Research Article

Hacettepe University Journal of the Faculty of Pharmacy
Volume 43 / Number 2 / June 2023 / pp. 142-149

Investigation on Some Biological Activities of Different Parts of Vincetoxicum hirundinaria Medik

Zehra ÖKSÜZ[1*]
ORCID: 0000-0002-1542-0556
Sevda GÜZEL KARA[2]
ORCID: 0000-0002-6642-5824

[1]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Mersin University, Mersin, Turkey

[2]Department of Pharmacognosy, Faculty of Pharmacy, Mersin University, Mersin, Turkey

Corresponding author:
Zehra ÖKSÜZ
Adress: Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Mersin University, Mersin, Turkey
E-mail: zehraoksz@gmail.com; zehraoksuz@mersin.edu.tr
Tel.: 03243412815/12160

Received date :10.06.2022
Accepted date : 24.08.2022

DOI: 10.52794/hujpharm.1128462

ABSTRACT

In this study, it was aimed to determine the antimicrobial, antibiofilm activities and synergistic effects of ethanol extracts obtained from aerial part, root, seed and seed coat of *Vincetoxicum hirundinaria* Medik. Antimicrobial activity was performed against 7 bacterial and 3 fungal reference strains by microdilution method and minimum inhibition concentrations (MIC) were determined. The crystal violet method was applied to determine the prevention of biofilm formation and inhibition of preformed biofilm activities on *Pseudomonas aeruginosa* biofilm. In addition, the synergistic effects of aerial part and seed extracts against both *Escherichia coli* and *Enterococcus faecalis* were examined by the microdilution checkerboard method. According to the antimicrobial results extracts had moderate to low efficacy against bacterial and yeast strains. The synergy test showed that the aerial part and seed extracts had additive effect against both *E.coli* and *E.faecalis*. The extracts also showed the potential to inhibit biofilm formation and preformed biofilms. Especially root and seed pod extracts showed strong antibiofilm activity. In conclusion, the literature search indicated that the antimicrobial and antibiofilm activities of *V. hirundinaria* was evaluated for the first time in the current study, therefore; our findings provide important preliminary data to the literature in terms of antibiofilm activity of *V. hirundinaria*.

Keywords: Antimicrobial, Antibiofilm, Synergy Test, *Vincetoxicum hirundinaria*

1. Introduction

Microorganisms can be life-threatening by causing infections all over the world which are difficult to treat. In addition, many microorganisms form biofilms in order to survive and increase their virulence in stressful environments such as nutrient-limited and unsuitable temperature conditions [1]. Biofilms that play an important role in the persistence of bacterial infections are organized bacterial communities embedded in a surface-bound extracellular polymeric matrix [2]. Extracellular matrix (ECM), a complex mixture of exopolysaccharides, nucleic acids, proteins, and other compounds, makes up 90% of the biofilm biomass [3,4].

It is known that biofilms typically play a greater role in chronic infections than planktonic bacteria and that 65-80% of all infections are associated with biofilm formation [5,6]. Many microorganisms may contribute to the etiology of infections by forming biofilm. Particularly in recent years, interest has been increased on the biofilm formed by a group of bacteria called ESKAPE (*Enterococcus faecalis, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* spp.), which is associated with high mortality [7]. It is known that microbial biofilms play an important role in the pathogenesis of a number of infectious diseases associated with these microorganisms, including infection of permanent medical devices such as prostheses, catheters, tracheal tubes, and heart valves, endocarditis, otitis media, wound infections and lung infections of cystic fibrosis patients [7,8]. In addition, it has been shown that biofilm-producing bacteria are better able to evade the host's immune response and are up to 1000-fold less sensitive to antibiotics compared to planktonic cells [1,4]. Mechanisms responsible for decreased antimicrobial susceptibility of biofilm include; the decrease in antibiotic penetration, different growth rates, and nutrient gradients within the biofilm, persister phenomenon, induction of resistance mechanisms, mutational resistance, gene transfer [5].

Increasing antimicrobial resistance is an important public health problem as it causes serious morbidity and mortality all over the world. Due to the lack of approved therapeutics in the treatment of biofilm-associated infections, which is one of the most important causes of antimicrobial resistance, the potential of the developed antimicrobials to prevent biofilm formation as well as to eliminate the causative microorganism is of great importance. In recent years, it has been proven that natural products obtained from plants are effective in the treatment of various diseases and inhibit ECM formation by suppressing cell adhesion/attachment during the biofilm formation process, and have potential as anti-biofilm agents [9,10].

The genus *Vincetoxicum* N.M. Wolf (Apocynaceae: subfamily Asclepiadoideae) [11] is represented by approximately 100 species which are distributed from Europe and the Mediterranean, to eastern Asia [12]. Some species of the genus have been traditionally used to treat neurosis, malaria, scrofula, scabies, internal fever, [13], external cancers, injuries, and wounds [14]. *Vincetoxicum* is one of the largest genera of the subfamily in Anatolia and is represented by 10 taxa [15]. *V. hirundinaria* Medik., commonly known as white swallow-wort, is a long-lived herbaceous perennial plant [16]. The presence of flavonoids, lipophilic compounds, chlorogenic acid, catechin derivatives, phenanthroindolizidine alkaloids, and antofine in leaves [16] and acetophenones, pregnane glycosides, and sterols in roots [17] of the plant were determined previously. The plant has been traditionally used in European medicinal system as expectorant [18,19], diuretic, emetic [11,17,18], antitumoral, laxative, and diaphoretic agents [11]. In the field of veterinary medicine, *V. hirundinaria* roots have been used for the treatment of dropsy and some other illnesses [11]. In France the plant is known as "dompte-venin" and traditionally used as an emetic as well as an expectorant [19], in Italy infusion and decoction of the roots and whole plant are used as antidote for poisons [20], and in Turkey, the plant is known as "Kırlangıçkuyruğu or/and Panzehir otu" and emetic properties of the roots were reported [21]. *V. hirundinaria* also included into homeopathic preparation Engystol [16]. A review of the literature data revealed no studies on antimicrobial and antibiofilm activities of different parts of *V. hirundinaria*, therefore; the aim of this study was to evaluate potential antimicrobial, antibiofilm activities and synergistic effects of ethanol extracts obtained from aerial parts, roots, seeds, and seed pods of *V. hirundinaria* growing wild in Turkey.

ISSN: 2458 - 8806

2. Material and Methods

2.1 Plant Material

V. hirundinaria was collected from Kızılgecit, Mersin, Turkey and identified/confirmed by Dr. S. Güzel Kara (Department of Pharmacognosy, Faculty of Pharmacy, Mersin University, Mersin, Turkey) and Dr. A. Kahraman (Department of Biology, Faculty of Arts and Science, Uşak University, Uşak, Turkey). The dried voucher specimens were deposited in the Plant Systematics and Phylogenetics Research Laboratory, Uşak University (A. Kahraman 2557).

2.2 Preparation of Plant Extract

The air-dried and powdered aerial parts, roots, seed pods, and seeds of the plant were macerated three times with ethanol [20 mL of EtOH per 1 g of sample, 96 %] at room temperature and filtered using filter paper (Whatman Grade No.1). Then, the solvent was evaporated using a vacuum evaporator (Heidolph Instruments GmbH & CO. KG, Germany) and obtained extracts were stored in the dark at 4°C until further use [22].

2.3 Biological Studies

2.3.1 Antimicrobial Activity Assay

Antimicrobial susceptibility testing was performed with minor modifications in the standard microdilution method as previously reported [23,24]. In this study 7 reference bacterial strains (*Escherichia coli* ATCC 35150, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, *Klebsiella pneumoniae* ATCC 100031, *Acinetobacter baumannii* ATCC 02026, *Bacillus subtilis* ATCC 6633), and 3 reference yeast strains (*Candida albicans* ATCC 90028, *Candida glabrata* ATCC 15126 and, *Candida parapsilosis* ATCC 90018) were included. Microorganism suspension concentrations; for yeasts on sabouraud dextrose agar (Merck, Germany) for 24 hours at 28°C and for bacteria on Mueller-Hinton agar (Merck, Germany) for 24 hours at 37°C, from stock cultures grown, were adjusted McFarland 0.5 ($5x10^5$ CFU/mL). Stock solutions of the extracts were prepared in dimethyl sulfoxide (DMSO) at 1000 µg/mL. For the microdilution test, 100 µL of medium [Sabouraud dextrose broth (Merck, Germany) for yeasts, Mueller-Hinton broth (MHB) (Merck, Germany) for bacteria] was dispensed into each well of the 96-well microplate. 100 µL of the stock solution of the extracts was added to the first wells and two-fold dilutions were made from the first well. Then, 5 µL of bacteria or yeast suspension was added to each well. Microorganism suspension was not added to some wells to create the medium control well, while only 5 µL of yeast or bacterial suspension was added to some wells without the tested extracts for microbial growth control. The minimum inhibitory concentration (MIC) was determined visually and using a microplate reader (BioTek Inc., USA) at a wavelength of 630 nm. MIC values were evaluated as the lowest concentration at which the tested extracts inhibited the growth of the microorganism. As a reference drug; Ampicillin (Sigma, USA) for bacteria and fluconazole (Sigma, USA) for yeasts were used. It was tested that DMSO had no effect on the growth of microorganisms included in the study and all experiments were repeated 2 times.

2.3.2 Determining of Biofilm Formation

Biofilm formation of strains was determined by modifying the crystal violet (CV) staining method [25]. Biofilm tests were performed on the biofilm of *P. aeruginosa.* Briefly, 100 µL of MHB was transferred to 96-well microplates and 10 µL of microorganism cell suspensions ($5x10^5$ cells) were added. Microplates were incubated at 37°C for 24 hours. After incubation, cell suspensions were gently aspirated and rinsed three times with sterile phosphate-buffered saline (PBS). Subsequently, the biofilms formed were fixed with 150 µL of methanol for 15 minutes. At the end of the time, the methanol in the wells was aspirated and the microplates were air-dried. Afterward, 150 µL of 0.5% CV solution was added to the microplate wells and incubated at 25°C for 15 minutes. The CV solution in the wells was aspirated, then washed with PBS, and the microplate wells were air-dried. In the last step, 150 µL of 95% ethanol was added to the microplate wells and maintained for 15 minutes. The biofilm formation was determined by measuring the absorbance at optical density (OD) at 550 nm using a microtiter plate spectrophotometer (BioTek Inc., USA). The OD values of the wells without microorganism inoculum were used as the negative control. All tests were performed in duplicate.

2.3.3 Biofilm-Prevention Assay

Biofilm-Prevention assay of the extracts were performed with minor modifications of the CV staining assay [26]. Briefly, serial dilutions were performed

at concentrations of sub-MICs of extracts (0.5X and 0.25X MIC) in microplate wells. A suspension of *P. aeruginosa* adjusted to McFarland 0.5 ($5x10^5$ CFU/mL) was then seeded into 96-well microplates and incubated at 37°C for 24 hours. Then, the potential of the tested extracts to inhibit the formation of *P. aeruginosa* biofilm was determined by following the CV staining assay steps mentioned in the "Determining of Biofilm Formation" section. PBS was used as the negative control. The effects of extracts on biofilm formation were evaluated by measuring the OD of the wells at 550 nm using a microplate reader (BioTek Inc., USA). The lowest extracts concentration at which biofilm formation was inhibited by at least 50% was defined as the minimum biofilm inhibition concentration ($MBIC_{50}$).

2.3.4 Biofilm-Eradication Assay

The effect of the extracts on the preformed biofilm was performed by minor modifying the CV staining assay [26]. Briefly, 100 µL of MHB medium and 5 µL of *P. aeruginosa* suspension adjusted to McFarland 0.5 ($5x10^5$ CFU/mL) were seeded into each well of 96-well plates and incubated at 37°C for 24 hours. After incubation, the supernatants were gently aspirated and 100 µL of each extract diluted to 0.5X, 1X and 2X MIC concentrations was added to each well. The plates were then incubated again at 37°C for 24 hours. The potential of the extracts to inhibit preformed biofilm by *P. aeruginosa* was then determined by following the CV staining test steps mentioned in the "Determination of Biofilm Formation" section [25]. PBS was used as negative control. The preformed biofilm effects of the extracts were evaluated by measuring the OD of the wells at 550 nm using a microplate reader (BioTek Inc., USA). The lowest concentration of the extracts required to destroy at least 50% of the preformed biofilm was defined as the minimum biofilm reduction concentration ($MBRC_{50}$).

2.3.5 Microdilution Checkerboard Method

The Checkerboard broth microdilution test method was used to evaluate the synergy between aerial part and seed extracts against both *E. coli* and *E. faecalis* [27]. The checkerboard synergy test was performed in duplicate with 96-well microplates. Extract-free wells were prepared as positive controls. Serial dilutions of ethanol extract obtained from aerial parts (250-1.95 µg/mL) were dispensed from left to right into the first eight wells of the microplate and serial dilutions (250-1.95 µg/mL) of ethanol extract obtained from seeds were dispensed from top to bottom into the first eight wells of another microplate. The concentration range of the compounds used was determined according to the MIC values (Table 1). The contents of the two microplates were combined in another microplate. Bacterial inoculum prepared at a density of 5×10^5 CFU/mL was added to each well and the microplates were incubated at 37ºC for 24 hours. Evaluation of the checkerboard test was made according to the fractional inhibitory concentration (FIC) index. FIC index were interpreted as follows: FIC $\leq$0.5, synergy; FIC>0.5 and <1, additive; FIC >1 and $\leq$4, indifference; and $\geq$4, antagonism [27,28].

FIC index was calculated according to the equation given below;

A/MICA + B/MICB = FICA + FICB = FIC index, where A and B are the MICs of drug A and drug B in the combination, MICA and MICB are the MICs of drug A and drug B alone, and FICA and FICB are the FICs of drug A and drug B.

Table 1. MIC values (µg/mL) of the tested extracts, and reference drugs against microbial strains.

Used part/ Reference antimicrobials	*K. pneumoniae* ATCC 100031	*B. subtilis ATCC 6633*	*P. aeruginosa* ATCC 27853	*S. aureus* ATCC 29213	*E. coli* ATCC 35150	*E. f*aecalis ATCC 29212	*A. baumannii* ATCC 02026	*C. albicans* ATCC 90028	*C. glabrata* ATCC 15126	*C. parapsilosis* ATCC 90018
Aerial part	62,5	125	62,5	125	62,5	62,5	125	125	62,5	62,5
Seed	125	125	125	250	62,5	62,5	125	125	62,5	62,5
Seed pod	125	125	125	250	125	125	125	125	62,5	62,5
Root	125	125	125	250	125	125	125	125	62,5	62,5
Ampicillin	*	*	31,25	*	3,90	*	31,25	-	-	-
Fluconazole	-	-	-	-	-	-	-	*	8	*

- Not tested; * Effective at all tested concentrations

ISSN: 2458 - 8806

3. Results and Discussion

In the current study, antibacterial, antifungal, and antibiofilm activities of ethanol extracts obtained from different parts (aerial part, root, seed, and seed pod) of *V. hirundinaria* were investigated. The yields of aerial part, root, seed pod, and seed extracts were 26.1%, 20.3%, 28.5%, and 18.9% (w/w), respectively.

The antimicrobial activities of the extracts against bacteria and yeast strains included in the study are shown in Table 1. Compared with reference antibacterial agents (ampicillin and fluconazole), the extracts showed moderate to low antimicrobial activity (MIC range: 250-62,5 µg/mL) against both bacteria and yeasts. When evaluated in terms of antibacterial and antifungal activities, there was no big difference between the extracts, but the antimicrobial activity of the aerial part extract was found to be higher than the other tested extracts, especially on *K. pneumoniae* and *P. aeruginosa*. When evaluated in terms of yeast strains, the antifungal activity of all extracts included in the study against *C. albicans* was lower than that of *C. glabrata* and *C. parapsilosis* (Table 1). Antimicrobial activities of ethanol extracts of five *Vinxetoxicum* taxa growing Turkey were investigated against *S. aureus, B. subtilis, E. coli, A. baumannii, A. hydrophila, M. tuberculosis, C. glabrata, C. parapsilosis*, and *C. tropicalis* in our previous studies [29,30] and tested extracts showed different antimicrobial activities (MIC range: 31.25-250 µg/mL). However, there is no antimicrobial study on *V. hirundinaria* growing in Turkey. The results of the study are consistent with our previous studies.

Biofilm prevention test determined that tested extracts obtained from aerial parts, seeds, seed pods, and roots inhibited biofilm formation by approximately 34%, 41%, 47%, 48%, and 30%, 34%, 44%, 43% at 0.5X and 0.25X MIC, respectively (Figure 1). None of the extracts included in the study were able to prevent biofilm formation by 50% at the concentrations tested. Therefore, although the extracts have no $MBIC_{50}$ value, seed pod and rood extracts (47%, 48%) at 0.5X MIC concentration prevented biofilm formation with a value close to 50%. In addition, using the biofilm eradication test, it was determined that tested extracts obtained from aerial parts, seeds, seed pods, and roots inhibited biofilm formation by approximately 27%, 40%, 47%, and 47%, respectively, at 0.5X MIC. This rate was approximately 33%, 42%, 48%, 51%, and 37%, 50%, 50%, 54% in 1X and 2X MIC, respectively (Figure 2). According to these data, the $MBRC_{50}$ value of seed and seed pod extracts was 250 µg/mL, but the seed pod extract was able to eradicate biofilm with a value close to 50% at 1X MIC (µg/mL). The $MBRC_{50}$ value of the root extract is 125 µg/mL. In general, when we compared the all tested extracts included in the study in terms of preventing biofilm formation, it was determined that seed pod and root extracts were more effective than the others, although there was not much difference. In addition, when we compared their activities on the formed bio-

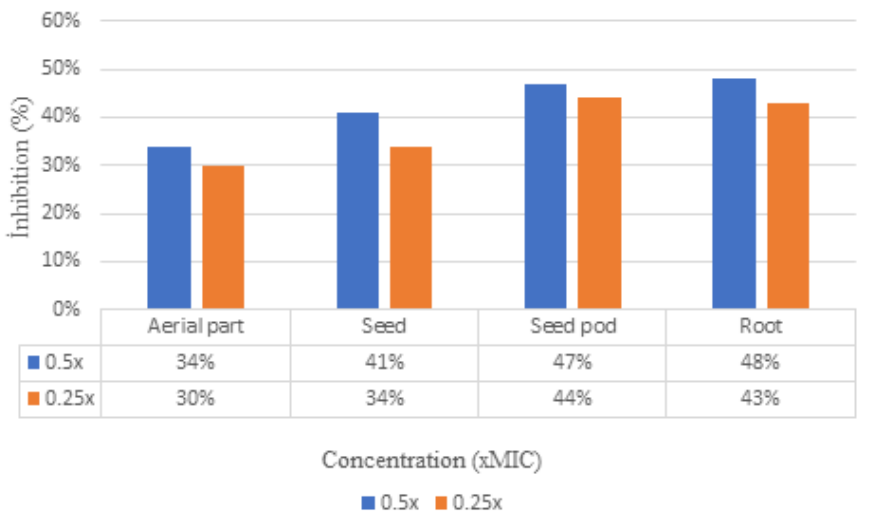

Figure 1. Prevention of biofilm formation of V. hirundinaria. Inhibition (%) of biofilm formation by extracts at 0.5X and 0.25X concentrations.

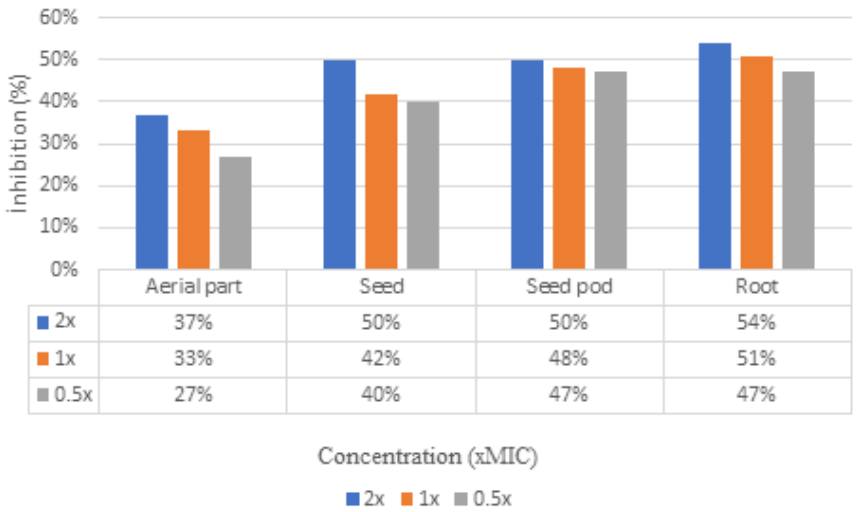

Figure 2. Eradication of biofilm formation of V. hirundinaria. Preformed biofilm inhibition (%) of extracts at 0.5X, 1X, and 2X concentrations.

film, it was found that root extract was more effective at lower concentrations (MIC) compared to the other extracts. When the extracts included in the study were compared, both in preventing biofilm formation and in removing the formed biofilm, seed pod and root extract showed the best efficiency, while aerial part extract showed the lowest efficiency.

Aerial part and seed extracts showed relatively better antimicrobial activity against *E. coli* and *E. faecalis* than the other extracts included in the study (Table 1). Therefore, the synergistic effects of these two extracts against both *E. coli* and *E. faecalis* were investigated. As a result of checkerboard experiments, it was determined that aerial part and seed extracts, whose synergistic effects were calculated according to the value of the FIC index, showed additive effects against both *E. coli* (FIC=0.74) and *E. faecalis* (FIC=1). This indicates that the antimicrobial effects of extracts obtained from aerial parts and seeds of *V. hirundinaria* on *E. coli* and *E. faecalis* are not different when used separately than when used together.

4. Conclusions

Different *Vincetoxicum* taxa (*V. parviflorum* Decne., *V. fuscatum* (Hornem.) Reichb. subsp. *boissieri* (Kusn) Browicz, *V. fuscatum* subsp. *fuscatum* (Hornem.) Reichb., *V. canescens* (Willd.) Decne. subsp. *pedunculata* Browicz, and *V. canescens* (Willd.) Decne. subsp. *canescens*) growing Turkey were studied for their antifungal [31], antifeedant [32,33], antimicrobial [29,30], and antiproliferative [30] activities previously while there is no study on *V. hirundinaria* growing wild Turkey. Therefore; this is the first study on antimicrobial and antibiofilm activities of aerial part, root, seed, and seed pod of *V. hirundinaria*. Moreover, this is the first study on antibiofilm activity of *Vincetoxicum* species. Our data show that *V. hirundinaria* extracts have moderate to low antimicrobial activity in the strains tested. In addition, these extracts have the potential to both inhibit biofilm formation and inhibit preformed biofilm. Investigation of the antibiofilm potential of *V. hirundinaria*, which grows easily in various conditions in Turkey and is not an expensive raw material, will contribute to the fields of health and economy in the treatment of biofilm-induced difficult infections. In conclusion, although our findings show the antibiofilm activity of *V. hirundinaria*, there is still a need to support this data with detailed further studies on different microorganisms, animal studies, action mechanisms, comparison of the efficiency of extracts, active components, and toxicity tests.

Acknowledgements

The authors thank to Dr. Ahmet Kahraman (Department of Biology, Faculty of Arts and Science, Uşak University).

ISSN: 2458 - 8806

Conflict of Interest

The authors declare that there is no conflict of interest.

Statement of Contribution of Researchers

Z.Ö: supervisor, concept, design, experiments of biological activity, implementation and interpretation of results; S.G.K.: Concept, plant extract experiments, implementation of results, collection of data. Both authors participated in the preparation, writing and final approval of the version to be published.

References

1. Bjarnsholt T. The role of bacterial biofilms in chronic infections. APMIS Suppl. 2013;136:1-51. doi: 10.1111/apm.12099
2. Berlanga M and Guerrero R. Living together in biofilms: the microbial cell factory and its biotechnological implications. Microb Cell Fact. 2016;15(1):165. doi: 10.1186/s12934-016-0569-5.
3. Whitchurch CB, Tolker-Nielsen T, Ragas PC, and Mattick JS. Extracellular DNA required for bacterial biofilm formation, Science. 2002;295(5559):1487. doi: 10.1126/science.295.5559.1487.
4. Melandera RJ, Basaka AK, Melandera C. Natural Products as Inspiration for the Development of Bacterial Antibiofilm Agents. Nat Prod Rep. 2020;37(11):1454–77. doi:10.1039/d0np00022a.
5. Macia MD, Rojo-Molinero E, Oliver A. Antimicrobial susceptibility testing in biofilm-growing bacteria. Clin Microbiol Infect. 2014;20(10):981-90. doi: 10.1111/1469-0691.12651.
6. Wolcott RD, Rhoads DD, Bennett ME, Wolcott BM, Gogokhia L, Costerton JW, Dowd SE. Chronic wounds and the medical biofilm paradigm. J Wound Care. 2010;19(2):45-6. doi: 10.12968/jowc.2010.19.2.46966.
7. Rabin N, Zheng Y, Opoku-Temeng C, Du Y, Bonsu E, Sintim HO. Biofilm formation mechanisms and targets for developing antibiofilm agents. Future Med Chem. 2015;7(4):493-512. doi: 10.4155/fmc.15.6.
8. Del Pozo JL. Biofilm-related disease. Expert Rev Anti Infect Ther. 2018;16(1):51-65. doi: 10.1080/14787210.2018.1417036.
9. Guzzo F, Scognamiglio M, Fiorentino A, Buommino E, D'Abrosca B. Plant Derived Natural Products against Pseudomonas aeruginosa and Staphylococcus aureus: Antibiofilm Activity and Molecular Mechanisms. Molecules. 2020;25(21):5024. doi: 10.3390/molecules25215024.
10. Lagha R, Abdallah FB, Al-Sarhan BO, Al-Sodany Y. Antibacterial and Biofilm Inhibitory Activity of Medicinal Plant Essential Oils Against Escherichia coli Isolated from UTI Patients. Molecules. 2019;24(6):1161. doi: 10.3390/molecules24061161.
11. 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(1): 243-263.
12. Güven S, Makbul S, Coskuncelebi K, Pınar NM. Pollinarium morphology of Vincetoxicum (Apocynaceae: Asclepiadoideae) in Turkey. Phytotaxa. 2015;230(1): 22-38
13. Sliumpaite I, Murkovic M, Zeb A, Venskutonisa PR. Antioxidant properties and phenolic composition of swallowwort (Vincetoxicum lutea L.) leaves. Ind Crops Prod. 2013;45:74-82.
14. Mansoor A, Ibrahim MA, Zaidi MA, Ahmed M. Antiprotozoal activities of Vincetoxicum stocksii and Carum copticum. Bangladesh J Pharmacol. 2011;6(1): 51-54.
15. Browicz K. Vincetoxicum N.M. Wolf. In: Davis PH, eds. Flora of Turkey and the East Aegean Islands. Edinburgh, Scotland; 1978. p. 163-173.
16. Slapšytė G, Dedonytė V, Adomėnienė A, Lazutka JR, Kazlauskaitė J, Ragažinskienė O, Venskutonis PR. Genotoxic properties of Betonica officinalis, Gratiola officinalis, Vincetoxicum luteum and Vincetoxicum hirundinaria extracts. Food and Chemical Toxicology. 2019;134:110815. doi: 10.1016/j.fct.2019.110815. Epub 2019 Sep 11.
17. Lavault M, Richomme P, Bruneton J. Acetophenones and new pregnane glycosides from the roots of Vincetoxicum hirundinaria. Fitoterapia. 1999;70(2): 216-20.
18. Pavela R. Antifeedant activity of plant extracts on Leptinotarsa decemlineata Say. and Spodoptera littoralis Bois. larvae. Ind Crops Prod. 2010;32(3): 213-19.
19. Lavault M, Richomme P, Bruneton J. New phenanthroindolizidine N-oxides alkaloids isolated from Vincetoxicum hirundinaria Medik. Pharm Acta Helv. 1994;68(4): 225-27.
20. Coassini-Lokar L, Poldini L. Herbal remedies in the traditional medicine of the Venezia Giulia Region (North East Italy). J Ethnopharmacol. 1988;22(3): 231-79.
21. Baytop T. Türkiye'de Bitkiler ile Tedavi Geçmişte ve Bugün. İlaveli 2. Baskı; İstanbul Üniversitesi Eczacılık Fakültesi, Nobel Tıp Kitap Evleri, İstanbul, Türkiye 1984.
22. Güzel S, Ülger M, Kahraman A. Chemical composition and some biological activities of Salvia longipedicellata Hedge mericarps. Chemistry of Natural Compounds. 2020;56(5): 788-92.
23. Woods GL, Washington JA. Antibacterial susceptibility tests: dilution and disk diffusion methods. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, eds. Manual of clinical

microbiology. 6th ed, Washington, DC: American Society for Microbiology; 1995. p. 1327–41.

24. Jorgensen JH, Ferraro MJ. Antimicrobial susceptibility testing: general principles and contemporary practice. Clin Infect Dis. 1998;26:973–80.

25. O'Toole GA. Microtiter dish biofilm formation assay. J Vis Exp. 2011;30(47):2437. doi: 10.3791/2437.

26. H. Zhong, Z. Xie, H. Wei, S. Zhang, Y. Song, M. Wang, and Y. Zhang. Antibacterial and Antibiofilm Activity of Temporin-GHc and Temporin-GHd Against Cariogenic Bacteria, Streptococcus mutans. Front Microbiol. 2019;10:2854. doi: 10.3389/fmicb.2019.02854.

27. Moody J. Synergism testing: broth microdilution checkerboard and broth microdilution methods. In: Garcia LS, eds. Clinical Microbiology Procedures Handbook. Washington, DC: ASM Press; 2010. P. 5.12.11-5.12.23.

28. Chin NX, Weitzman I & Della-Latta P. In vitro activityof fluvastatin, a cholesterol-lowering agent, and synergy with fluconazole and itraconazole against Candida species and Cryptococcus neoformans. Antimicrobial Agents and Chemotherapy. 1997;41(4), 850-52.

29. Güzel S, Ülger M, Aslan G, Kökdil G. Evaluation of antimicrobial activity of five Vincetoxicum taxa growing in Turkey. Marmara Pharmaceutical Journal. 2018;22(3): 365-373.

30. Güzel S, Ülger M, Özay Y, Yumrutaş Ö, Bozgeyik I, Sarıkaya Ö. Vincetoxicum canescens subsp. canescens ve Vincetoxicum cancescens subsp. pedunculata tohumlarının antimikrobiyal ve antiproliferatif aktiviteleri. Lokman Hekim Dergisi 2019;9: 367-75.

31. Güzel S, Pavela R, Kökdil G. Evaluation of antifungal effect of different polarity extracts from five Vincetoxicum taxa against Aspergillus fumigatus. International Anatolian Academic Online Journal/Health Science. 2015;3(2): 1-9.

32. Güzel S, Pavela R, Kökdil G. Phytochemistry and antifeedant activity of root extracts from some Vincetoxicum taxa against Leptinotarsa decemlineata and Spodoptera littoralis. JBiopest. 2015;8(2):128-40.

33. Güzel S, Pavela R, İlçim A, Kökdil G. Phytochemical composition and antifeedant activity of five Vincetoxicum taxa against Spodoptera littoralis and Leptinotarsa decemlineata. Marmara Pharm J. 2017;21(4):872-80.

ISSN: 2458 - 8806