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The Effect of Some *Verbascum* Plant Extracts on Cytoplasmic Membrane of Multidrug Resistant Bacteria by Flow Cytometry

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Abstract**—***Verbascum species have been the focus of antioxidant and antimicrobial studies thanks to their secondary metabolites, especially saponins. While disk diffusion and dilution methods are generally used within the scope of antimicrobial studies, the Flow cytometry method is not well known. The ability of six Verbascum species to increase the permeability of multidrug resistant bacterial cells was conducted by flow cytometric assay on Listeria innocua and Escherichia coli. Fluorescence based flow cytometry is a technique for measuring characteristics of cells or biological particles and using flourescent dye as fluorogenic substrate provides. Propidium iodide (PI) is a fluorescent molecule and it can be used to stain cells. The SYTO dyes can be used to stain nucleic acids in both live and dead cells, as well as in Gram-positive and Gram-negative bacteria.*

Keywords**— Verbascum, flow cytometry, antibacterial, Lischeria Innocua, Escherichia Coli. Propidium iodide, SYTO.**

I. INTRODUCTION

The natural compounds also known as secondary found in medicinal plants belong to various chemical structures and some of these compounds have anticancer, antioxidant, and antimicrobial activity. However, little is known about the antibacterial drug resistance mechanisms of these compounds.. The *Verbascum* genus is known as “Mullein” has more than 2500 species in the world. This plant species in Turkey has about 233 units and 196 of these types indicate an endemic feature. Verbascum is a plant species used for a variety of medical studies in Turkey. Leaves and flowers of Verbascum species are used as an expectorant and diuretic as sweaty [1]. The antibacterial antioxidant, anticancer, antimalarial and cytotoxic activities of Verbascum species have been the subject of many studies. [1–5]. Mirjalili et al reported the antibacterial and antioxidant properties of *Verbascum* species belonging to various regions of Iran [4]. Soltaninejad and Akhgar have obtained a report on these components by isolating some secondary metabolites from a *Verbascum* genus. Biologically active components of plants of the *Verbascum* genus are saponins, iridoid, and phenylethanoid glycosides, neolignan and monoterpene glucosides, phenolic and fatty acids, spermine alkaloids and steroids, and flavonoids [6–10]. Since verbascum seeds contain saponin, they show a poisonous effect. For this reason, it is used especially in fishing on the Black Sea coast. In the same study, antimicrobial properties of three *Verbascum* species (*Verbascum Olympicum* Boiss., *Verbascum Prussianum* Boiss. And *Verbascum Bombyciferum* Bois) were reported. [11]. The antioxidant and phenolic structure of the extract of *Verbascum Glomeratum Linneus* species

was observed by HPLC [12]. Anticarcinogenic and antioxidant properties of Verbascum thapsus L. species have been reported in the literatüre. *Verbascum thapsus L*. leaf was extracted with different solvents, and its antibacterial activity was investigated. In addition, in these studies, it was stated that microbial biofilms are also very important in ensuring that microorganisms live in a complex structure. [13-16]. It has been reported that the leaves of Verbascum Thapsus have antibacterial properties against Escherichia Coli, Yersinia Pestis, Bacillus Cereus, Pseudomonas Aeruginosa, Listeria Monocytogenes and Staphylococcus Aureus bacteria. Similarly, it has been reported in the literature that the roots of Verbascum Undulatum also exhibit antibacterial properties. [17-18]. CuO nanoparticles synthesized from Verbascum thapsus leaves showed both photocatalytic and antibacterial activity against Gram-positive Staphylococcus aureus and drug-resistant Gram-negative Escherichia coli bacteria. Such approaches also help to develop new biofilm strategies from plants. [19-20]. The pharmacological properties of Verbascum species have been revealed in many studies such as those described above and took their place in the literature. In conclusion, Verbascum species have an important place in the treatment of diseases due to their antioxidant and antibacterial properties [16,21-23]. The pharmacological properties of *Verbascum* species have been revealed in many studies such as those described above and took their place in the literature. In conclusion, *Verbascum* species have an important place in the treatment of diseases due to their antioxidant, antibacterial and biofilm activity properties [3,16,21-26,33-40]. In this study, the antibacterial effects of six Verbascum species (*Verbascum tripolitanum, Verbascum sinuatum, Verbascum caesareum, Verbascum gaillardotti, Verbascum pinetorum* and *Verbascum antiochium*) against *Listeria innocua* and *Escherichia coli* bacteria were investigated by Flow cytometry.

II. MATERIALS AND METHOD

Plant Material

Verbascum plant samples (*Verbascum tripolitanum, Verbascum sinuatum, Verbascum caesareum, Verbascum gaillardotti, Verbascum pinetorum,* and *Verbascum antiochium)* were collected by Dr. Yelda Guzel. The voucher specimen is stored in the fungarium at the Biology Department of Mustafa Kemal University. *Verbascum tripolitanum* was collected from Yayladağ road, Şenköy pasture,in Hatay (Turkey). *Verbascum sinuatum L* was collected from Aşağıokçular, Antakya in Hatay (Turkey). *Verbascum caesareum* was collected from Musa Mountain, Çevlik-Arsuz, Antakya in Hatay (Turkey). *Verbascum antiochium* and *Verbascum gaillardotti* were collected from near St. Pierre Church,Antakya in Hatay (Turkey). *Verbascum pinetorum* was collected from Samandağ, Antakya in Hatay (Turkey).

Preparation of the methanol extracts

The plant sample, weighing about 100 g was extracted with methanol at 40-45ºC for 2 hours (3 times). The filtrates were combined and concentrated in vacuo at 45ºC. Finally, the extracts were then lyophilized and kept in the dark at 4ºC until tested.

Experimental Stage

Initially optimum conditions were determined. *Verbascum* concentrations were arranged 6 mg/ml-3mg/ml-1,5mg/ml in 10% dimethylsulfoxide. 200 µl of the amount of extract for added in bacterial culture. PI (propidium iodide) or Fluorophores SYTO 9 concentration was applied at 10% in PBS buffer in *Listeria Innocua* bacteria, while PI was applied as 10% SYTO undiluted in *Escherichia coli*. In the experiments for *Listeria Innocua* bacteria, 2.5 µL PI (diluted) and 2.5 µl SYTO (diluted) were applied in a 500 µl bacteria culture. Operations were carried out in a 1-hour time unit at 70° C. The incubation process is for positive control. In the negative control, 200 µl of 10% dimethylsulfoxide was added. This application was applied in a period of 25°C. 2.5 µl PI (diluted) and 2.5 µl SYTO (undiluted) was applied in 500 µL bacteria culture for *Escherichia coli*. The incubation process was applied in one hour at 70°C. This process is for positive control. In the negative control, 200 µl 10% dimethylsulfoxide was added. The incubation was carried out at 25°C for 1 hour. All these trials were performed by incubating 6 mg/ml-3mg/ml-1,5mg/ml 10% Dimethylsulfoxide at 25°C for 1 hour for *Listeria Innocua* and *Escherichia coli* bacteria.

III. RESULTS AND DISCUSSION

Taking positive and negative controls of six species of *Verbascum* plants *(Verbascum Tripolitanum, Verbascum Sinuatum L, Verbascum Caesareum, Verbascum Antiochium, Verbascum Gaillardotti, and Verbascum Pinetorum)* against *Listeria Innocua* and *Escherichia Coli* bacteria, their antibacterial efficacy was demonstrated. After exposure incubated at 25°C for one hour, the herb extracts mediated staining of PI and SYTO 13 combined system were brighter and more uniform when the cell was treated at the increasing concentration. The negative and positive control of *Verbascum* species against *Listeria Innocua* bacteria is shown in figures (1, 3, 5, 7, 9 and 11) and the antibacterial activity of the extracts against

Listeria Innocua bacteria is shown as the percentage of dead cells in figures (2, 4, 6, 8, 10 and 12). As understood in all figures, all Verbascum species show antbacterial activity. When the results are examined, the right side shows live cells, while the left side shows dead cells. With reference to these figures, the lowest concentration of *Verbascum* species reveals that the number of viable cells is greater than the higher concentration (Figures 2-24, even-numbered figures). When the extract concentration in the bacterial culture reduced, the number of living cells increased.

Lischeria Innocua tests

Verbascum tripolitanum

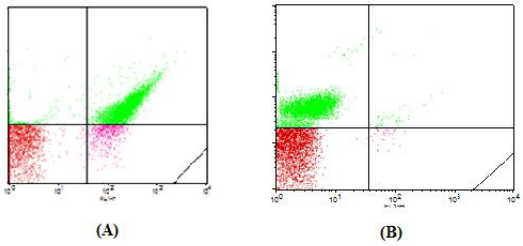

Fig.1: Negative (A) and positive (B) control of Listeria innocua

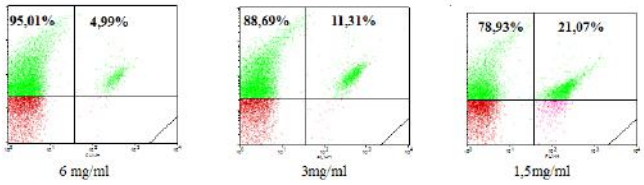

Fig.2: Percentage of dead cell in Listeria innocua bacterial culture.

According to Figure 2, when the drug concentration is 6 mg/mL, it is understood that 95.01% of the living *Listeria innocua* cells die and this ratio decreases as the concentration decreases. This ratio is 78.93% while the concentration is 1,5 mg/mL.

Verbascum sinuatum L

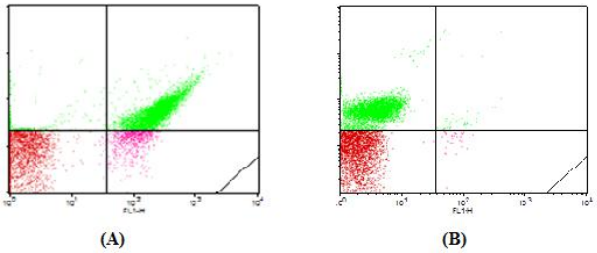

Fig.3: Negative (A) and positive (B) control of Listeria innocua

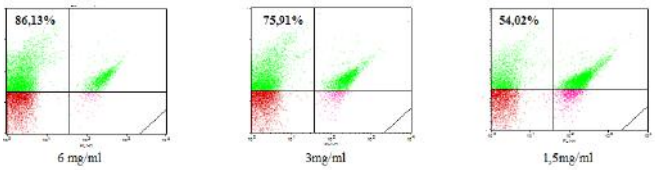

Fig.4: Percentage of dead cell in Listeria innocua bacterial culture.

According to Figure 4, when the drug concentration is 6 mg/mL, it is understood that 86,13% of the living *Listeria innocua* cells die and this ratio is 54,02% while the concentration is 1.5 mg/mL.

Verbascum caesareum

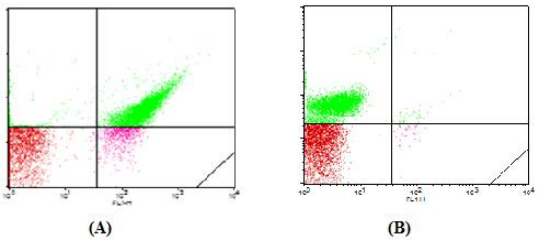

Fig.5: Negative (A) and positive (B) control of Listeria innocua.

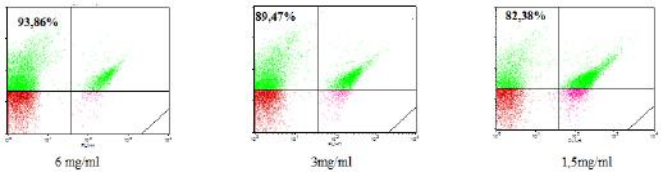

Fig. 6: Percentage of dead cell in Listeria innocua bacterial culture.

According to Figure 6, when the drug concentration is 6 mg/mL, it is understood that 93,86% of the living *Listeria innocua* cells die and this ratio is 82,38% while the concentration is 1.5 mg/mL.

Verbascum antiochium

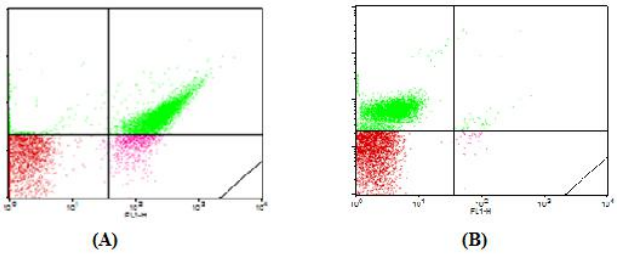

Fig.7: Negative (A) and positive (B) control of Listeria innocua

According to Figure 8, when the drug concentration is 6 mg/mL, it is understood that 93,15% of the living *Listeria*

innocua cells die and this ratio is 70,97% while the concentration is 1.5 mg/mL.

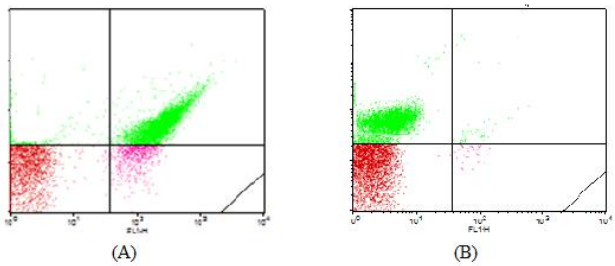

Fig.9. Negative (A) and positive (B) control of Listeria innocua

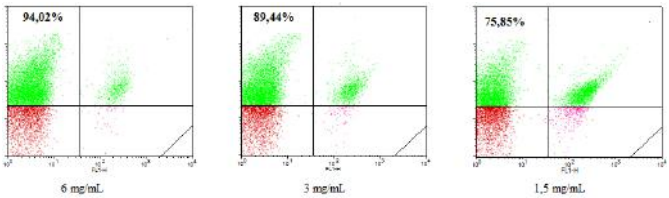

Fig.10. Percentage of dead cell in Listeria innocua bacterial culture

According to Figure 10, when the drug concentration is 6 mg/mL, it is understood that 94,02% of the living *Listeria innocua* cells die and this ratio is 75,85% while the concentration is 1.5 mg/mL.

Verbascum pinetorum

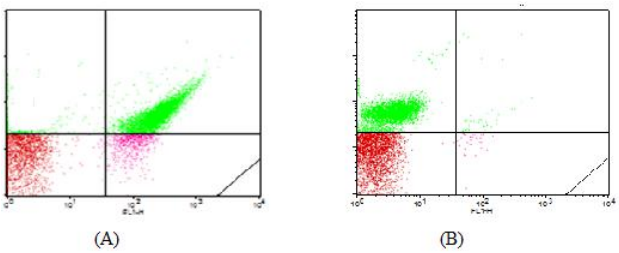

Fig.11: Negative (A) and positive (B) control of Listeria innocua

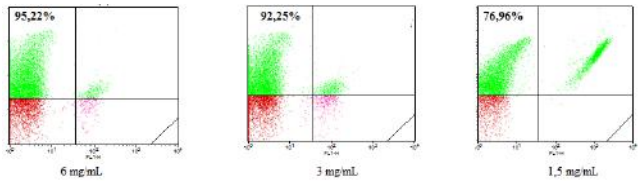

Fig.12: Percentage of dead cell in Listeria innocua bacterial culture.

According to Figure 12, when the drug concentration is 6 mg/mL, it is understood that 95,22% of the living *Listeria innocua* cells die and this ratio is 76,96% while the concentration is 1.5 mg/mL.

Table: Percentage of dead Listeria innocua bacteria cells for Verbascum species.

	6 mg/mL	3 mg/mL	1,5 mg/mL
V.tripolitanum	95,01	88,69	78,93
V.sinuatum L	86,13	75,91	54,02
V.caesareum	93,86	89,47	82,38
V.antiochium	93,15	89,33	70,97
V.gaillardotti	94,02	89,44	75.85
V.pinetorum	95,22	92,25	76,96

Escherichia coli tests

Verbascum tripolitanum

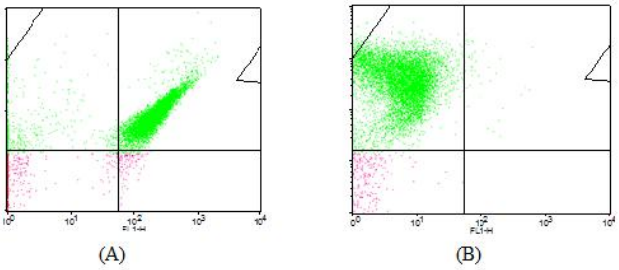

Fig.13: Negative (A) and positive (B) control of Escherichia coli.

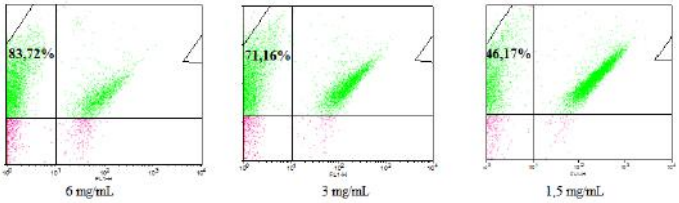

Fig.14: Percentage of dead cell in Escherichia coli bacterial culture.

According to Figure 14, when the drug concentration is 6 mg/mL, it is understood that 83,72% of the living *Escherichia coli* cells die and this ratio is 46,17% while the concentration is 1.5 mg/mL

Verbascum sinuatum L

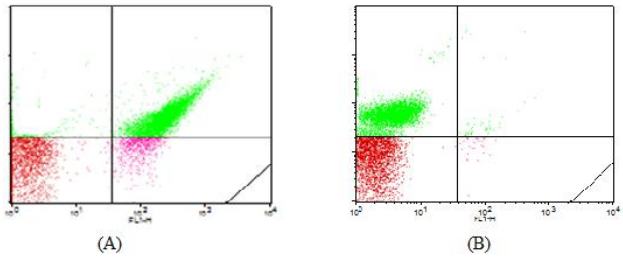

Fig.15: Negative (A) and positive (B) control of Escherichia coli

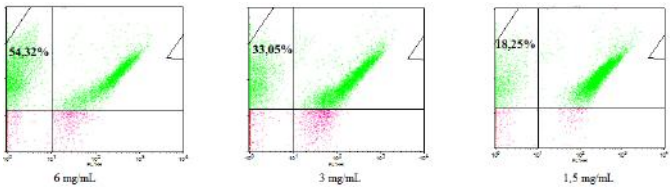

Fig.16: Percentage of dead cell in Escherichia coli bacterial culture

According to Figure 16, when the drug concentration is 6 mg/mL, it is understood that 54,32% of the living *Escherichia coli* cells die and this ratio is 18,25% while the concentration is 1.5 mg/mL.

Verbascum caesareum

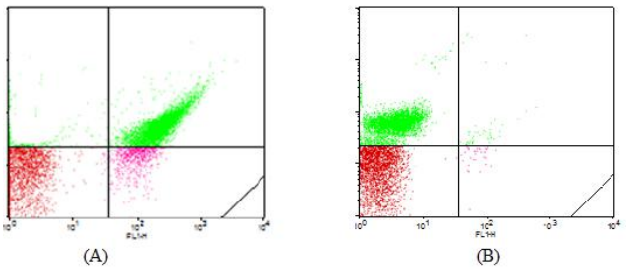

Fig.17. Negative (A) and positive (B) control of Escherichia coli

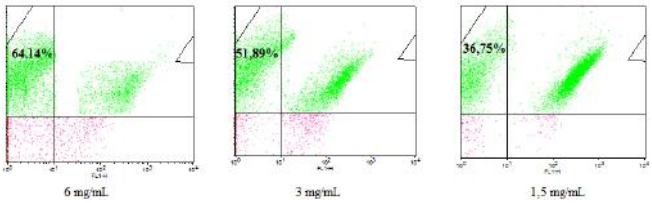

Fig.18: Percentage of dead cell in Escherichia coli bacterial culture

According to Figure 14, when the drug concentration is 6 mg/mL, it is understood that 64,14% of the living *Escherichia coli* cells die and this ratio is 36,75% while the concentration is 1.5 mg/mL.

Verbascum antiochium

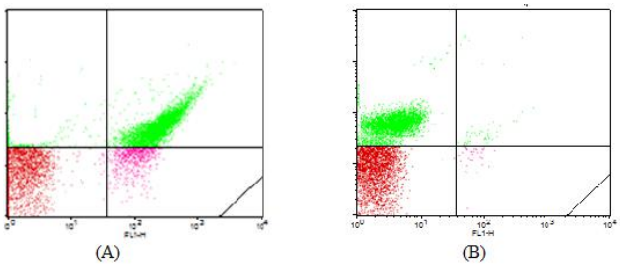

Fig.19: Negative (A) and positive (B) control of Escherichia coli

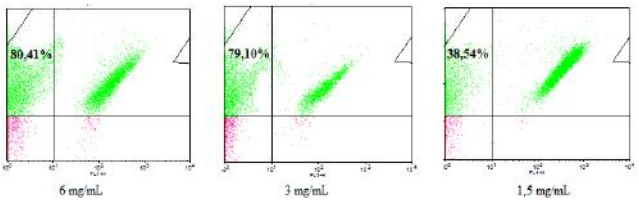

Fig.20. Percentage of dead cell in Escherichia coli bacterial culture.

According to Figure 20, when the drug concentration is 6 mg/mL, it is understood that 80,41% of the living *Escherichia coli* cells die and this ratio is 38,54% while the concentration is 1.5 mg/mL.

Verbascum gaillardotti

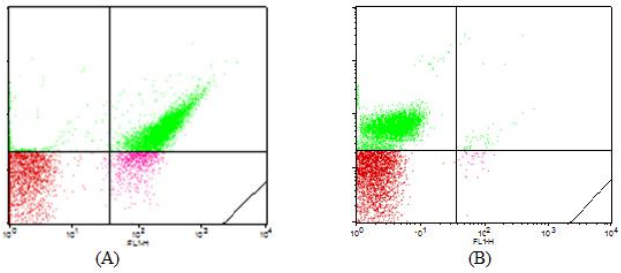

Fig.21: Negative (A) and positive (B) control of Escherichia coli

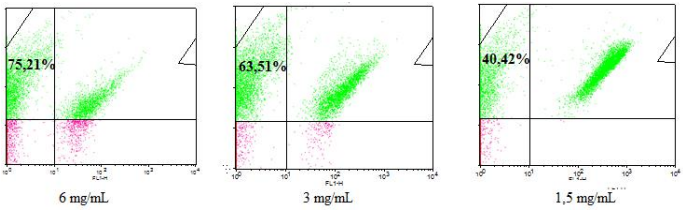

Fig.22: Percentage of dead cell in Escherichia coli bacterial culture

According to Figure 22, when the drug concentration is 6 mg/mL, it is understood that 75,21% of the living *Escherichia coli* cells die and this ratio is 40,42% while the concentration is 1.5 mg/mL.

Verbascum pinetorum

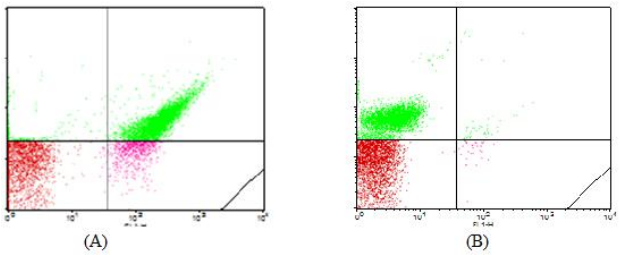

Fig.23: Negative (A) and positive (B) control of Escherichia coli

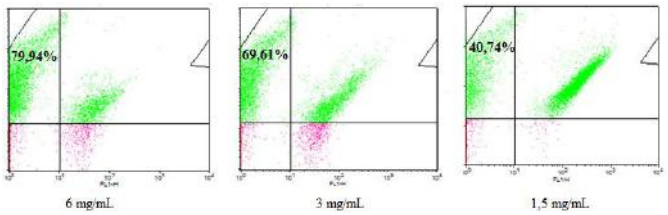

Fig.24: Percentage of dead cell in Escherichia coli bacterial culture

According to Figure 24, when the drug concentration is 6 mg/mL, it is understood that 79,94% of the living *Escherichia coli* cells die and this ratio is 40,74% while the concentration is 1.5 mg/mL.

Table 2: Percentage of dead Escherichia coli bacteria cells for Verbascum species

	6 g/mL	3 mg/mL	1,5 mg/mL
V. ripolitanum	83,72	71,16	46,17
V. sinuatum L	54,32	33,05	18,25
V.caesareum	64.14	51,89	36,75
V.antiochium	80,41	79,10	38.54
V.gaillardotti	75,21	63,51	40,42
V.pinetorum	79,94	69,61	40,74

Verbascum species studied in this study showed antibacterial effects for both *Listeria innocua* and *Escherichia coli*. However, when looking at the results given in Table 1 and Table 2, its resistance against *Listeria innocua* is higher than that of *Escherichia coli*. While *Verbascum pinetorum (*95,22%) showed the antibacterial effect against *Listeria innocua* at the highest concentration, it showed the lowest effect against *Verbascum sinuatum L. (*86,13%). While *Verbascum tripolitanum* (83,72%) showed the antibacterial effect against *Escherichia coli* at the highest concentration, it showed the lowest effect against *Verbascum sinuatum L*. (54,32%). *Verbascum sinuatum* showed the least resistant effect on both bacterial species.

IV. CONCLUSION

It can be said that the *Verbascum* species extract may disrupt the membrane barrier and allow exogeneous solutes such as PI and SYTO 13 to permeabilize into the bacterial cells. Secondary metabolites in the *Verbascum* species may have the ability to increase the permeability of bacterial membranes and allow antibiotics to access the bacterial targets. These results show that the flow cytometry method is more advantageous than traditional methods, such as disk diffusion and mikro dilution, for quickly generating a large amount of data. This technique can serve as a powerful tool in optimally combining different preservative factors in order to design an effective antimicrobial system for selected foods. As a result, This studyshows that methanolic extracts of *Verbascum* species is a potential source of natural antioxidants and antimicrobial agent and can form the basis for pharmacological studies.

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