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The Effect of Some Macrofungi from Turkey Extracts on Cytoplasmic Membrane of Multidrug Resistant Bacteria by Flow Cytometry

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ABSTRACT: In this study; some macrofungi extracts were investigated for their abilities to enhance bacterial permeability by flow cytometry. These experiments exhibited the enhancement of these extracts to disrupt the cytoplasmic membrane of living bacterial (Listeria innocua and Escherichia coli) cells. Antibacterial activity of various macrofungi samples (Pholiota Lucifera, Pleurotus Ostreatus, and Ganoderma Applanatum) against resistant Escherichia Coli and Listeria innocua bacteria was measured by Flow Cytometry. The samples were collected from various regions of Fungi Antakya, Alahan, and Derince of Hatay city of Turkey. These experiments were designed to detect uptake of PI & SYTO by enhancing with a ranged concentration of macrofungi extracts. For this purpose, macrofungi extracts were diluted in the presence of 10% dimethyl sulfoxide in the bacterial culture and incubated at 70 ^{0}C for positive control at 70 ^{0}C for positive control and at 25 ^{0}C for 1 hour for the negative control, and various samples were prepared. According to the results, the macrofungi Ganaderma applanatum, which shows the most antibacterial activity in the negative direction in Listeria innocua bacteria, is the least effective Pleurotus Ostreatus. In Escherichia coli bacteria, the most negative activity is Ganoderma Applanatum, while the least effective is Pholiota Lucifera

KEYWORDS: Macrofungi , flow cytometry, antibacterial effects, Lischeria Innocua, Escherichia Coli.

I. INTRODUCTION

The natural active compounds found in medicinal plants belong to various chemical structures including polyphenolic compounds, flavonoids, essential oils, and vitamins and some of these compounds have anticancer, antioxidant, and antimicrobial activity. However, these compounds have been little known about mechanisms to confer antibacterial drug resistance. In recent years, research on antimicrobial, anticancer, and antioxidant properties of plant research has increased by researchers. Phenolic compounds, which include functional derivatives such as phenolic acids, flavonoids, anthocyanins, and stilbenes, are functional structures that make up the largest family of all secondary metabolite classes[1]. Polyphenols act as antioxidant compounds on oxidative stress. Thus, they play an important role in scavenging reactive oxygen species (ROS), which is another equivalent of toxic free radicals and radicals [1,2]. Many antibacterial studies on various macrofungus species have been reported in the literatüre [3,4]. Pathogenic microorganisms have gained resistance to traditionally used antibacterial drugs. Therefore, the researchers studied plants, fungi, algae etc, and many natural sources investigated their antibacterial activities against microorganisms [15-21]. Naturally, in recent years, antibacterial drug studies and functional food studies related to macrofungi have taken place a lot in the literatüre [22]. It has been reported in the literature that wild mushrooms accumulate a wide variety of pharmacological agents in their bodies. These agents are known to have anticancer, immunostimulating, hypotensive, hypocholesterolemic and antibacterial effects [23–25]. Cultured mycelial macrofungi are prescribed to treat a variety of ailments in many countries, particularly China. The macrofungi species are Phellinus linteus (Teng), Phellinus ignarius (L)

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Quel. and Phellinus robusticus P. Karst. It has an important area of use in China, Phellinus rimosus (Berk.) Pilat. (Hymenochaetaceae), Ganoderma lucidum (Curt: Fr.) P. Karst. (Ganodermataceae) and Navesporus floccosa (Bres.) (Polyporaceae) macrofungi grow on trees. Phellinus rimosus, one of these macrofungi, has antioxidant, antitumor and anti-inflammatory propertie [25-29]. Likewise, this applies to Navesporus floccosa macrofungi. Cortinarius type fungi have about 2000 species in the world and are the most abundant macrofungi in the world. Taxonomic studies have been carried out on such macrofungi in Australia, distinguishing new species [30]. Beattie et al. studied the antibacterial metabolites of fungi of the Cortinarius type [31]. In the Philippines, macrofungi are generally found in the mountainous regions of the country, and approximately 4698 macrofungi species and 1031 of them have been described [32,33]. Studies on the antibacterial activity of wild Basidiomycota and Ascomycota fungi (Cyclocybe aegerita, Cortinarius traganus, Gyroporus castaneus, Neoboletus luridiformis, Rubroboletus Lupinus, Gyromitra esculenta and Helvella crispa) were conducted in France. Of these, the Cyclohexanic extract of Gyromitra esculenta was found to have the strongest efficacy [34]. Yamaç et al investigated mycelial and culture antioxidant activities of various mushrooms from Turkey [35]. In these and similar studies, antibacterial and antioxidant activities of many fungi from various countries have been reported [36–41]. In this study, antibacterial activities of some macrofungi (Pholiota Lucifera, Ganoderma Applanatum, and Pleurotus Ostreatus) belonging to some regions in Hatay province were tested against Listeria innocua and Escherichia coli bacteria using flow cytometry test measurements.

II. EXPERIMENTATION

Materials and methods

Macrofungi samples (Pholiota Lucifera, Ganoderma Applanatum, and Pleurotus Ostreatus)were collected by Baba. The voucher specimen is stored in the Fungarium at the Biology Department of Mustafa Kemal University. Pholiota Lucifera was collected from Derince road (200 m), Antakya in Hatay city Derince in Turkey, Ganoderma Applanatum was collected from Alahan road (100 m) Antakya in Hatay city in Turkey, and Pleurotus Ostreatus was collected from Derince road (100 m) Antakya in Hatay city in Turkey.

Preparation of the methanol extracts

The macrofungi 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 4 ºC until tested.

Extraction can also be done with the supercritical CO_2 method. This method can also be used, especially in order to prevent secondary metabolites from being affected by test conditions [42].

Experimental Stage

Initially optimum conditions were determined. Macrofungi samples (6 mg/ml-3mg/ml-1,5mg/ml, respectively) has been mixed in 10 % dimethyl sulfoxide, respectively. Then 200µL of extract for added to bacterial culture. PI (10%) and SYTO (10%) concentrations were prepared for Listeria innocua and PI (10%) and SYTO (undiluted) concentrations were prepared for Escherichia coli**.** PBS buffer was used for both Listeria innocua and 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. The samples were incubated in a 1-hour time unit at 70 °C for the positive control. In the negative control, 200 µl of 10% dimethylsulfoxide was added. This application was incubated for a period of 25 ° C for a 1-hour. In experiments for Escherichia coli, 2.5 µL PI (diluted) and 2.5 µl SYTO (undiluted) were applied in a 500 µl bacteria culture and incubated in a 1-hour at 70 ° C for the positive control. In the negative control, 200 µl of 10% dimethylsulfoxide was added and incubated for a 1-hour at 25 °C. Finally, in the presence of 10% dimethylsulfoxide, 6 mg/ml, 3mg/ml, and 1.5mg/ml macrofungi extracts were added separately and incubated at 25 °C for 1 hour, respectively.

III. RESULTS AND DISCUSSION

Taking the positive and negative controls of three species of macrofungi samples (Pholiota Lucifera, Ganoderma Applanatum, and Pleurotus Ostreatus) against Listeria Innocua and Escherichia Coli bacteria, their antibacterial efficacy was demonstrated.

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Listeria Innocua tests

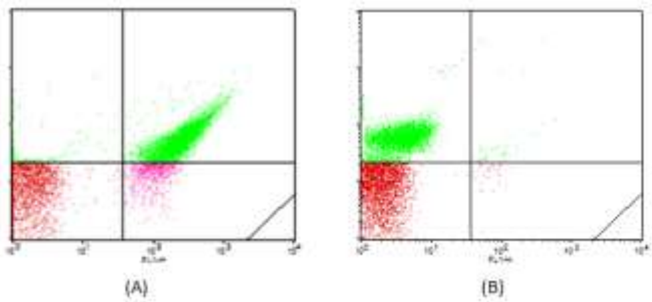

Fig. 1 Negative (A) and positive (B) control of Listeria innocua

Pholiota Lucifera (effect of Listeria innocua)

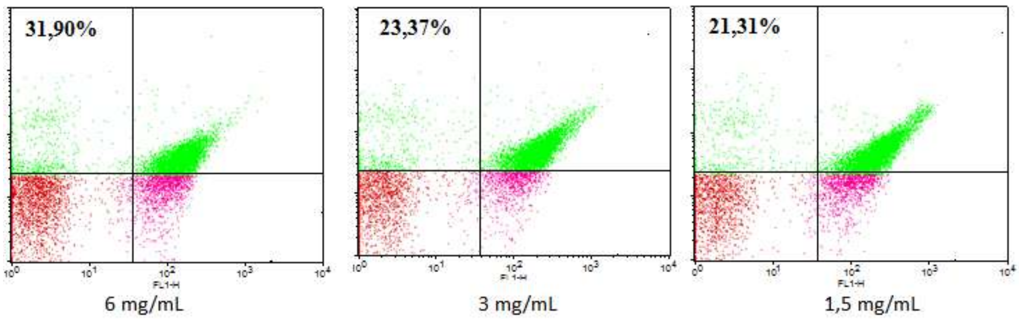

Fig. 2 Percentage of dead cell in Listeria innocua bacterial culture

The negative and positive control of macrofungi samples against Listeria Innocua bacteria is shown in fig 1. and the antibacterial activity of Pholiota Lucifera against Listeria Innocua bacteria is shown in fig. 2. According to fig. 2, when the drug concentration is 6 mg/mL, it is understood that 31,90% of the living Listeria innocua cells die and this ratio decreases as the concentration decreases. This ratio is 21,31% while the concentration is 1,5 mg/mL.

Pleurotus Ostreatus (effect of Listeria innocua)

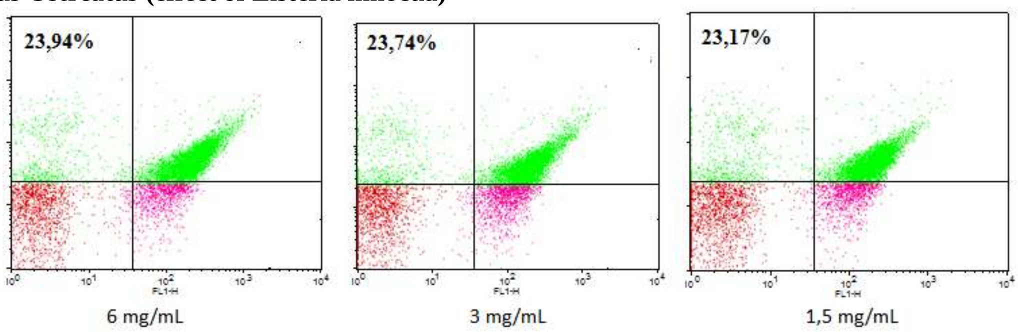

Fig.3 Percentage of dead cell in Listeria innocua bacterial culture

According to fig. 3, when the drug concentration is 6 mg/mL, it is understood that 23,94% of the living Listeria innocua cells die and this ratio decreases as the concentration decreases. This ratio is 23,17% while the concentration is 1,5 mg/mL.

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Gnaoderma Applanatum (effect of Listeria innocua)

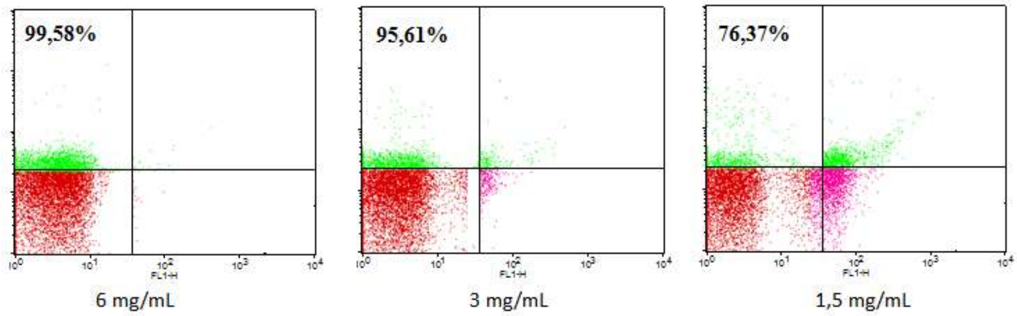

Fig 4. Percentage of dead cell in Listeria innocua bacterial culture

Gnaoderma Applanatum was a very interesting fungus in this study. In fig. 4, when the drug concentration is 6 mg/mL, it is understood that 99,58% of the living Listeria innocua cells die and this ratio decreases as the concentration decreases. This ratio is 76,37% while the concentration is 1,5 mg/mL.

	6 mg/mL	3 mg/mL	1,5 mg/mL
Pholiota Lucifera	31,90	23,37	21,31
Pleurotus Ostreatus	23,94	23,74	23,17
Gnaoderma Applanatum	99,58	95,61	76,37

Escherichia coli tests

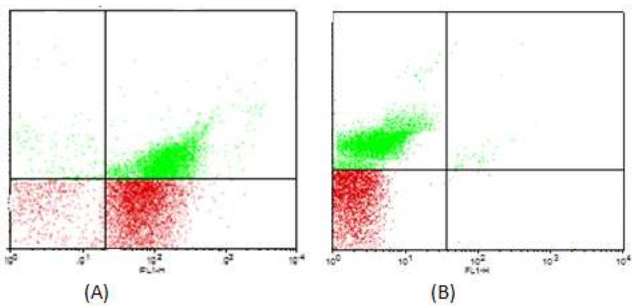

Fig. 5 Negative (A) and positive (B) control of Escherichia coli

Pholiota Lucifera (effect of E-coli)

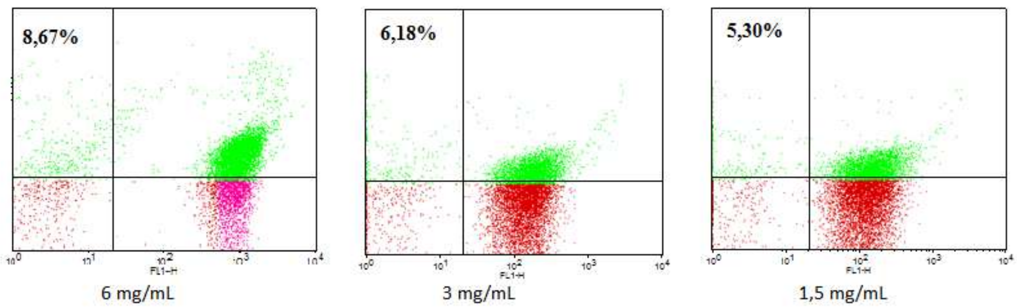

Fig. 6 Percentage of dead cell in E-coli bacterial culture

According to fig.6, when the drug concentration is 6 mg/mL, it is understood that 8,67% of the living Listeria innocua cells die and this ratio decreases as the concentration decreases. This ratio is 5,30% while the concentration is 1,5 mg/mL.

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Pleurotus Ostreatus (effect of E-coli)

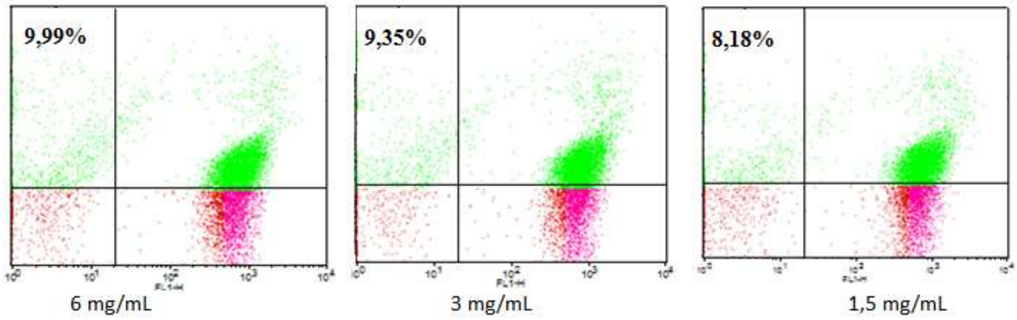

Fig. 7 Percentage of dead cell in E-coli bacterial culture

Fig. 7 showed that when the drug concentration is 6 mg/mL, it is understood that 9,99% of the living Listeria innocua cells die and this ratio decreases as the concentration decreases. This ratio is 8,18% while the concentration is 1,5 mg/mL.

Gnaoderma Applanatum (effect of E-coli)

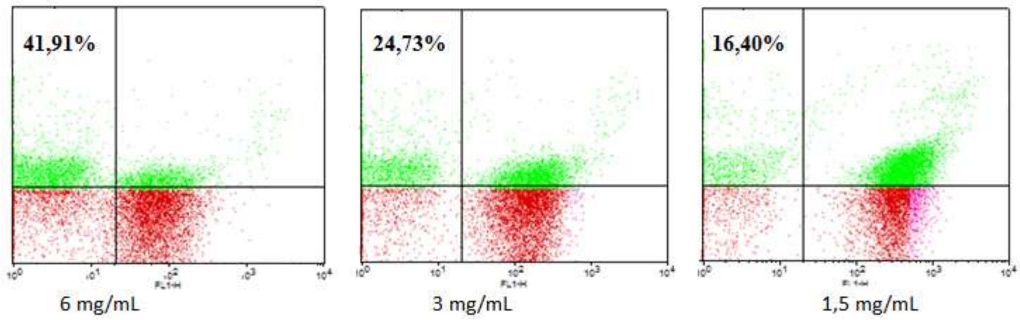

Fig. 8 Percentage of dead cell in E-coli bacterial culture

Ganoderma Applanatum showed remarkable results against E-coli bacteria, just like in Listeria innocua. According to fig. 8, when the drug concentration is 6 mg/mL, it is understood that 41,910% of the living Listeria innocua cells die and this ratio decreases as the concentration decreases. This ratio is 16,40% while the concentration is 1,5 mg/mL.

	6 mg/mL	3 mg/mL	1,5 mg/mL
Pholiota Lucifera	8,67	6,18	5,30
Pleurotus Ostreatus	9,99	9,35	8,18
Gnaoderma Applanatum	41,91	24,73	16,40

Listeria monocytogenes is a widespread pathogen that can be found in water, silage, sewage, slaughterhouse waste, cow's milk, human and animal feces. Just like E-coli, it is a bacterium that poses a danger to human health [41]. In this study, especially the effect of Gnaoderma Applanatum on Listeria innocua is quite remarkable. It appears to kill 99.58% of Listeria innocua bacteria at the highest concentration (6mg/mL) (Table 1). It was determined that it was effective at a rate of 41.91% on E-coli. It has been understood that while it has an effect of approximately 20 percent on Pholiota Lucifera and Pleurotus Ostreatus Listeria innocua at all three concentrations, it is not very effective on E-coli and has an effect below ten percent. Looking at these results, Gnaoderma Applanatum showed remarkable differences. With this feature, it is understood that it can have an important function especially in food control and protection and can be evaluated in the food industry.

IV. CONCLUSION

The antibacterial test activity was observed against bacteria in the cytoplasmic membrane (Lischeria Innocua and Escherichia Coli) by flow cytometry of 3 species of macrofungi (Pholiota Lucifera, Pleurotus Ostreatus, and Ganoderma applanatum). In the results obtained, the macrofungi species Ganoderma Applanatum shows the highest negative efficiency in Lischeria

Innocua and Escherichia Coli bacteria. The macrofungi species that show the least negative activity in Lischeria Innocua bacteria is the Pleurotus Ostreatus genus. In Escherichia Coli bacteria, the macrofungi genus showing the least negative activity is Pholitota Lucifer. In antibacterial activity tests in these macrofungi species, it showed a more resistant property against Escherichia Coli bacteria against Lischeria Innocua bacteria.

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