DOI: HTTP://DOI.ORG/10.31925/FARMACIA.2018.6.14 **ORIGINAL ARTICLE**

AN EVALUATION OF ANTIOXIDANT, ANTIMICROBIAL, ANTIBIOFILM AND CYTOTOXIC ACTIVITIES OF FIVE *VERBASCUM* SPECIES IN TURKEY

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Abstract

Verbascum L. species are used for different medicinal purposes in Turkish folk medicine such as diuretic, expectorant, laxative, mucolytic, sedative, sudorific, and wound healer. Five *Verbascum* species, two of which are used in medicine, were investigated for their antimicrobial, antioxidant and cytotoxic activities. Determination of the total phenolic compounds, DPPH and ABTS methods were preferred for the antioxidant tests. The antimicrobial and antibiofilm activities of the extracts, which were not studied previously, were investigated against pathogenic and potential pathogenic agents. The cytotoxicity was assessed by MTT and LDH tests. This study is very important for finding new therapeutic sources and also new, natural sources for food preservation.

Rezumat

Speciile *Verbascum* L. sunt utilizate în scopuri medicinale diferite în medicina populară turcă, cum ar fi diuretic, expectorant, laxativ, mucolitic, sedativ, sudorific și cicatrizant. Cinci specii *Verbascum*, dintre care două sunt utilizate în scop medicinal, au fost investigate pentru activitățile lor antimicrobiene, antioxidante și citotoxice. Pentru evaluarea capacității antioxidante s-au determinat compușii fenolici totali și testele specifice prin metoda DPPH și ABTS. Activitățile antimicrobiană și antibiofilm ale extractelor, care nu au fost studiate anterior, au fost investigate împotriva agenților patogeni și potențiali patogeni. Citotoxicitatea a fost evaluată prin teste MTT și LDH. Acest studiu are o importanță deosebită pentru găsirea de noi surse terapeutice și, de asemenea, pentru noi surse naturale de conservanți alimentari.

Keywords: *Verbascum* species, antioxidant, antimicrobial, antibiofilm, cytotoxicity, food preservation

Introduction

Due to the appearance of undesirable side effects of certain commercial antioxidants, an increased interest in antioxidant activity of plants has been registered in recent years. A great number of bioactive compounds with antioxidant activity have important roles in free radical chain reactions [1]. Similarly, studies on the antimicrobial activity have increased over the years. Researchers also focus on finding out new antimicrobial agents from plant sources. Based on drug-resistant infections 25,000 people die every year in Europe. The antibiotic resistance has reached a critical point, as human and economic costs escalate. For some pathogens, the choice of available drugs is now greatly reduced [36]. Beside of this, the antimicrobial agents have an important role in food conservation. The food industry tries to find alternative natural sources, especially green chemicals, instead of the chemical preservatives [4]. The tendency to green chemicals increase day after day. Therefore, the investigations on antioxidant and antimicrobial activities of plants became crucial. Also, the cytotoxicity of new drugs/ sources is very important from the standpoint of cell damage.

The genus *Verbascum* L. is represented by 360 species, approximately, worldwide. In Turkey, it comprises about 249 species, of which 191 are endemic [9, 13, 15, 18, 23-27]. In traditional Turkish folk medicine, *Verbascum* species, commonly known as "*sigirkuyrugu*", have been used as anti-diarrheic, diuretic, mucolytic, expectorant, sedative, sudorific and wound healer [2, 33, 38]. The species of this genus contain flavonoids, iridoid glucosides, neolignan glucosides, phenylethanoid glycosides, saponins, spermine alkaloids, steroids, etc. In modern phytotherapy, *V. densiflorum*, *V. phlomoides* and *V. thapsus* L. are recognized as medicinal plants. *V. lagurus* Fisch. & C. A. Mey (VL) is wide spread in the Marmara Region, Turkey. Previously, we investigated

the antimicrobial activity of some extracts of this species and the chemical content of the active extract. Three flavonoids, three phenylethanoid compounds and two phenolic acids were isolated from the EtOAc (ethylacetate) extract [30]. Furthermore, we have compared the antimicrobial activities of different extracts from *V. densiflorum* Bertol. (VD), *V. gnaphalodes* Bieb. (VG), *V. lagurus* (VL), *V. phlomoides* L. (VP), *V. xanthophoeniceum* Griseb. (VX) [31].

Additionally, three studies on *V. xanthophoeniceum* exist in literature. The antioxidant and cholinesterases inhibitory activities of *Verbascum xanthophoeniceum* and its phenylethanoid glycosides were investigated by various methods. The anti-inflamatory effect of crude methanol extract, its subfraction and isolated compounds (iridoid glycosides and phenylethanoid glycosides) were also determined on human keratinocytes. Beside of these, the cytotoxicity and inhibitory effect on DHT-induced PSA secretion of crude methanol extract and its isolated phenylethanoid glycoside (verbascoside) in an *in vitro* model of human prostate epithelium were designated [15, 16, 20].

Because of the few studies on these *Verbascum* species and because a comparison between their activities and those of the medicinal species, it is aimed to improve and develop the previous studies and to determine the activity potentials of different extracts from these species.

Materials and Methods

Plant material: *Verbascum xanthophoeniceum* (VX) *Verbascum densiflorum* (VD) and *Verbascum lagurus* (VL) were collected from Kirklareli-Demirkoy, Turkey and *Verbascum gnaphalodes* Bieb. (VG) from Yalova-Armutlu, Turkey, in May 2009 and identified by Prof. Emine Akalin (Istanbul, Turkey). The voucher specimen has been deposited in the Herbarium of the Faculty of Pharmacy, Istanbul University (ISTE 91932; 91869; 91931; 92498). *Verbascum phlomoides* (VP) were provided from "Kurtsan Holding".

Preparation of extracts: The dried and powdered aerial parts of 5 *Verbascum* species were extracted in a Soxhlet apparatus with methanol (MeOH). The concentrated extract was diluted with water and extracted with petroleum ether (PE) in a separating funnel. The aqueous phase was successively extracted with toluene, chloroform and ethyl acetate (EtOAc). There were obtained several extracts: Me-OH, PE, Tol, $CHCl_3$ and EtOAc. All extracts were stored at ± 4°C after preparation.

Antioxidant assay:

Chemicals and reagent for antioxidant activity: 2,2-di-phenyl-1-picrylhydrazyl (DPPH•), Folin-Ciocâlteu's phenol reagent 2 N, gallic acid and ascorbic acid were obtained from Sigma Chemical Co., USA. All other reagents were of analytical grade.

DPPH• radical scavenging activity: The DPPH radical scavenging activities of different extracts were measured by the DPPH• method proposed by Wei *et al*. [39]. A DPPH solution (0.1 mM, 3.9 mL) was added to extracts (0.1 mL) prepared at different concentrations. Then the mixture was allowed to stand at room temperature for 30 min. The absorbance of the mixture was measured against the reference using a spectro-photometer at 517 nm.

$ABTS^{\cdot+}$ radical-scavenging activity: The $ABTS^{\cdot+}$ assay was performed according to the method developed by Re *et al*. [29]. 40 µL of extracts prepared from plant material, 3960 µL of $ABTS^{\cdot+}$ working solution were combined. The absorbance of the mixture was measured against the reference at 734 nm for 6 min. The data obtained in this study were expressed as mM trolox/mg extract.

Determination of total phenolic compounds: The different extracts were placed in 0.1 mL tubes and 4.5 mL of water was added to them. Then Folin-Ciocâlteu reagent (diluted 1/3 with distilled water) and 0.3 mL of 2% sodium carbonate solution were added to the mixture. The mixture was allowed to stand at room conditions for 2 hours, and then the absorbance was measured at 760 nm against the reference. The total phenolic content in the extracts was expressed as mg gallic acid equivalents /mg extract [34].

Antimicrobial assay: *In vitro* antibacterial activities of the toluene extracts against *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212; *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* ATCC 14153 and antifungal activities against *Candida albicans* ATCC 10231 were investigated, as like as the other extracts, which were tested in our previous study. Minimum inhibitory concentrations (MICs) of compounds were determined by micro-broth dilution technique as described by the Clinical and Laboratory Standards Institute (CLSI) [5, 6].

For this purpose, serial two-fold dilutions of extracts ranging from 5000 µg/mL to 9.76 µg/mL were prepared in Mueller-Hinton Broth (MHB) (Difco, Detroid, USA) for bacteria and RPMI-1640 (Sigma) medium for yeast. The inoculums were diluted in broth media to give a final concentration of 5 x 10^5 CFU/mL for bacteria and 0.5 × 10^3 to 2.5 × 10^3 CFU/mL for yeast in the test tray. The trays were incubated at 37°C for 18 - 24 h. The MIC was defined as the lowest concentration of compound giving complete inhibition of visible growth. To verify the standardization of test procedure, ciprofloxacin and fluconazole were used as reference substances for bacteria and yeast, respectively.

Antibiofilm Assay: For determination of anti-biofilm activities of extracts, the measurements of the minimum biofilm eradication concentration (MBEC) values were assessed against bacterial and *C. albicans* biofilms as

previously described [21]. Serial 2-fold dilutions of extracts ranging from 10000 to 625 mg/L were prepared in MHB, and added to the 24 h biofilms in a 96 well tissue culture microtiter plates. After the 24 h incubation at 37°C, the plates were washed, sonicated for disrupting the biofilms and samples were plated on TSA for colony counts. MBEC was defined as the lowest concentration of extracts which microorganism fails to regrow after exposure. All experiments were performed in two independent assays [12].

Cytotoxic Assay

The cytotoxic effects of all extracts on NRK-52E rat kidney cell line (NRK-52E) (ATCC, USA) were evaluated using MTT and LDH assays. The cells were seeded into 96-well plates at a density of 10^4 cells/ well, and incubated at 37°C and 5% CO_2 for 24 h. Then, the cells exposed to the extracts in the range of 0.005 - 0.5 mg/mL concentrations. Dimethylsulfoxide (DMSO) was used as solvent control, non-exposed cells as growth control and 10% triton-X100 as positive control. Assays were performed in triplicates, and each test was repeated three times (n = 9). For both tests, IC_{50} values were defined as the concentrations of compounds required to reduce the absorbance to 50% of the control values.

For MTT test, 20 µL of MTT solution (5 mg/mL in phosphate buffered saline) was added to each well, and incubated at 37°C for 2 h. The formed formazan crystals were solubilized in 100 µL of DMSO, and then measured the absorbance at 570 nm. The percentage cell viability was calculated with respect to solvent control as follows [22]:

$$\% \text{ Cell viability} = \text{Abs}_{\text{Compounds}}/\text{Abs}_{\text{Solvent Control}} \times 100.$$

Lactate dehydrogenase (LDH) is a stable cytoplasmic enzyme present in all cells. It is rapidly released into the cell culture supernatant upon damage of the plasma membrane. LDH activity was evaluated using a Roche Cytotoxicity Detection Kit (Roche diagnostics GMBH, Mannheim, Germany) according to the manufacturer instructions. In brief, the mediums of the exposed cells were mixed with the assay reagent prepared by mixing two separate solutions (diaphorase/NAD^+ mixture and iodotetrazolium chloride/sodium lactate mixture). After incubation for 30 min at room the temperature and away from the light, the reaction was stopped, and the absorbance was read at 490 nm. The cell viability was calculated according to the following equation:

$$\% \text{ Cell viability} = 100 - (\text{Abs}_{\text{Compounds}}/\text{Abs}_{\text{Positive Control}} \times 100).$$

Results and Discussion

As a continuation of our previous studies on the antimicrobial effects, of different extracts from 5 *Verbascum* species and on the chemical content from the most active extract regarding its antimicrobial activity. We aimed to develop the studies and to determine the activity potentials of different extracts from these species. The number of studies on these 3 species, common in the Marmara Region, is quite small, especially on *V. gnaphalodes* and there are no studies, comprising a comparison between their activities and those of the medicinal species. Differently to the previously performed study, the antimicrobial activities of toluene extracts were determined. Furthermore, the antioxidant and cytotoxic activities of various extracts from the aerial parts of 5 *Verbascum* species and also the antibiofilm activities of the extracts, which showed antimicrobial activity, were investigated.

Antioxidant activity: The antioxidant activities were investigated by DPPH and ABTS methods and the contents of the total phenolic compounds were determined (Table I). It was designated that the ethylacetate extracts of VL, VD, VX, VG and VP showed stronger DPPH and $ABTS^{\cdot+}$ radical scavenging activity than the other extracts. In addition, the ethylacetate extract of VL and all ethylacetate extracts from plants exhibited stronger DPPH radical scavenging and $ABTS^{\cdot+}$ radical scavenging activity than BHA, respectively. The antioxidant activities of toluene extracts, shown in our study, are remarkable. Additionally, the PE extracts exhibited less antioxidant activity than the other ones. The EtOAc extracts exhibited the highest total phenolic contents (TPC) among other extracts. The EtOAc extracts of VD, VL, VX are remarkable with their TPC (0.201 ± 0.009 mg GAE/mg extract, 0.194 ± 0.016 mg GAE/mg extract, 0.170 ± 0.005 mg GAE/mg extract; respectively). Also, the MeOH extracts had higher TPC, especially the VD-MeOH (0.140 ± 0.009 mg GAE/mg extract). According to these results, there is a linearity between the antioxidant activities and the total phenolic contents.

Table I

DPPH radical scavenging, ABTS radical cation scavenging activities and the amount of total phenolic compounds (TPC) (as gallic acid equivalents) of the different extracts
(These values were the mean values of three replicates ± standard deviation.)

Extracts/Standards	DPPH (IC_{50}: µg/mL)	ABTS (mM trolox/mg extract)	TPC (mg GAE/mg extract)
VL-MeOH	25 ± 0.001	83.810 ± 1.826	0.096 ± 0.007
VL-PE	1036 ± 0.216	7.555 ± 0.112	0.016 ± 0.002
VL-Tol	79 ± 0.039	54.302 ± 1.379	0.044 ± 0.003
VL-$CHCl_3$	84.8 ± 0.0027	79.843 ± 0.114	0.057 ± 0.004
VL-EtOAc	**5 ± 0.002**	**131.291 ± 0.10**	**0.194 ± 0.016**
VL-AQ	79 ± 0.002	48.334 ± 0.094	0.042 ± 0.002

Extracts/Standards	DPPH (IC_{50}: µg/mL)	ABTS (mM trolox/mg extract)	TPC (mg GAE/mg extract)
VG-MeOH	38 ± 0.002	62.529 ± 0.1	0.069 ± 0.003
VG-PE	378 ± 0.051	12.623 ± 0.284	0.018 ± 0.001
VG-Tol	33 ± 0.001	52.555 ± 2.439	0.045 ± 0.003
VG-$CHCl_3$	53 ± 0.001	83.257 ± 0.591	0.057 ± 0.004
VG-EtOAc	17 ± 0.001	100.852 ± 0.19	**0.101 ± 0.006**
VG-AQ	77 ± 0.002	41.486 ± 0.496	0.014 ± 0.017
VX-MeOH	13 ± 0.003	109.514 ± 0.12	0.124 ± 0.003
VX-PE	326 ± 0.023	18.772 ± 0.825	0.019 ± 0.001
VX-Tol	70 ± 0.001	59.247 ± 1.08	0.043 ± 0.001
VX-$CHCl_3$	92 ± 0.002	60.014 ± 0.59	0.042 ± 0.001
VX-EtOAc	**9 ± 0.002**	**131.102 ± 0.29**	**0.170 ± 0.005**
VX-AQ	30 ± 0.003	86.139 ± 0.188	0.069 ± 0.002
VP-MeOH	28 ± 0.001	77.824 ± 0.101	0.081 ± 0.004
VP-PE	623 ± 0.060	10.636 ± 0.001	0.017 ± 0.003
VP-Tol	40 ± 0.001	72.912 ± 0.47	0.065 ± 0.003
VP-$CHCl_3$	70 ± 0.001	58.865 ± 1.44	0.039 ± 0.002
VP-EtOAc	26 ± 0.001	93.321 ± 0.001	**0.091 ± 0.005**
VP-AQ	49 ± 0.001	56.214 ± 0.66	0.047 ± 0.001
VD-MeOH	**7.2 ± 0.0003**	122.167 ± 1.90	**0.140 ± 0.009**
VD-PE	523 ± 0.008	10.022 ± 0.285	0.015 ± 0.002
VD-Tol	49 ± 0.001	72.818 ± 2.2516	0.063 ± 0.001
VD-$CHCl_3$	85.3 ± 0.001	77.840 ± 1.477	0.005 ± 0.001
VD-EtOAc	**7.4 ± 0.001**	**131.479 ± 0.10**	**0.201 ± 0.009**
VD-AQ	33 ± 0.001	74.225 ± 0.281	0.051 ± 0.041
BHA	6 ± 0.60	52.63 ± 0.01	-
Ascorbic acid	4 ± 0.90	-	-

VD = *V. densiflorum* Bertol.,VG = *V. gnaphalodes* Bieb.,VL = *V. lagurus*,VP = *V. phlomoides* L., VX = *V. xanthophoeniceum* Griseb., MeOH = methanol, PE = petroleum ether, Tol = toluene, EtOAc = ethyl acetate, AQ = water, BHA = buthylhydroxyanisol

Antimicrobial activity: All extracts were tested for their antimicrobial activity against Gram positive/Gram negative bacteria and yeast. While no antibacterial activity was registered by VX-PE against all bacteria, the other extracts of VL, VX and VG, except VL-AQ, exhibited good antibacterial activity against *S. aureus*, *S. epidermidis* and *E. faecalis* with MIC values between 156 and 1250 µg/mL. VL-AQ showed an activity only against *S. aureus* and *E. faecalis*. The extracts of VP and VD were active against all Gram positive bacteria (*S. aureus*, *S. epidermidis*, *E. faecalis*), generally. VP-MeOH and VD-MeOH were found active against *S. aureus* and *S. epidermidis* as well as VD-PE against *S. epidermidis* and *E. faecalis*. The extracts of VP were more active against Gram positive bacteria than the VD-extracts.

The VL-EtOAc was found the most active against Gram positive bacteria with the concentrations of 156 and 625 µg/mL. Also, VL-EtOAc was the only active extract against *K. pneumoniae*. Against *P. aeruginosa*, only 3 VL-extracts (VL-MeOH, VL-EtOAc and VL-PE) possessed important activity. The most active antifungal extract was VL-EtOAc (156 µg/mL). Overall, the best results were given by the VL-extracts, especially VL-EtOAc, against all bacteria and yeast, even better than the extracts of medicinal species (Table II).

Table II

In vitro antimicrobial activities of the different extracts

	MIC values (µg/mL)							
Extracts	**Sa**	**Se**	**Ef**	**Ec**	**Kp**	**Pm**	**Pa**	**Ca**
VL- MeOH	++	+	+	-	-	-	++	++
VL-PE	+	+++	+	-	-	-	++	++
VL-Tol	+	++	+	-	-	-	-	++
VL-$CHCl_3$	++	+	+	-	-	-	-	++
VL-EtOAc	++++	++	++	-	+++	-	+++	++++
VL-AQ	+++	-	++	-	-	-	-	+++
VG- MeOH	+	+	+	-	-	-	-	++
VG-PE	+	+	+	-	-	-	-	++
VG-Tol	+	++	+	-	-	-	-	++
VG- $CHCl_3$	+	+	+	-	-	-	-	++
VG- EtOAc	+	+	+	-	-	-	-	++
VG- AQ	+	+	+	-	-	-	-	-
VX- MeOH	+	+	+	-	-	-	-	++

MIC values (µg/mL)								
Extracts	**Sa**	**Se**	**Ef**	**Ec**	**Kp**	**Pm**	**Pa**	**Ca**
VX-PE	-	-	-	-	-	-	-	++
VX-Tol	+	++	+	-	-	-	-	++
VX- $CHCl_3$	+	+	+	-	-	-	-	-
VX- EtOAc	+	+	+	-	-	-	-	++
VX- AQ	+	+	+	-	-	++	-	-
VP- MeOH	+	+	-	-	-	++	-	++
VP-PE	+	++	+	-	-	++	-	++
VP-Tol	+	++	++	-	-	-	-	++
VP- $CHCl_3$	+	++	+	-	-	-	-	++
VP- EtOAc	+	+	+	-	-	-	-	++
VP- AQ	+	+	+	-	-	++	-	++
VD- MeOH	+	+	-	-	-	++	-	-
VD-PE	-	+	+	-	-	-	-	++
VD-Tol	+	++	+	-	-	-	-	++
VD- $CHCl_3$	+	+	+	-	-	-	-	++
VD- EtOAc	+	+	+	-	-	-	-	++
VD- AQ	+	++	+	-	-	-	-	-

Sa: *S. aureus* ATCC 6538; **Se**: *S. epidermidis* ATCC 12228; **Ef**: *E. faecalis* ATCC; **Ec**: *E. coli* ATCC 25922; **Kp**: *K. pneumoniae* ATCC 4352; **Pm**: *P. mirabilis* ATCC 14153; **Pa**: *P. aeruginosa* ATCC 27853; **Ca:** *C. albicans* ATCC 10231; 1250 µg/mL = +; 625 µg/mL = ++; 312.5 µg/mL = +++; 156 µg/mL = ++++; – : no inhibition

Antibiofilm activity: All extracts, which were found to have antimicrobial activity, were tested for their anti-biofilm effect. It was determined that none of the extracts were active against the biofilm even at the concentration of 10.000 µg/mL.

Cytotoxicity potentials: The cytotoxic potentials of the extracts were investigated on the normal NRK-52E rat kidney cell line at the effective concentrations in the antioxidant and antimicrobial tests. The VD-extracts (except VD-$CHCl_3$ and VD-TOL), VG-AQ, VL-EtOAc, VL-MeOH and VX-AQ were non-toxic at the tested concentrations by MTT and LDH methods. The nonpolar extracts exhibited higher cytotoxicity than the polar extracts. The extracts of the medicinal species, VP and VG, showed toxicity at higher concentrations in contrast to the other extracts. However, it is designated that, the samples were much more active in LDH test than in MTT test (Table III).

Table III

The IC_{50} values (µg/mL) of the extracts in MTT and LDH tests

Extracts	**MTT IC_{50} (µg/mL)**	**LDH IC_{50} (µg/mL)**
VL- MeOH	-	-
VL-PE	0.224	-
VL-TOL	0.006	0.048
VL-$CHCl_3$	0.108	-
VL-EtOAc	-	-
VL-AQ	0.138	-
VG- MeOH	0.092	-
VG-PE	0.041	0.198
VG-TOL	0.064	0.065
VG- $CHCl_3$	0.197	-
VG- EtOAc	0.099	-
VG- AQ	-	-
VX- MeOH	0.019	-
VX-PE	0.215	-
VX-TOL	0.024	0.042
VX- $CHCl_3$	0.023	0.198
VX- EtOAc	0.006	-
VX- AQ	-	-
VP- MeOH	0.113	0.230
VP-PE	0.017	-
VP-TOL	0.089	0.095
VP- $CHCl_3$	0.037	-
VP- EtOAc	0.035	-
VP- AQ	-	0.141
VD- MeOH	-	-
VD-PE	-	-
VD-TOL	0.038	0.078
VD- $CHCl_3$	0.057	0.219
VD- EtOAc	-	-
VD- AQ	-	-

Nowadays, the antioxidant and antimicrobial agents have an important place in everyday life. Also, the food preservation is very important for a healthy life. The organic biomolecules are one of the methods for food preservation. The consumers concern about foods without/with lower levels of chemical preservatives because of their possible toxic effects and demand for the long shelf-life of food and absence of risk of causing foodborne diseases. Therefore, the food industry tries to find alternative natural sources instead of the chemical preservatives. The usage of chemical preservatives can result in obtaining resistant microbial strains to classic anti-microbial agents. At this point, the natural alternatives take important place in food conservation, especially the plants. There are many studies on plants, which present excellent antimicrobial properties, but the usage to enhance the shelf stability of foods were restricted.

The plants have excellent antimicrobial activity and contain many compounds, which have a role in their antimicrobial activity and are collectively called green chemicals. Wilkins and Board reported over 1389 plants as potential green chemical sources, and more specifically by the identification of over 250 new antifungal metabolites in plants between 1982 and 1993. It has been recognized that, investigations are now focused on the potential use of phytoalexins, organic acids, and phenols [4, 10, 11, 17, 19, 35, 40]. In literature, some articles investigated the antioxidant activities of *V. wiedemannianum* Fisch. *et* Mey. (the inhibition rate of 52.5% ± 3.11) and *V. cheiranthifolium* Boiss. [3, 7]. Dalar *et al*. investigated the lyophilized hydrophilic extract of *V. cheiranthifolium* Boiss. var. *cheiranthifolium* Boiss. at non-toxic concentrations and it exhibited the suppression of the accumulation of nitric oxide (NO) in lipopolysaccharide (LPS)-activated murine macrophages (RAW 264.7) and hepatocellular carcinoma (HepG2) cells [8]. In another study, in which 2,2-diphenyl-1-picrylhydrazyl (DPPH), oxygen radical absorbance capacity (ORAC) and total phenolic content (TPC) assays together with MTT cell viability assay (performed on Hep-G2 and MRC-5) were used, 18 Ethiopian medicinal plants were investigated for their antioxidant and antiproliferative activity. The extract of *V. sinaiticum* Benth. showed one of the most potent cytotoxic results [37]. The methanolic and hydrophilic extracts of *Verbascum* species showed antioxidant activity. Thusly, *Verbascum* species in this study indicate an important anti-oxidant activity.

Quave *et al*. tested *V. sinuatum* L. and *V. thapsus*, for their inhibition of growth and biofilms in methicillin-resistant *S. aureus* (MRSA) and found the extracts of these two species inactive [28]. In addition, a study on the antibacterial activity of the methanolic extract from *V. sinuatum* inflorescences and its chemical constituents were reported in the literature [32]. In spite of the antimicrobial results of studied *Verbascum* species, they did not exhibit an antibiofilm activity.

According to all previously mentioned information and results, the importance of antimicrobial and anti-oxidant activities in food preservation come in view. From the standpoint of the role of phenolic compounds in food preservation and the results regarding the phenolic content and the activities of *Verbascum* species, these species can have a food preservative potential. Likewise, studied *Verbascum* species shown good antioxidant and antimicrobial activities. Considering all reviewed aspects, the *Verbascum* species can be used as food preservative agents; the VL-EtOAc as an antioxidant agent as well as antimicrobial agent and as food preservative; the extract VD-EtOAc as an agent with good antioxidant activity.

Conclusions

This study represents a comparison of the activity profiles of 5 *Verbascum* species. It is remarkable that, the VL-EtOAc was found nontoxic at the effective concentrations in the antioxidant and antimicrobial tests. This extract can be assigned as an antioxidant agent as well as antimicrobial agent. This extract can also be used for food preservation. Beside of this, the extract VD-EtOAc can be designated as a good anti-oxidant agent.

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