksik, antifeedant ve büyüme inhibisyonu gibi bir çok biyolojik aktivite bilimsel çalışmalarda gösterilmektedir.

Bu çalışmada beş *Vincetoxicum* taksonu (*V. canescens* subsp. *canescens, V. canescens* subsp. *pedunculata, V. fuscatum* subsp. *fuscatum, V. fuscatum* subsp. *boissieri* and *V. parviflorum*) Türkiye'nin farklı bölgelerinde, doğal habitatlarından toplanmış ve kök ve toprak üstü kısımlarıdan farklı polaritede otuz ekstre (diklorometan, metanol:diklorometan (1:1), metanol) ve on total etanol ekstresi hazırlanmıştır. Bu ekstreler muhtemel antifungal aktiviteleri için yaygın filamentöz fungus *Aspergillus fumigatus*'a karşı agar dilüsyon yöntemi kullanılarak değerlendirilmiştir.

Test edilen 40 ekstre içerisinde, *A. fumigatus*'a karşı en yüksek antifungal etki *V. parviflorum*'un kök ve toprak üstü kısımlarından ve *V. canescens* subsp. *canescens*'in köklerinden elde edilen diklorometanlı ekstrelerde saptanmıştır (45.86 % inhibisyon değeri). Bu, *A. fumigatus*'a karşı *Vincetoxicum* türlerinin antifungal etkileri üzerine yapılmış ilk çalışmadır.

Anahtar kelimeler: Antifungal aktivite, *Aspergillus fumigatus*, Agar dilüsyon yöntemi, *Vincetoxicum*

**Bu çalışma XX. Bitkisel İlaç Hammaddeleri Toplantısı (BİHAT), Antalya, 10-13 Ekim 2012, P40 poster numarası ile sunulmuştur.

INTRODUCTION

Aspergillus species are ubiquitous saprophytes in nature[1-3] included approximately 182 species[4] and found in soil and on various dead organic matter[2]. One of the most common species *A. fumigatus*[2,5] is an environmentally widespread filamentous fungus[5,6] which is the reason of over than 90 % of human infections[7]. *A. fumigatus* especially infects immunocompromised patients[3,8] and intensive care units patients[9] by leading life-threatening infections such as aspergillosis[3,8]. Additionally, healthy individuals may under risk of asthma and allergic sinusitis because of this fungus[5].

Some of antifungal agents used in fungal infections have toxic effects with risk of developing resistance on human beings[6,10]. Therefore, it is needed to explore new and effective therapies for treatment of fungal infections[6,8]. It seems that one of the popular ways of exploring new antifungal drugs is using plant extracts or various types of natural compounds[10,11]. Previous studies indicated that approximately 40% of antifungal agents were naturally originated[10].

The genus *Vincetoxicum* N.M. Wolf, which is belonging to Apocynaceae, subfamily Asclepiadoideae[12,13], comprises nearly 100 species distributed throughout Asia, Japan, Europe[14,15] and North America[16,17]. Some species of the genus have been used in different traditional medicinal systems[12,18-20]. Poultice of *V. stocksii* Ali & Khatoon has been used for the treatment of wounds and injuries in Pakistan and also in the same area traditionally known as antileishmanial plant. Antileishmanial and antimalarial effects of *V. stocksii* were comfirmed in scientific reports[19]. *V. canescens* (Willd.) Decne. subsp. *canescens* is locally known as "Zilasur" and it has been used for the treatment of scabies and fungal infections in east part of Turkey[21]. Furthermore, crushed fruits and leaves of the *V. canescens* subsp. *canescens* was externally used for treatment of fungal infections in Ovacık (Tunceli)[22]. Antifungal and antibacterial activities of *V. rossicum* (Kleo.) Barb., *V. nigrum* (L.) Moench[23,24] and *V. stocksii*[19,25] have been reported in previous studies. *Vincetoxicum* is one of the largest genera of the subfamily Asclepiadoideae in Turkey which is represented by 10 taxa that three of them (*V. canescens* subsp. *pedunculata* Browicz, *V. fuscatum* subsp. *boissieri* (Kusn) Browicz and *V. parviflorum* Decne.) being endemic to Turkey[26,27]. The aim of the study was to investigate antifungal activities of four different polarity extracts from five *Vincetoxicum* taxa (*V. canescens* subsp. *canescens*, *V. canescens* subsp. *pedunculata*, *V. fuscatum* subsp. *fuscatum* (Hornem) Reichb., *V. fuscatum* subsp. *boissieri* and *V. parviflorum*) against *A. fumigatus*.

MATERIAL AND METHODS

Plant material

The plant materials were obtained from their natural habitats in different regions of Turkey during the summer of 2009. The samples identity was done by one of the authors (S. Güzel) and authenticated by Dr. Ahmet İlçim. (Department of Biology, Faculty of Arts and Science, Mustafa Kemal University, Antakya, Turkey). The dried voucher specimens were preserved in the herbarium (Herbarium of the Mustafa Kemal University-MKUH). The locality of plant materials were shown in Figure 1 and the voucher numbers were noted in paranthesis.

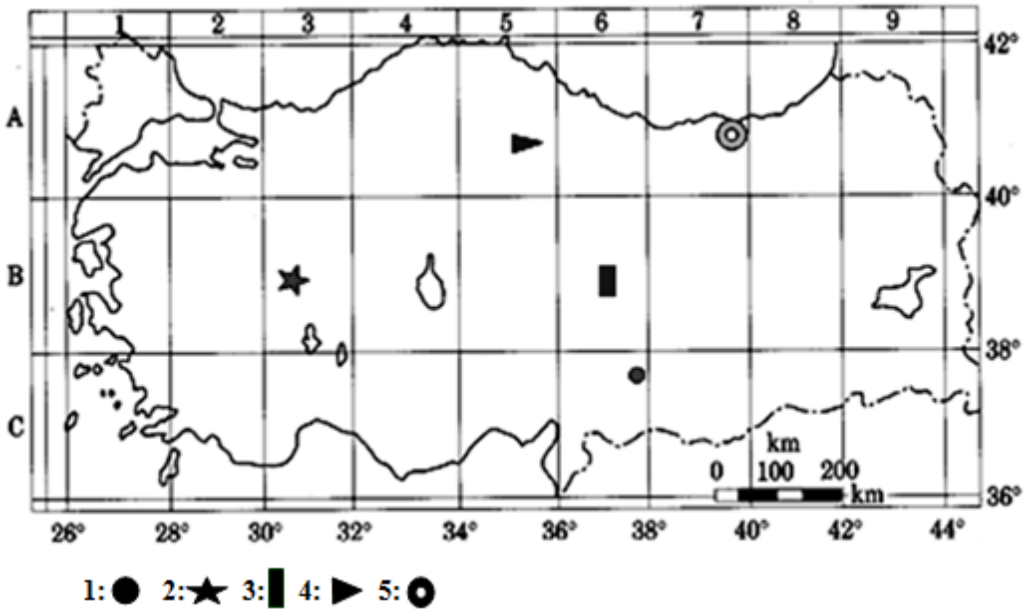

Figure 1: Locality of collected *Vincetoxicum* taxa.
(1:*V. canescens subsp. canescens* (MKUH 1283); 2: *V. canescens* subsp. *pedunculata* (MKUH 1284); 3: *V. fuscatum* subsp. *fuscatum* (MKUH 1315); 4: *V. fuscatum* subsp. *boissieri* (MKUH 1316); 5: *V. parviflorum* (MKUH 1334))

Chemicals and reagents

Analytical-reagent grade chemicals were used in the study. Dichloromethane was obtained from Sigma Chemical Company (USA) and ethanol and methanol were obtained from Merck (Germany). All the solutions were done by using distilled water.

Extraction procedure

The roots and aerial parts of the plants were air-dried at room temperature then mechanically powdered. 100 g of each plant material was sequentially extracted three times with 600 ml of CH_2Cl_2, MeOH: CH_2Cl_2 (1:1) and MeOH at room temperature to give crude extracts. For total ethanol extracts 100 g of plant powders were macerated twice with 720 ml of 96 % EtOH then sonicated for 30 min. and left a night to shake at room temperature. Whatman No:1 filter papers were used for separately filtration procedures of all suspensions and filtrates were collected to evaporate for removing solvents under reduce pressure by using vacuum evaporator (Heidolph-Rotar TLR 1000) at 35-40 °C. The all extracts were stored in the dark at 4 °C.

Antifungal Activity

A. fumigatus: The tested fungus were isolated from contaminated stored corn from pathogenic fungi collection of The Crop Research Institute, Prague, Czech Republic. Fungal strains were maintained on potato carrot agar (slant agar) at 4 °C. Subcultivations and following application of the strains were performed in the Bio Security Level 2 laboratory (BSL 2) by acting in accordance with the instruction of the BSL procedure of the tested fungi.

Inhibitory effects of the forty extracts obtained from different parts of studied *Vincetoxicum* taxa on growth of *A. fumigatus* at 1 mg/ml concentration were evaluated by using the agar dilution method[28]. The extracts were dissolved in appropriate solvent with an equal volume then each dissolved sample was completely diluated in potato dextrose agar (PDA) at 1 mg/mL concentration. 0.75 % (v/v) was the last concentration of the solvent in PDA. Before inoculation of the petri dishes with assay discs (0.4 cm), the dishes with 9.0 cm diam. were prepared and after inoculation they cut from the periphery of the target fungi with 7-day-old culture. These applications were performed at steril conditions. Also an equal volume of appropriate solvents with no extracts were used as a control. Incubation of all the control and the treated sets were done at 21°C in the dark for 7 days. The formula given below was used for measuring the percent inhibition of the *A. fumigatus*'s radial growth[28].

Percent inhibition = (DC − DT)/DC × 100

DC: Control sets' colony diam.

DT: Treated sets' colony diam (28).

Statistical analysis

Percentages were transformed to arcsine square root values for analysis of variance (ANOVA). Tukey's test ($P < 0.05$) was used to analyze for significant differences among the test extracts for inhibition efficiency. Means (±standard errors) of untransformed data are reported.

RESULTS

The aerial part and root extracts of five *Vincetoxicum* taxa were screened for antifungal activity against *A. fumigatus* at 1 mg/ml concentrations by agar dilution method. In all tested extracts, the CH_2Cl_2 extracts of both tested parts of *V. parviflorum* and root of *V. canescens* subsp. *canescens* were displayed the highest inhibitory effect with 45.86 % against the target fungus (Table 1-2). The CH_2Cl_2 and total EtOH extracts of roots of *V. fuscatum* subsp. *fuscatum*, CH_2Cl_2 extract of roots of *V. fuscatum* subsp. *boissieri* and MeOH:CH_2Cl_2 (1:1) extract of roots of *V. parviflorum* displayed inhibitory effect with 44.36 %, 41.35 %, 42.86 % and 39.85 %, respectively. Inhibitory effect of other fourteen root extracts were in the range of 36.84-12.03 % (Table 1).

The aerial parts of total EtOH and CH_2Cl_2 extracts of *V. canescens* subsp. *pedunculata* and total EtOH extract of *V. parviflorum* showed inhibitory effect with 41.35 %, 39.85 % and 39.10 %, respectively. Inhibitory effect of other sixteen extracts obtained from aerial parts were in the range of 36.84-3.01 %. (Table 2). Additionally, MeOH extracts of the roots and aerial parts were less active than the other tested extracts.

Table 1: Comparison of % inhibition values of root extracts of tested plants (1 mg/mL concentration).

Extracts	*V. canescens* subsp. *canescens*	*V. canescens* subsp. *pedunculata* *	*V. fuscatum* subsp. *fuscatum*	*V. fuscatum* subsp. *boissieri**	*V. parviflorum**
CH_2Cl_2	45.86±0.5a	32.33±0.6a	44.36±0.8a	42.86±0.7a	45.86±0.3a
MeOH:CH_2Cl_2 (1:1)	36.84±1.8ab	25.56±0.3b	33.83±0.4b	27.82±0.8c	39.85±0.7b
MeOH	16.54±0.9c	22.56±0.5b	12.03±0.3c	14.29±0.3d	13.53±0.2d
Total EtOH	32.33±0.8b	34.32±0.7a	41.35±0.6a	33.83±0.3b	23.31±0.5c
ANOVA *F, P***	84.22; 0.0001	42.62; 0.035	54.12; 0.0001	125.35; 0.0001	81.32; 0.0001

* Endemic taxon.
Mean growth inhibition (± S.E) within a column follower by the same letter do not differ significantly according to the least significant difference (Turkey's HSD test, P<0.05). Numbers represent the average efficiency (in %) of the extract compared with the control. **ANOVA parameters - F-value, P-significantly level

Table 2: Comparison of % inhibition values of aerial part extracts of tested plants (1 mg/mL concentration).

Extracts	*V. canescens* subsp. *canescens*	*V. canescens* subsp. *pedunculata**	*V. fuscatum* subsp. *fuscatum*	*V. fuscatum* subsp. *boissieri**	*V. parviflorum**
CH_2Cl_2	32.33±0.3a	39.85±0.8a	27.82±2.1ab	36.84±1.3a	45.86±2.3a
MeOH:CH_2Cl_2 (1:1)	18.88±2.5b	25.56±0.6b	34.59±0.5a	23.31±0.5b	27.82±0.8b
MeOH	3.01±0.6c	16.54±0.5c	7.52±0.5c	6.77±0.8c	6.02±0.7c
Total EtOH	26.56±1.7ab	41.35±1.5a	25.56±1.5b	28.57±2.5ab	39.10±3.5a
ANOVA *F, P***	79.55; 0.0001	98.52; 0.0001	95.72; 0.0001	67.88; 0.0001	69.52; 0.0001

* Endemic taxon.
Mean growth inhibition (± S.E) within a column follower by the same letter do not differ significantly according to the least significant difference (Turkey's HSD test, P<0.05). Numbers represent the average efficiency (in %) of the extract compared with the control. **ANOVA parameters - F-value, P-significantly level

DISCUSSION

Five *Vincetoxicum* taxa were screened for their antifungal activity against *A. fumigatus* at 1 mg/ml concentrations. In all tested extracts, the CH_2Cl_2 extracts of roots and aerial parts of *V. parviflorum* and root of *V. canescens* subsp. *canescens* were showed the highest inhibitory effect against the target fungus (45.86 % inhibition value) (Table 1-2).

Antimicrobial activity of *Vincetoxicum* species showed in a few study[23-25]. Zaidi and Crow (2005) reported that five fractions (hexane, ethyl acetate, chloroform, butanol and water) of methanol extract of *V. stocksii* had been investigated for antimicrobial activity against 12 fungal strains including *A. flavus* and *A. niger* and 12 bacterial strains. They found that *V. stocksii* exhibited significantly effectiveness against *C. albicans*, *B. subtilis* and *B. cereus*[25]. Mogg et al. (2008) demonstrated that ethanolic extracts of roots, leaves and fruits of *V. rossicum* had antifungal and antibacterial activities against some filamentous and yeast-like fungi and gram (-) and gram (+) bacteria[23].

Literature data indicated that the all parts of *Vincetoxicum* species contain triterpenoids[29,30], alkaloids[31,32] and steroids[29,30,33]; aerial parts contain alkanols[30] and phenolics[25,34,35] and roots contain volatile compounds, acetophenone[29], saponins[27] and sugars[33]. Previous studies showed that plant extracts including essential oils, alkaloids, terpenes and flavonoids had antimicrobial activities[11].

Medicinal plants used traditionally in several medicinal systems are important source of plant extracts, natural compounds and their derivatives. Their natural abundance, with high availability, broad range effectiviness and being less expensive than other options are another properties of plants and plant derived products to why evaluating to find safer, cheaper and less toxic novel fungal agents for treatment of fungal infectious[10]. Literature data indicated that different part of *Vincetoxicum* species had antifungal activity against some fungus[23,25], while there is no study on *A. fumigatus*. According to the literature this is the first study that investigation on antifungal effect of *Vincetoxicum* species against *A. fumigatus*. Our results showed that effect of tested plants were not enough to design further studies. Therefore, in future *Vincetoxicum* species studied in this research will be tested against various fungus for their antifungal activities.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge to the Dr. Ahmet Ilcim (Department of Biology, Faculty of Arts and Science, Mustafa Kemal University, Antakya, Turkey) for authentication of the plant samples. The Ministry of Agriculture of the Czech Republic supported this work with Project No. RO0415.

REFERENCES

1. Walker CA, Fitzharris P, Longbottom JL, Newman Taylor AJ. Lymphocyte sensitization to *Aspergillus fumigatus* in allergic bronchopulmonary aspergillosis. Clin Exp Immunol 1989;76:34-40.
2. Mahmoud YAG, Al-Ghamdi AY, Abd El-Zaher EHF. A protective mechanism in lungs of rats experimentally infected with *Aspergillus fumigatus*. Mycobiology 2011;39(1):40-44.
3. Kuboi S, Ishimaru T, Tamada S, Bernard EM, Perlin DS, Armstrong D. Molecular characterization of AfuFleA, an L-fucose-specific lectin from *Aspergillus fumigatus*. J Infect Chemother 2013;19:1021-1028.
4. Samarasinghe AE, Hoselton SA, Schuh JM. A comparison between intratracheal and inhalation delivery of *Aspergillus fumigatus* conidia in the development of fungal allergic asthma in C57BL/6 mice. Fungal Biol 2011;115:21-29.
5. Kim JR, Michielsen S. Photodynamic antifungal activities of nanostructured fabrics grafted with rose bengal and phloxine B against *Aspergillus fumigatus*. J Appl Polym Sci 2015; DOI:10.1002/APP.42114.
6. Lei G, Dan H, Jinhua L, Wei Y, So G, Li W. Berberine and Itraconazole are not synergistic *in Vitro* against *Aspergillus fumigatus* isolated from clinical patients. Molecules 2011;16:9218-9233.
7. Perkhofer S, Zenzmaier C, Frealle E, Blatzer M, Hackl H, Sartori B, et. al. Differential gene expression in *Aspergillus fumigatus* induced by human platelets in vitro. Int J Med Microbiol 2015;305:327-338.
8. Kumari S, Jain P, Sharma B, Kadyan P, Dabur R. In vitro antifungal activity and probable fungicidal mechanism of aqueous extract of *Barleria grandiflora*. Appl Biochem Biotechnol 2015;175:3571-3584.
9. Taccone FS, Abeele AV, Bulpa P, Misset B, Meersseman W, Cardoso T, et. al. Epidemiology of invasive aspergillosis in critically ill patients: clinical presentation, underlying conditions, and outcomes. Critical Care 2015;19:7. DOI 10.1186/s13054-014-0722-7.
10. Ayoub IM, El-Shazly M, Lu MC, Singab ANB. Antimicrobial and cytotoxic activities of the crude extracts of Dietes bicolor leaves, flowers and rhizomes. S Afr *J* Bot 2014;95:97-101.
11. Boligon AA, Piana M, Kubiça TF, Mario DN, Dalmolin TV, Bonez PC, et. al. HPLC analysis and antimicrobial, antimycobacterial and antiviral activities of *Tabernaemontana catharinensis* A. DC. J Appl Biomed 2015;13:7-18.
12. DiTommaso A, Lawlor FM, Darbyshire SJ. The biology of invasive alien plants in Canada 2. *Cynanchum rossicum* (Kleopow) Borhidi [=*Vincetoxicum rossicum* (Kleopow) Barbar.] and *Cynanchum louseae* (L.) Kartesz&Gandhi [= *Vincetoxicum nigrum* (L.) Moench]. Can J Plant Sci 2004;85:243-263.
13. Heywood VH, Brummitt RK, Culham A, Seberg O. Flowering plant families of the world, Firefly Books, 2007:38-40.
14. Liede S. *Cynanchum-Rhodostegiella-Vincetoxicum-Tylophora* (Asclepiadaceae): new considerations on an old problem. Taxon 1996;45:193-211.
15. Yamashiro T, Yamashiro A, Yokoyama J, Maki M. Morphological aspects and phylogenetic analyses of pollination systems in the *Tylophora–Vincetoxicum* complex (Apocynaceae-Asclepiadoideae) in Japan. Biol J Linn Soc 2008;93:325-341.
16. Sheeley SE, Raynal DJ. The distribution and status of species of *Vincetoxicum* in eastern North America. Bull Torrey Bot Club 1996;123(2):148-156.
17. Leimu R. Variation in the mating system of *Vincetoxicum hirundinaria* (Asclepiadaceae) in peripherial island populations. Ann of Bot 2004;93:107-113.
18. Weston LA, Barney JN, DiTommaso A. A review of the biology and ecology of three invasive perennials in New York State: Japanese knotweed (*Polygonum cuspidatum*), mugwort (*Artemisia vulgaris*) and pale swallow-wort (*Vincetoxicum rossicum*). Plant Soil 2005;277:53-69.

19. Mansoor A, Ibrahim MA, Zaidi MA, Ahmed M. Antiprotozoal activities of *Vincetoxicum stocksii* and *Carum copticum*. Bangl J Pharmacol 2011;6:51-54.

20. Sliumpaite I, Murkovic M, Zeb A, Venskutonis PR. Antioxidant properties and phenolic composition of swallow-wort (*Vincetoxicum lutea* L.) leaves. Ind Crop Prod 2013;45:74-82.

21. Altundag E, Ozturk M. Ethnomedicinal studies on the plant resources of east Anatolia, Turkey. Procedia Soc Behav Sci 2011;19:756-777.

22. Tuzlacı E, Dogan A. Turkish folk medicinal plants, IX: Ovacık (Tunceli). Marmara Pharmaceutical Journal 2010;14:136-143.

23. Mogg C, Petit P, Cappuccino N, Durst T, McKague C, Foster M, et. al. Test of the antibiotic properties of the invasive vine *Vincetoxicum rossicum* against bacteria, fungi and insects. Biochem Syst and Ecol 2008;36:383-391.

24. Gibson DM, Krasnoff SB, Biazzo J, Milbrath L. Phytotoxicity of antofine from invasive swallow-worts. J Chem Ecol 2011;37:871-879.

25. Zaidi MA, Crow JSA. Biologically active traditional medicinal herbs from Balochistan, Pakistan. J Ethnopharmacol 2005;96:331-334.

26. Browicz K. *Vincetoxicum* N.M. Wolf. In: Davis P.H., ed., Flora of Turkey and the East Aegean Islands, Edinburgh 1978: 6:163-173.

27. Tanker N, Koyuncu M, Coskun M. Farmasotik Botanik, Ankara Universitesi Eczacılık Fakültesi, Ankara: 2004:294.

28. Zabka M, Pavela R, Gabrielova-Slezakova L. Promising antifungal effect of some Euro-Asiatic plants against dangerous pathogenic and toxinogenic fungi. J Sci Food Agric 2011;91: 492-497.

29. Lavault M, Richomme P, Bruneton J. Acetophenones and new pregnane glycosides from the roots of *Vincetoxicum hirundinaria*. Fitoterapia 1999;70:216-220.

30. Nowak R, Kisiel W. Hancokinol from *Vincetoxicum officinale*. Fitoterapia 2000;71: 584-586.

31. Staerk D, Lykkeberg AL, Christensen J, Budnik BA, Abe F, Jaroszewski JW. In vitro cytotoxic activity of phenanthroindolizidine alkaloids from *Cynanchum vincetoxicum* and *Tylophora tanakae* against drug-sensitive and multidrug-resistant cancer cells. J Nat Prod 2002;65:1299-1302.

32. Stærk D, Nezhad KB, Asili J, Emami SA, Ahi A, Sairafianpour M, et. al. Phenanthroindolizidine alkaloids from *Vincetoxicum pumilum*. Biochem Syst Ecol 2005;33:957-960.

33. Stöckel K, Stöcklin W, Reichstein T. Die glykoside von *Vincetoxicum hirundinaria* MEDIKUS. 1. Mitteilung: isolierungen und spaltprodukte. Glykoside und aglykone, 316. Mitteilung. Helv Chim Acta 1969;52(5):1175-1202.

34. Pavela R. Antifeedant activity of plant extracts on *Leptinotarsa decemlineata* Say. and *Spodoptera littoralis* Bois. larvae. Ind Crop Prod 2010;32:213-219.

35. Shah AJ, Zaidi MA, Sajjad H, Gilani HAH. Antidiarrheal and antispasmodic activities of *Vincetoxicum stocksii* are mediated through calcium channel blockade. Bangl J Pharmacol 2011;6:46-50.