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Three species of *Verbascum* L. from Northwest Anatolia of Turkey as a source of biological activities

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Abstract

Phytochemical constituents and some biological activities i.e., antimutagenicity, DNA damage protecting, antioxidant, antibacterial, and antibiofilm of ethanolic extracts of three *Verbascum* plants (*Verbascum mucronatum* Lam., *V. bombyciferum* Boiss., *V. vacillans* Murb.) were studied. This paper is the first comprehensive research on *V. mucronatum*, *V. bombyciferum*, *V. vacillans* biological activities. *V. vacillans* ethanol extract has been determined to be the lowest plant for phytochemical contents. In 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity and the Cu(II) ion reducing antioxidant capacity (CUPRAC) of three plant extracts showed concentration-dependent antioxidant capacity. *V. mucronatum* and *V. bombyciferum* extracts exhibited a strong antimutagenic effect on *Salmonella typhimurium* TA98 and TA100 strains. *Verbascum* extracts showed DNA damage protection potential in tested concentrations. However, the lowest concentration (0.5 µM) of the *V. bombyciferum* species Form III were observed and almost completely disintegrate DNA in this concentration. Three *Verbascum* plants were showed strong antibacterial activities with inhibition zones at 9.0 - 19.0 mm and a significant reduction in biofilm formation. It was observed that these plants are potential sources of various biological activities.

Keywords: Antibacterial, antimutagenicity, antioxidant activity, *Verbascum mucronatum*, *V. bombyciferum*, *V. vacillans*

1. Introduction

The genus *Verbascum* L. (Scrophulariaceae), knowing as mullein, has approximately 360 species, which are distributed in Europe, Asia, and Northeast Africa [1]. Turkey is one of the richest countries in the world with circa 250 species, and nearly 80% of this species is endemic to Anatolia [2,3,4]. The genus *Verbascum* has been divided in the flora of Turkey into thirteen artificial groups from A to M. The species which have chosen for this study belong to group E (*Verbascum bombyciferum* Boiss.) and group K (*V. mucronatum* Lam., *V. vacillans* Murb.). Except *V. mucronatum*, they have local distributions. *V. mucronatum* has a wide distribution, but to be found rare in their habitats, in west Anatolia. Also, this species is growing only in Crete, outside Turkey. While *V. bombyciferum* is an endemic around to Bursa and Yalova provinces, *V. vacillans* is confined to the south part of Kazdağı Mountain in Balıkesir, and also has been reported from Lesbos [5-6].

Verbascum plant species are a group used in traditional and modern medicine for respiratory diseases, dysentery infection antimicrobial, anti-inflammatory, sedative, diuretic, sudorific, expectorant, and antidiarrheal [7,8,9]. It is very important to investigate the medicinal components and effects of herbs as natural and alternative medicine sources. Some of the biological activities such as antimicrobial, antimalarial, antiviral, antioxidant, anti-inflammatory, antitumoral, cytotoxic, antinociceptive, immunomodulatory, antiulcerogenic, antihepatotoxic, antihyperlipidemic, antinociceptive, antitussive, anthelminthic, and antigermination of genus *Verbascum* have been also previously reviewed [7,10,11,12,13,14,15]. However, in the literature, it has been determined that biological activity studies especially from these three plant species are insufficient. As far as we know, there are not comprehensive investigations on phytochemical content, antioxidant, antimutagenicity, DNA damage protection, antibacterial and antibiofilm activities with the *V. mucronatum*, *V. bombyciferum*, and *V. vacillans* plant species. Therefore, the present study is about the comparison of three *Verbascum* species in terms of these biological activities.

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2. Material and Methods

2.1. Plant materials

The specimens belong to *V. mucronatum*, *V. bombyciferum*, and *V. vacillans* were collected from Bursa and Balıkesir provinces (NW Anatolia) in 2017, respectively. The specimens were identified with the aid of flora of Turkey [2,5,16,17] and other relevant publications [18,19,20] by Dr. Özer Yılmaz. Also samples of the three species kept in the herbarium BULU (University of Uludağ, Bursa, Turkey) (Table 1).

2.2. Preparation of plant extracts

Dried plant materials (15 g) were ground mechanically in aseptic condition and extracted with 150 mL ethanol (80%) by using Soxhlet (Wisd-WiseTherm) [21], then filtered extracts were evaporated with rotary evaporator equipment.

2.3. Qualitative phytochemical screening

The phytochemical screening was performed for the presence of seven phytochemicals; coumarins, cardiac glycosides, phlabotannins, quinones, flavanones, anthocyanins, and proteins using the standard procedures as described by Harborne (1973), Raaman (2006), Evans (2009) [22,23,24].

2.4. Evaluation of antioxidant activity

2.4.1. DPPH free-radical scavenging assay

The antioxidant activity of the extracts was measured by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) following the procedure described by Brand-Williams [25]. The absorbance values of the samples were measured at 517 nm. The radical scavenging activity of each sample was calculated using the following formula and the results were expressed as % inhibition.

$$Inhibition\ (\%) = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100 \qquad (1)$$

2.4.2. Cu(II) ion reducing antioxidant capacity (CUPRAC)

The Cu(II) ion reducing the antioxidant capacity of *Verbascum* species was performed using Apak [26] method. The samples were incubated for half an hour at room temperature; absorbance was measured at 450 nm by spectrophotometer.

2.5. Antimutagenicity assay

The antimutagenic activity of *Verbascum* extracts has been investigated by Ames/*Salmonella* test system [27]. The experiment was carried out using *Salmonella typhimurium* auxotroph mutant strains TA98 (frame-shift mutation) and TA100 (base-pair substitution) strains. The positive controls, 4-nitro-*o*-phenylenediamine (NPD, 10 µg/plate) for TA98 strain and the sodium azide (SA, 1 µg/plate) for the TA100 strain were used. In the negative control, the solvents of the extracts were used for both strains. The inhibition rates of extracts were calculated according to the formula given by Hong and Lyu [28].

$$Inhibition\ Rate\ (\%) = \frac{A - B}{A - C} \times 100 \qquad (2)$$

A is the number of revertant colonies in the presence of mutagen/plate, B is the number of revertant colonies in the presence of extract/plate, C is the number of spontaneous colony/plate.

As a result of antimutagenicity studies, it was considered non-antimutagenic when the inhibitory effect of the extracts tested was below 25%. The 25 - 40% inhibition rate is moderate and more than 40% is defined as a strong antimutagenic effect [29, 30]

2.6. DNA damage protecting activity

DNA damage protecting activity was performed using supercoiled pBR322 DNA plasmid by agarose gel electrophoresis. Plasmid DNA in Tris-HCl buffer was treated with the extracts of different *Verbascum* species at 37 °C for 3 h. To determine the mechanism of damage protecting activity H_2O_2 was added to the mixture as an oxidant. After incubation samples were electrophoresis for 1 h at 60 V on 1% agarose gel in TAE buffer according to Russo et al. [31] with some modifications. Then, the DNA bands were visualized under UV light and photographed (Quantum ST4 gel imagining system, Vilbar Lourmat).

2.7. Screening antibacterial activities

The standard disc diffusion method [32] was used for screening antibacterial activities of three *Verbascum* extracts against some Gram-negative and positive bacteria (Table 4). Reference antibiotic [Penicillin (P10)] was used to compare afterward with plant extracts. Minimal inhibition concentration (MIC) and minimal bactericidal concentration (MBC) were investigated as recommended instruction of the Clinical and Laboratory Standards Institute [33,34].

2.8. Biofilm inhibition assay

The microplate biofilm method [35] was used to evaluate the inhibition of biofilm formation by *Verbascum* plant extracts against test bacteria. The measurement of the antibiotic effect of the extracts was made by the percentage reduction formulation.

Table 1. Voucher specimens used in this study.

Species	Locality	Collectors
V. mucronatum	Balıkesir: Edremit, Akçay-Küçükkuyu, 39°35′27′′N-26°53′37′′E, 29 July 2017.	A. Yılmaz and Ö. Yılmaz 1933 (BULU)
V. bombyciferum	Bursa: Nilüfer, Çalı-Atlas, 242 m, 40°09′28′′N-28°54′58′′E, 26 April 2017.	Ö. Yılmaz 1860 (BULU)
V. vacillans	Balıkesir: Edremit, Zeytinli-Beyoba, 39°38′07′′N-26°55′52′′E, 30 July 2017.	A. Yılmaz and Ö. Yılmaz 1934 (BULU)

$$Inhibition\ (\%) = \frac{A_{control} - A_{sample}}{A_{sample}} \times 100 \qquad (3)$$

$A_{control}$: Absorbance of the control (containing 100 µL Mueller Hinton Broth instead of plant extract) reaction, A_{sample}: Absorbance of the test compounds

3. Results and discussions

3.1. Qualitative phytochemical analysis

This study revealed that the ethanol extracts of three *Verbascum* species contained coumarins, cardiac glycosides, phlorotannins quinones, flavanones, anthocyanins, and proteins (Table 2).

Table 2. Phytochemical analysis of *Verbascum* species

Phytochemical contents	*Verbascum* plant species		
	V1	V2	V3
Coumarins	-	+	+
Cardiac glycosides	+	-	-
Phlabotannins	+	++	-
Quinones	+	-	-
Flavanones	+	+	++
Anthocyanins	+	-	-
Proteins (Biuret test)	-	+	-

V1: *V. mucronatum*, V2: *V. bombyciferum*, V3: *V. vacillans*; +: low intensity reaction, ++: strong intensity reaction; -: Not Detected

However, cardiac glycosides, quinones anthocyanins were detected only in *V. mucronatum* (V1); proteins were detected only in *V. bombyciferum* (V2); only flavanones were detected in all three *Verbascum* species. *V. vacillans* ethanol extract also has been determined to be the lowest plant for phytochemical contents.

Phytochemicals are natural bioactive including alkaloids, terpenoids, steroids, polyphenols, and flavonoids have rational uses and are found in varying amounts in different organisms [36]. Plants are an important source of these bioactive compounds and elucidation of these phytochemicals is important in revealing the benefits for human health [37].

The members of the *Verbascum* species are known to be rich in saponins, tannins, terpenoids, phenylethanoid glycosides, flavonoids contained in the *Verbascum* species are responsible for biological activities [38,39]. It can be said that not only a single substance but the different substances they contain affect the activity [39]. The presence of alkaloids, tannins, flavonoids, flavones, anthraquinones, cardiac glycosides *V. thapsus* were determined by phytochemical analysis [40]. In the present study, phytochemical analysis of *Verbascum* species showed the presence of various bioactive constituents. This variety can be explained by that the secondary metabolite profile in plants can be determined by different factors. The genotypic characteristics and phenology of the species are the basic elements that determine the phytochemical profile [41]. Biotic (pathogens and herbivorous organisms) and abiotic factors (light, temperature, nutrients, water condition, and geographical conditions) can directly affect the secondary metabolite chemical composition of the plant [42,43].

There is no report about the phytochemical contents of three *Verbascum* species except *V. mucronatum* [44]. This is the first study that report phytochemical contents of *V. mucronatum*, *V. bombyciferum*, and *V. vacillans* ethanol extracts. Flavonoids were detected as common the phytochemical content in three plant extracts. It is important that flavonoids constitute the largest plant phenolic group that makes up more than half of the natural phenolic compounds [45].

3.2. Antioxidant activity

3.2.1. DPPH free radical scavenging activity

Free radical scavenging activity of the ethanolic extracts of *Verbascum* species was measured by DPPH assay at five varying concentrations (10, 20, 40, 60, and 80 µg/mL). DPPH free scavenging activity of each plant extracts was increased in a concentration-dependent. The highest concentration of 80 µg/mL extract of *V. mucronatum* shown the best antioxidant activity (75.06%), followed by *V. vacillans* (73.16%), *V. bombyciferum* (72.79%), and synthetic antioxidant butylhydroxytoluene (BHT) (68.89%), respectively (Fig. 1). The IC_{50} value, which indicates the amount of sample needed to inhibit 50% of the radical. According to the results, all the extract was found strongly active in the range of 10-50 µg/mL [46]. The IC_{50} value of *Verbascum* extracts were; *V. vacillans* (24.84 µg/mL), *V. bombyciferum* (27.84 µg/mL), and *V. mucronatum* (35.22 µg/mL).

3.2.2. Cu(II) ion reducing antioxidant capacity

The Cu(II) ion reducing the antioxidant capacity of *Verbascum* plant species, especially *V. vacillans* showed strong antioxidant capacity at the highest concentration of 80 µg/mL as same as a positive control (Fig. 2).

Free radicals are drawn in many disorders like neurodegenerative diseases, cancer, and AIDS. Antioxidants due to their scavenging activity are useful for the management of these diseases and this explains

the curative effects of medicinal plants having antioxidant effect [24].

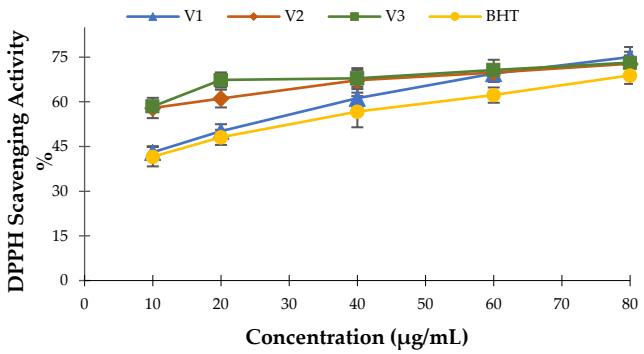

Figure 1. DPPH free scavenging activity of *Verbascum* species. V1: *V. mucronatum*, V2: *V. bombyciferum*, V3: *V. vacillans*. Values are means of three experiments ± SD

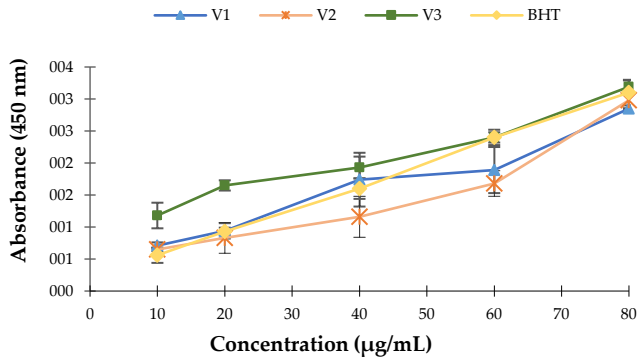

Figure 2. The Cu(II) ion reducing antioxidant capacity of *Verbascum* species. V1: *V. mucronatum*, V2: *V. bombyciferum*, V3: *V. vacillans*. Values are means of three experiments ± SD

It has been determined that the biological activities of the extracts can vary depending on the season, the type of the solvent, the dose, and the application time. Studies have emphasized that there is a linear correlation between the total phenolic content of plant extracts and antioxidant efficiency values. Considering the crude extracts of these plants have plenty of constituents it becomes tough to praise the antioxidant property selectively to any group of them without more investigations [47].

DPPH is a stable free radical. It is purple in color and this color can be absorbed in 517 nm. When DPPH free radicals are captured by an antioxidant, their color changes from purple to yellow. This color change is observed because DPPH transforms into 2,2-diphenyl-1-picryl hydrazine by interacting with antioxidant substances [25]. The antioxidant activity of *Verbascum* species was evaluated using different extracts such as the methanol and acetone extracts of *V. pinetorum* besides methanol (IC_{50} = 65.4 ± 0.5 µg/mL) and water (IC_{50} = 235.6 ± 0.5 µg/mL) extracts of *V. mucronatum* showed high free radical scavenging activity by DPPH assay [48,49] and *V. pinetorum* acetone, methanol, and water extracts showed high activity by CUPRAC test [49]. Georgiev et al. [8] studied *V. xanthophoeniceum* leaves and reported that have strong antioxidant activities and active constituents (forsythoside B, verbascoside and leucosceptoside B) showed IC_{50} values of 21-44 µg/mL DPPH radical scavenging activities. In this study, the ethanolic extracts of *Verbascum* species were evaluated for the antioxidant activity for DPPH free radical scavenging and Cu(II) ion reducing antioxidant capacity. Our results supported the previous findings that *Verbascum* extracts had concentration-dependent antioxidant capacity.

3.3. Antimutagenicity assay

In this study, the antimutagenicity of the *Verbascum* extracts was investigated using *S. typhimurium* TA98 and TA100 mutant strains. Findings obtained as a result of antimutagenicity activity were shown in Table 3.

V. mucronatum extract showed a strong antimutagenic effect (inhibition rate > 40 %) at all concentrations on *S. typhimurium* TA98 strain. *V. bombyciferum* was found to have a moderate antimutagenic effect (32.83 %) at 0.5 ppm concentration and strong antimutagenic activity (45.48 % and 48.03 %) at concentrations of 1 and 2 ppm, respectively on TA98 strains. *V. vacillans* extract did not prevent frameshift mutation except the higher concentration of 2 ppm (28.37%). The results obtained from the TA100 strain showed strong antimutagenic effect of *V. mucronatum* and *V. bombyciferum* extracts of all concentrations while the *V. vacillans* extract did not prevent base-pair mutation at any concentrations.

It is known that many plants or plant products consumed contain a variety of the antimutagenic agents and are also capable of inactivating environmental mutagens or carcinogens. Therefore, it is important to determine antimutagenic activities of extracts obtained from plants. In this study, the potential *in vitro* effects of *Verbascum* extracts on genetic material was investigated by the Ames test. As a result of the antimutagenicity study, *Verbascum* species generally found to have moderate or strong antimutagenic effects at different concentrations. Probably chemical contents of the *Verbascum* species play an active role in the antimutagenic activity. There is no report about *Verbascum* plant species antimutagenic effects. Makhafola et al. [50] have examined 31 plant extracts for antimutagenic activity, and it was found that most plants have potential antimutagenic activity and also a close relationship between antioxidant activities. Mutagenic and antimutagenic activities of medically used *Salacia crassifolia* root shell fractions (hexane, ethyl acetate, and hydroalcoholic) were investigated using *S. typhimurium* TA98 and TA100 strains. There was no mutagenic effect

and high antimutagenic activity was present in hexane of strain TA100 [51].

Table 3. The antimutagenic effects of different concentration of *Verbascum* extract on *S. typhimurium* TA98 and TA100 strains

Extracts	Conc. (ppm)	Number of his+ revertant colony/Plate TA98 Mean ± SD	TA98 Inh. %	TA100 Mean ± SD	TA100 Inh. %
Positive control NPD		894± 17.1			
Positive control SA				1033± 21.1	
V1	0.5	545±9.8	40.6	461±6.9	61.6
	1	520±6.3	43.5	453±9.2	62.4
	2	514±7.6	44.2	422±11.0	65.8
V2	0.5	480±8.3	32.8	568±8.5	50.1
	1	502±10.2	45.5	556±6.5	51.4
	2	611±9.6	48.0	515±10.3	55.8
V3	0.5	776±13.6	13.7	857±4.9	18.9
	1	701±11.2	22.4	804±7.5	24.7
	2	650±14.1	28.4	812±8.3	23.8
Negative control		43±2.9		112±4.3	
Spontaneous control		34±3.9		104±6.1	

V1: *V. mucronatum*, V2: *V. bombyciferum*, V3: *V. Vacillans*, NPD: 4-nitro-o-phenylene-diamine, SA: sodium azide, Conc.: Concentration, Inh.: Inhibition, Values were expressed as mean ± standard deviation of three experiments.

Sutherlandia frutescens (L.) is an endemic species used in the treatment of cancer and diabetes in Southern Africa. Mutagenic and antimutagenic activities were tested with Ames test using ethyl acetate and 50% methanol extracts. No mutagenic activity was observed, ethyl acetate extract showed an antimutagenic effect in all concentrations and bacterial strains (TA97a, TA98, TA100, and TA102) [52]. The antimutagenicity findings obtained in our study support previous studies.

3.4. DNA damage protection potential

In the presence of an oxidizing agent (H_2O_2), *Verbascum* plant ethanol extracts showed DNA damage protection potential in tested concentrations. However, the lowest concentration (0.5 µM) of the *V. bombyciferum* species Form III were observed and almost completely disintegrate DNA in this concentration (Fig. 3). Form I indicated supercoiled, Form II relaxed circular, and Form III linear form of plasmid DNA. These forms act at different speeds in agarose gel electrophoresis. Because the load density is high and the volume is low, Form I moves fastest in the gel, and Form II is the slower moving band in gel.

The DNA damage protection potential results showed that the treatment of pBR322 plasmid DNA with H_2O_2 did not result in changes in plasmid conformation. Only, the lowest concentration (0.5 µM) of the *V. bomyciferum* species changed the plasmid DNA conformation. However, Boğa et al. [49] found that methanol extracts of *V. pinetorum* did not show a significant protection activity of DNA.

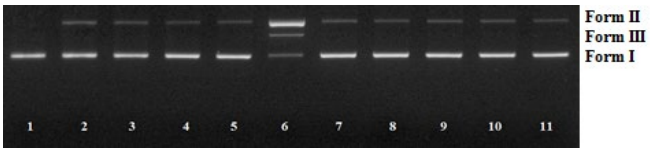

Figure 3. DNA damage protection potential of *Verbascum* species. 1. Plazmid DNA, 2. DNA+ H_2O_2, 3. DNA+ 0,5 µM V1+ H_2O_2, 4. DNA+ 1 µM V1+ H_2O_2, 5. DNA+ 2µM V1+ H_2O_2, 6. DNA+ 0,5 µM V2+ H_2O_2, 7. DNA+ 1 µM V2+ H_2O_2, 8. DNA+ 2 µM V2+ H_2O_2, 9. DNA+ 0,5 µM V3+ H_2O_2, 10. DNA+ 1 µM V3+ H_2O_2, 11. DNA+ 2 µM V3+ H_2O_2

3.5. Antibacterial activity

Results of antibacterial activity of three *Verbascum* plant extracts against the test bacteria were qualitatively and quantitatively assessed by the presence and diameters of the inhibition zones, MIC, and MBC (Table 4). The ethanolic extracts obtained from the three *Verbascum* plants were strong antibacterial activities against the test bacteria with inhibition zones at 9.0-19.0 mm. *V. vacillans* extract was more effective than comparative antibiotic P10 against *P. aeruginosa* ATCC 27853, *B. subtilis* ATCC 6633 and *S. haemolyticus* ATCC 43252. But *V. mucronatum* and *V. bombyciferum* are obtained in similar strong antibacterial activity only against *P. aeruginosa* ATCC 27853. The extracts have shown the weaker activity against *E. coli* NRRL B-3704, *E. aerogenes* ATCC 13048, *P. vulgaris* ATCC 13315, *A. baumanii* ATCC 19606, *S. aureus* ATCC 6538P as compared to control antibiotic P10.

Table 4. Disc Diffusion, MIC, MBC, and MBC/MIC ratios of the extracts of strains

Test bacteria	Plant extracts *Disc Diffusion[a] V1	V2	V3	Control P10	MIC (µg/mL) V1	V2	V3	Control ST10	MBC V1	V2	V3	MBC/MIC V1	V2	V3
E. coli NRRL B-3704	10.3±0.1	9.6±0.1	10.0±0.1	16.0	10±0	5±0	10±0	4.0	10±0	20±0	10±0	1	4	1
E. aerogenes ATCC 13048	11.3±0.1	10.0±0.1	10.0±0.1	14.0	10±0	5±0	1.25±0.01	2.0	10±0	10±0	1.25±0.01	1	2	1
P. aeruginosa ATCC 27853	11.3±0.5	12.3±0.2	10.3±0.2	8.0	20±0	5±0	10±0	1.0	20±0	20±0	10±0	1	4	1
P. vulgaris ATCC 13315	12.6±0.2	9.3±0.1	9.0±0.5	13.0	10±0	5±0	10±0.1	4.0	10±0	10±0	20±0	1	2	2
A. baumanii ATCC 19606	10.4±0.2	10.0±0.2	11.0±0.2	12.0	2.5±0	10±0	1.25±0.01	2.0	5±0	20±0	1.25±0.01	2	2	1
B. subtilis ATCC 6633	9.6±0.2	10.0±0.2	15.0±0.2	14.0	5±0	5±0	2.5±0.0	4.0	5±0	5±0	5±0	1	1	2
S. aureus ATCC 6538P	14.0±0.4	10.3±01	9.3±0.3	15.0	10±0	10±0	10±0	4.0	10±0	10±0	10±0	1	1	1
S. haemolyticus ATCC 43252	13.0±0.2	12.3±0.1	19.0±0.6	14.0	20±0	10±0	20±0	5.0	20±0	20±0	20±0	1	2	1

V1: V. mucronatum; V2: *V. bombyciferum;* V3: *V. vacillans;* *Inhibition zone (mm); a includes diameter of disk (6 mm); P10 = Penicillin (10 ug/disc); ST10: *Streptomycin (10 ug/disc)*

V. vacillans extract demonstrated good inhibitory activity against *E. aerogenes* ATCC 13048, *A. baumanii* ATCC 19606, and *B. subtilis* ATCC 6633, with MICs values of 1.25 mg/mL, 1.25 mg/mL, 2.5 mg/mL as compared to control antibiotic ST10, respectively. However, the three *Verbascum* plant extracts have a weak antibacterial effect against the other test bacteria with MICs and MBCs ranged from 20 (20) to 5 (5) mg/mL. These values are far below the standard antibiotic-Streptomycin (ST10).

The results of the present investigation show that three different *Verbascum* ethanol extracts have antibacterial and antibiofilm potential against test bacteria. In Table 3, MBC/MIC ratio was calculated to establish bacteriostatic or bactericidal effects of the plant extracts. According to Ocampo et al. [53] and Azman et al. [54] bacteriostatic can be defined as the agent that inhibit the growth of bacteria without killing effects, while bactericidal means agents that kill bacteria. An extract is considered bactericidal when the ratio of MBC/MIC is ≤ 4 and bacteriostatic when this ratio is > 4 [55]. It appears that all plant extracts have bactericidal activity on the test bacteria. Our antibacterial activity findings confirmed the observations of some other investigations about various *Verbascum* species antimicrobial activity [12,14,15, 38,56].

When examining previous studies about *V. mucronatum*, *V. bombyciferum*, *V. vacillans*, we found that Dülger et al. [56], Dülger and Hacıoğlu [57] and Kahraman et al. [12] revealed antibacterial activity against Gram (+) bacteria, respectively. Our findings are distinct from previous studies because of strong antibacterial activity against *P. aeruginosa* ATCC 27853 of three plant extracts. Ethanol was chosen as the solvent since it was reported that ethanol was the best solvent in previous studies [58].

3.6. Results of biofilm inhibition

Inhibition biofilm activity was performed with MIC concentrations of plant extracts. Treatment with *V. mucronatum*, *V. bombyciferum*, *V. vacillans* extracts have shown significant reduction in biofilm formation in *E. coli* NRRL B-3704, *P. aeruginosa* ATCC 27853, *A. baumanii* ATCC 19606, *B.subtilis* ATCC 6633, *S. aureus* ATCC 6538P (Fig. 4a, 4b, 4c).

Biofilm is assessed as significant virulence factor because of increasing the resistance of bacteria to antibiotics and host defense systems [59]. This necessitated the screening of new and natural antibiotic sources in the fight against biofilm. Studies of antibiofilm activity of *Verbascum* species are very limited. Moghaddam et al. [60] reported that *V. thapsus* extracts have inhibitory effects on biofilm formation of three oral streptococci. The high the biofilm inhibition activity we obtain from three *Verbascum* species is very important in this respect. Therefore, comprehensive investigations about these three *Verbascum* species antibacterial and antibiofilm activity have become a new strategy for the treatment of these bacterial infections.

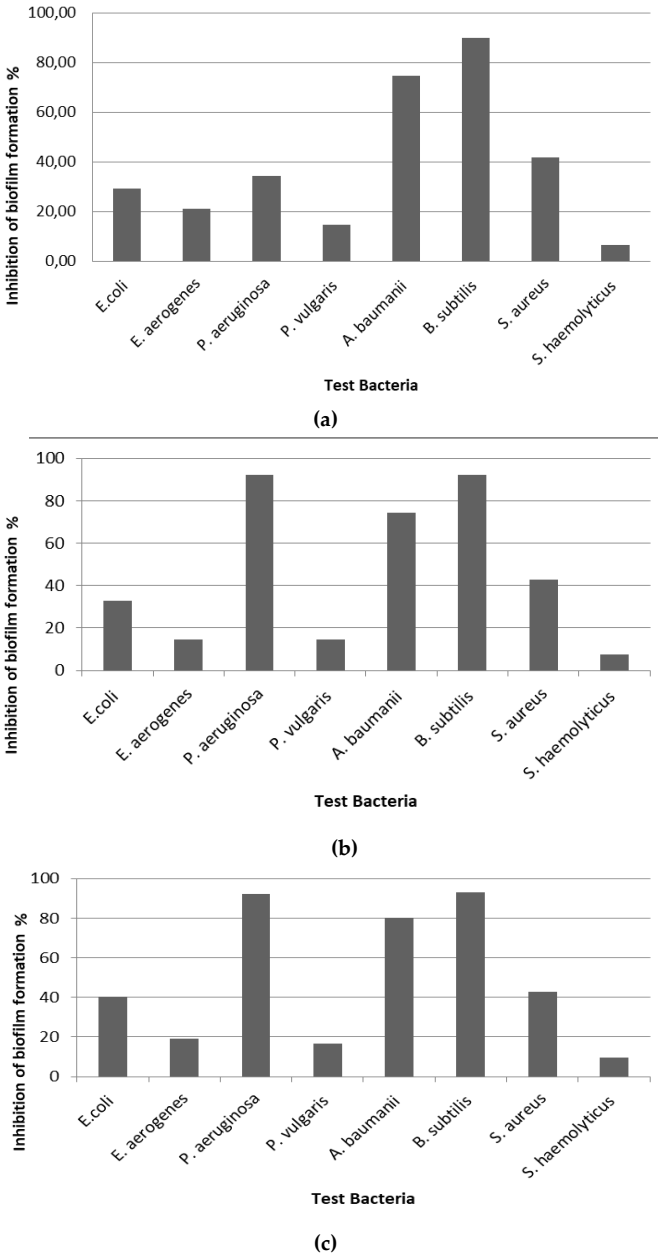

Figure 4. Inhibition of biofilms formation of three *Verbascum* plant extracts, **a)** *V. mucronatum* biofilm inhibition activity, **b)** *V. bombyciferum* biofilm inhibition activity, **c)** *V. vacillans* biofilm inhibition activity

4. Conclusion

Three *Verbascum* plant species demonstrated the presence of some phytochemical-especially flavonoids-as secondary metabolites with potential biological activities. It may be considered that these extracts are a mixture of substances with different characteristics and biological activities. Results reported in this study can be considered as the first comprehensive study on phytochemical contents, antioxidant, antimutagenicity, DNA damage protecting, the antibacterial and antibiofilm activity of ethanolic extracts of *V.*

mucronatum, *V. bombyciferum*, *V. vacillans*. Future studies should be done to define the biological active components of three *Verbascum* species, especially with different solvents.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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