© by PSP Volume 31– No. 09/2022 pages 9346-9354 Fresenius Environmental Bulletin

OBSERVATION OF THE ANTIBACTERIAL ACTIVITY AND TIME-KILL KINETICS OF SOME POLYPORES MACROFUNGI EXTRACTS

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ABSTRACT

Natural products produce compounds having the potential of being used against various diseases. It is important to investigate new antimicrobial compounds as a reason for the widespread drug resistance. In this study, the antibacterial potentials of three macrofungi extracts, namely *Fomes fomentarius* (L.) Fr., *Phellinus hartigii* (Allesch. & Schnabl) Pat., and *Fomitopsis pinicola* (Sw.) P. Karst., which were extracted by using six different solvents (water, methanol, chloroform, acetone, petroleum ether, and n-hexane), and examined against twelve different *Escherichia coli* strains, where one of these strains was a standard strain (*E. coli* ATCC 25922) and eleven strain were clinical isolates presenting different resistance profiles. The antibacterial activity of the extracts was determined by using minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) tests. In addition, a time-kill kinetics test was conducted for selected extracts. As a result, it was observed that extracts have either bacteriostatic or bacteriocidal activity against *E. coli* strains. Further research should be done to reveal the chemical composition of the extracts and their mode of action.

KEYWORDS:
Antimicrobial activity, *Fomes fomentarius*, *Phellinus hartigii*, *Fomitopsis pinicola*, minimum inhibition concentration, time-kill kinetics

INTRODUCTION

Wild macrofungi are natural resources that have economic and nutritional value. Several scientific studies show that they are one of the non-timber forest products having economic importance in the world, due to being consumed commonly by human beings as food [1]. On the other hand, wild macrofungi also have therapeutic properties, thus the pharmaceuticals obtained from wild macrofungi are commonly used in both developing and developed countries against several diseases [2].

The medicinal properties of macrofungi are known and used throughout the ages. Mushroom extracts are known to present biological activities, such as immuno-stimulating, anticancer, and anti-inflammatory activities, which were used in folk medicine in many ancient far east countries [3]. Today, they are still in use as complementary and alternative medicine in most of the developed countries [4].

World Health Organization (WHO) strictly proposed that antibacterial resistance, which has a tremendous increase, will be a major threat to human health in the 21st century. To avoid the spreading of antibiotic-resistant infections, scientists have been intensively working on discovering new antibacterial compounds [5-7], and macrofungi are one of the resources, which can be used to extract antibacterial compounds [8].

Fomes fomentarius is a macrofungi, which acts both as a decomposer and a parasite and is distributed mostly in the northern hemisphere of the world [9,10]. Several studies are presenting the medicinal uses of *F. fomentarius* in traditional Slavic and East Asian medicine [11]. Different preparations of *F. fomentarius* are known to be used against various cancer types, inflammation, hepatocyriosis, gastroenteric disorders, and oral ulcers. In addition, the antimicrobial property of this macrofungi was proven previously [12].

Phellinus hartigii is a saprophytic macrofungus like most other polypores. Several pharmaceutical uses of this fungus are known and due to its biological activities, *P. hartigii is* commonly used in traditional Asian medicine [13].

Fomitopsis species led to brown rotting in live or dead hardwoods and conifers [14]. Previous studies show that *F. pinicola* has antimicrobial activity [15,16].

In the present study, the antibacterial activity of three macrofungi extracts, namely *Fomes fomentarius* (L.) Fr., *Phellinus hartigii* (Allesch. & Schnabl) Pat., and *Fomitopsis pinicola* (Sw.) P. Karst., which was extracted by using six different solvents (water, methanol, chloroform, acetone, petroleum ether, and n-hexane), were examined against twelve different *Escherichia coli* strains.

MATERIALS AND METHODS

Collection of macrofungi samples. *Fomes fomentarius* (L.) Fr.*, Phellinus hartigii* (Allesch. & Schnabl) Pat., and *Fomitopsis pinicola* (Sw.) P. Karst samples were collected from Istanbul Belgrad Forest, Ilgaz Mountain National Park, and İzmit Yuvacık respectively.

Microorganisms. The antimicrobial activity of the extracts was tested against twelve different *Escherichia coli* strains, where one of them is a standard strain (*E. coli* ATCC 25922), and the rest of them are multidrug-resistant (MDR) clinical strains with different resistance profiles.

Inocula preparation. The inoculum of each bacteria was prepared by transferring the morphologically similar colonies of each bacteria to adjust the turbidity of a 0.9% (w/v) sterile NaCl solution by comparing a 0.5 McFarland standard.

Extraction process. Macrofungi samples were ground into a fine powder using a laboratory grinding mill. 20 g of *P. hartigii* was weighed into a flask and 200 mL of petroleum ether was added into the flask, and the flask was placed on a shaker (WiseShake, Korea) and shaken at room temperature with a speed of 100 rpm for 3 days [17,18]. After three days the mixture was filtrated into evaporating flasks. These flasks were attached to a rotary evaporator (Heidolph, Germany) and the solvents in the extract were removed by rotating samples at 35 to 45 ºC. The same procedure was repeated with n-hexane, chloroform, acetone, methanol, and distilled water (dH_2O) successively. The dH_2O in the extracts was removed by a freeze dryer (Hanil, Korea). The same series of steps was repeated for *F. fomentarius* and *F. pinicola*.

The remnants of the extracts were dissolved in 10 mL of 1% DMSO and transferred into air-tight containers, labeled, and stored at -18 °C until use. The stock extracts were filtered through syringe filters (0.45 µm) before use.

Determination of minimum inhibition concentration. Minimum inhibition concentrations (MICs) of each extract were determined by a serial microdilution method in 96-well plates as described in detail in previous studies [19]. The 96 well-plates were incubated at 37 °C for 24 hours and the MIC values were determined as the minimum extract concentrations, which inhibit the bacterial growth completely.

Determination of minimum bactericidal concentration. The minimum bactericidal concentrations (MBCs) were determined by transferring a loopful of samples from each well of 96-well plates presenting no growth onto nutrient agar plates and incubated for a further 24 hours at 37 °C. Then, the agar plates were examined for growth or no growth by the naked eye. MBC values were determined as the minimum extract concentrations that completely kill the bacteria.

Time-kill kinetics test. Time-kill kinetics of the selected extract presenting activity in the MIC test was observed by the protocol defined by Tsuji et al [20]. This test was used to show the bacteriostatic or bactericidal activities of the extracts on the *E. coli* strains over time. MIC, 2x MIC, and 4x MIC concentrations were used and the change in the absorbance at 600 nm of each well was recorded at half an hour time intervals for 24 hours, and a graph was plotted for absorbance vs time [21].

Statistics. All tests were performed in triplicate and with negative controls. The results were statistically analyzed using a one-way analysis of variance (ANOVA). Means were evaluated depending on a test of normality by the Duncan and Kruskal-Wallis multiple tests using SPSS (v.25). Values were considered significant at $P < 0.05$.

RESULTS

The efficiency of the extraction process. After removing all solvents in the extracts, the efficiency of the extraction process was observed to be between (0.0380 - 2.0235 %), (0.4955 - 5.4470 %), and (0.9000 - 5.5955 %) for *F. fomentarius*, *P. hartigii,* and *F. pinicola* respectively as given in Table 1.

TABLE 1
The efficiency of the extraction process (%)

	Mushrooms		
Extracts	***F. fomentarius***	***P. hartigii***	***F. pinicola***
Petroleum Ether	2.0235	0.9745	4.9750
n-Hexane	0.0380	0.6120	5.2545
Chloroform	0.8455	0.6935	5.0025
Acetone	0.6680	0.4955	5.5955
Methanol	1.2535	5.4470	5.3010
dH_2O	0.9400	3.3150	0.9000

TABLE 2
MIC values for *P. hartigii* extracts (mg/mL)

	Petroleum Ether	n-Hexane	Chloroform	Acetone	Methanol	dH_2O
E. coli 1*	2.43	3.06	3.47	2.48	6.81	16.58
E. coli 2	4.87	3.06	3.47	4.96	13.62	33.15
E. coli 3	4.87	3.06	3.47	2.48	27.24	16.58
E. coli 4	4.87	3.06	6.94	2.48	13.62	33.15
E. coli 5	4.87	6.12	3.47	2.48	6.81	16.58
E. coli 6	4.87	3.06	6.94	2.48	13.62	16.58
E. coli 7	4.87	6.12	6.94	2.48	13.62	33.15
E. coli 8	4.87	3.06	3.47	2.48	27.24	16.58
E. coli 9	4.87	3.06	3.47	4.96	13.62	16.58
E. coli 10	4.87	3.06	6.94	2.48	27.24	33.15
E. coli 11	4.87	3.06	3.47	2.48	27.24	33.15
E. coli 12	2.43	3.06	3.47	2.48	13.62	33.15

* *E. coli* ATCC 25922

MIC values of extracts. The data in Table 2 shows that the extract obtained from *P. hartigii* by petroleum ether had antibacterial activity against all *E. coli* strains with MIC values either 2.43 or 4.87 mg/mL, while the MIC values for n-hexane extract were either 3.06 or 6.12 mg/mL, chloroform extract was either 3.47 or 6.94 mg/mL, acetone extract was either 2.48 or 4.96 mg/mL, methanol extract was between 6.81 and 27.24 mg/mL and dH_2O extract was either 16.58 or 33.15 mg/mL.

MIC test results for *F. pinicola* extracts, which were given below in Table 3 clearly shows that the extract obtained from *F. pinicola* by petroleum ether had antibacterial activity against all *E. coli* strains with the MIC values ranging between 12.44 and 49.75 mg/mL, while the MIC values for n-hexane extract were either 26.27 or 52.55 mg/mL, chloroform extract was between 6.25 and 50.03 mg/mL, acetone extract was either 13.99 or 27.98 mg/mL, methanol extract was between 6.63 and 26.51 mg/mL and dH_2O extract was either 4.50 or 9.00 mg/mL.

While the MIC test results for *F. fomentarus* extracts, which were given below in Table 4 shows that the extract obtained from *F. fomentarus* by petroleum ether had antibacterial activity against all *E. coli* strains with MIC values either 5.06 or 10.12 mg/mL, while the MIC values for n-hexane extract were either 0.10 or 0.38 mg/mL, chloroform extract was 2.11 mg/mL against all bacteria, acetone extract was either 1.67 or 3.34 mg/mL, methanol extract was between 3.13 and 12.54 mg/mL and dH_2O extract was either 4.70 or 9.40 mg/mL.

MBC values of extracts. The results of the MBC test for *P. hartigii* extracts, which were given below in Table 5 shows that the extract obtained from *P. hartigii* by petroleum ether had antibacterial activity against all *E. coli* strains with MBC values either 9.75 or 19.49 mg/mL, while the MBC values for n-hexane extract were either 3.06 or 12.24 mg/mL, chloroform extract was between 3.47 and 13.87 mg/mL, acetone extract was either 4.96 or 9.91 mg/mL, methanol extract was between 13.62 and 54.47 mg/mL and dH_2O extract was ranging between 16.58 and 66.30 mg/mL.

TABLE 3
MIC values for *F. pinicola* extracts (mg/mL)

	Petroleum Ether	n-Hexane	Chloroform	Acetone	Methanol	dH_2O
E. coli 1*	24.88	26.27	25.01	13.99	6.63	9.00
E. coli 2	24.88	26.27	50.03	27.98	13.25	9.00
E. coli 3	24.88	52.55	50.03	27.98	26.51	4.50
E. coli 4	24.88	26.27	25.01	13.99	13.25	9.00
E. coli 5	49.75	52.55	25.01	27.98	13.25	9.00
E. coli 6	24.88	26.27	25.01	13.99	6.63	4.50
E. coli 7	24.88	52.55	50.03	13.99	13.25	9.00
E. coli 8	24.88	26.27	50.03	27.98	13.25	4.50
E. coli 9	12.44	52.55	25.01	13.99	6.63	9.00
E. coli 10	24.88	26.27	25.01	13.99	13.25	9.00
E. coli 11	24.88	26.27	6.25	13.99	6.63	9.00
E. coli 12	12.44	52.55	25.01	27.98	6.63	9.00

* *E. coli* ATCC 25922

TABLE 4
MIC values for *F. fomentarus* extracts (mg/mL)

	Petroleum Ether	n-Hexane	Chloroform	Acetone	Methanol	dH_2O
E. coli 1*	5.06	0.19	2.11	1.67	6.27	9.40
E. coli 2	10.12	0.19	2.11	1.67	12.54	9.40
E. coli 3	10.12	0.19	2.11	3.34	12.54	4.70
E. coli 4	10.12	0.19	2.11	3.34	6.27	9.40
E. coli 5	10.12	0.19	2.11	3.34	6.27	9.40
E. coli 6	10.12	0.19	2.11	3.34	6.27	4.70
E. coli 7	10.12	0.38	2.11	3.34	12.54	9.40
E. coli 8	10.12	0.19	2.11	3.34	6.27	9.40
E. coli 9	5.06	0.19	2.11	1.67	3.13	9.40
E. coli 10	10.12	0.19	2.11	3.34	3.13	9.40
E. coli 11	5.06	0.10	2.11	3.34	6.27	9.40
E. coli 12	10.12	0.19	2.11	3.34	6.27	9.40

* *E. coli* ATCC 25922

TABLE 5
MBC values for *P. hartigii* extracts (mg/mL)

	Petroleum Ether	n-Hexane	Chloroform	Acetone	Methanol	dH_2O
E. coli 1*	9.75	3.06	6.94	4.96	13.62	16.58
E. coli 2	19.49	12.24	13.87	9.91	54.47	33.15
E. coli 3	19.49	12.24	13.87	9.91	27.24	33.15
E. coli 4	19.49	12.24	13.87	9.91	54.47	66.30
E. coli 5	19.49	12.24	13.87	9.91	27.24	33.15
E. coli 6	9.75	12.24	13.87	9.91	54.47	33.15
E. coli 7	9.75	12.24	13.87	9.91	27.24	33.15
E. coli 8	19.49	12.24	13.87	9.91	54.47	16.58
E. coli 9	19.49	12.24	6.94	4.96	27.24	33.15
E. coli 10	19.49	12.24	13.87	9.91	27.24	66.30
E. coli 11	9.75	12.24	13.87	9.91	27.24	66.30
E. coli 12	19.49	12.24	3.47	4.96	27.24	33.15

* *E. coli* ATCC 25922

The results of the MBC test for *F. pinicola* extracts, which were given below in Table 6 shows that the extract obtained from *F. pinicola* by petroleum ether had antibacterial activity against all *E. coli* strains with MBC values ranging between 24.88 and 99.50 mg/mL, while the MBC values for n-hexane extract were either 26.27 or 105.09 mg/mL, chloroform extract was either 25.01 or 100.05 mg/mL, acetone extract was either 55.96 or 111.91 mg/mL, methanol extract was between 26.51 and 106.02 mg/mL and dH_2O extract was ranging between 4.50 and 18.00 mg/mL.

TABLE 6
MBC values for *F. pinicola* extracts (mg/mL)

	Petroleum Ether	n-Hexane	Chloroform	Acetone	Methanol	dH_2O
E. coli 1*	49.75	26.27	25.01	55.96	26.51	9.00
E. coli 2	99.50	105.09	100.05	111.91	106.02	18.00
E. coli 3	99.50	105.09	100.05	111.91	53.01	9.00
E. coli 4	99.50	105.09	100.05	111.91	106.02	18.00
E. coli 5	99.50	105.09	100.05	111.91	53.01	18.00
E. coli 6	99.50	105.09	100.05	111.91	106.02	18.00
E. coli 7	99.50	105.09	100.05	111.91	53.01	18.00
E. coli 8	99.50	105.09	100.05	111.91	26.51	4.50
E. coli 9	49.75	105.09	100.05	111.91	26.51	9.00
E. coli 10	99.50	105.09	100.05	111.91	26.51	9.00
E. coli 11	99.50	105.09	100.05	111.91	53.01	18.00
E. coli 12	24.88	105.09	100.05	111.91	53.01	9.00

* *E. coli* ATCC 25922

TABLE 7
MBC values for *F. fomentarus* extracts (mg/mL)

	Petroleum Ether	n-Hexane	Chloroform	Acetone	Methanol	dH_2O
E. coli 1*	10.12	0.38	8.46	3.34	6.27	9.40
E. coli 2	40.47	0.76	16.91	13.36	12.54	9.40
E. coli 3	20.24	0.76	16.91	6.68	12.54	4.70
E. coli 4	40.47	0.76	16.91	13.36	25.07	9.40
E. coli 5	40.47	0.76	16.91	6.68	12.54	9.40
E. coli 6	40.47	0.76	16.91	13.36	12.54	9.40
E. coli 7	20.24	0.38	16.91	6.68	12.54	9.40
E. coli 8	40.47	0.19	16.91	3.34	6.27	18.80
E. coli 9	20.24	0.76	8.46	6.68	3.13	9.40
E. coli 10	40.47	0.76	16.91	6.68	6.27	9.40
E. coli 11	10.12	0.76	16.91	13.36	12.54	9.40
E. coli 12	10.12	0.38	8.46	3.34	12.54	9.40

* *E. coli* ATCC 25922

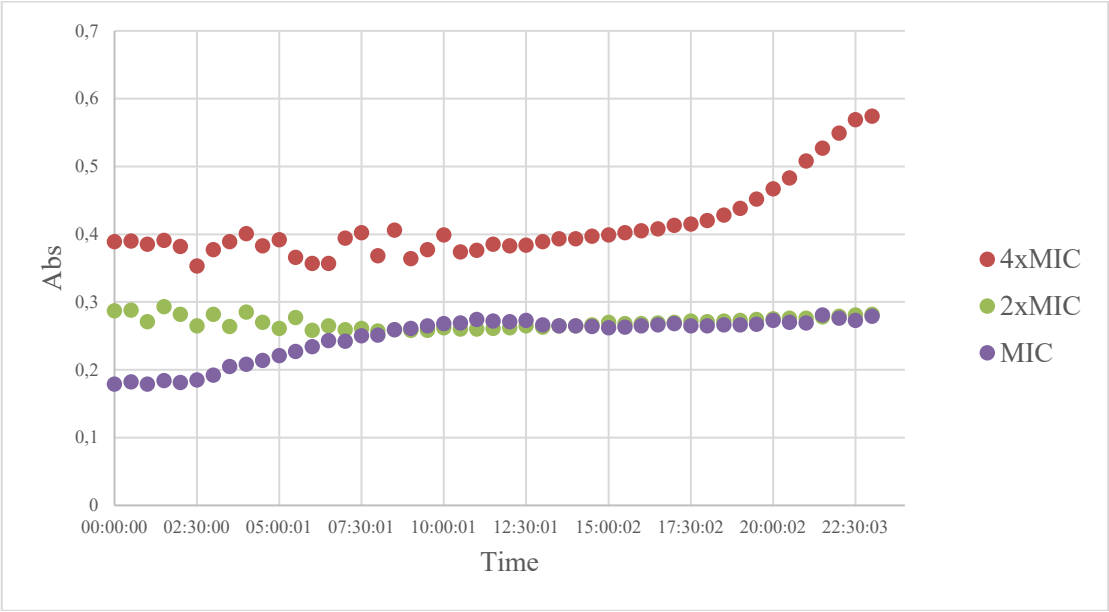

FIGURE 1
Time-kill kinetics results of *F. fomentarus* methanol extract against *E. coli* 9.

The results of the MBC test for *F. fomentarus* extracts, which were given below in Table 7 shows that the extract obtained from *F. fomentarus* by petroleum ether had antibacterial activity against all *E. coli* strains with MBC values ranging between 10.12 and 40.47 mg/mL, while the MBC values for n-hexane extract were between 0.19 and 0.76 mg/mL, chloroform extract was either 8.46 or 16.91 mg/mL, acetone extract was between 3.34 and 13.36 mg/mL, methanol extract was between 3.13 and 25.07 mg/mL and dH_2O extract was ranging between 4.70 and 18.80 mg/mL.

Time-kill kinetics test results. For the time-kill kinetics test, methanol extract of *F. fomentarius* against *E. coli* 9 was chosen, and the results are given in Figure 1.

The time-kill kinetics profile of *F. fomentarus* methanol extract against *E. coli* 9, at test concentrations of 1x MIC, 2x MIC, and 4x MIC showed that at 1x MIC concentration the absorbance was stable in the first 2 and a half hours. After 2 and a half hours a slight increase in the absorbance was observed until 10 hours of incubation, and after this point, the absorbance trend seems to be stable. 2x MIC concentration was observed to cause a slight decrease in the first 7 hours and after this point, the absorbance trend seems to be stable again in 1x MIC concentration. The pattern in the change of absorbance in 4x MIC was slightly different than other concentrations. In 4x MIC concentration a slight decrease was observed in the first 6 hours, between this point and 18 hours of incubation a minor increase trend was observed. But after this point, a sharp increase was observed.

Statistical analysis. The statistical analysis presented that there is no statistically significant difference was present between the triplicate results (p

> 0.05). The area under the curve (AUC) for methanol extract of *F. fomentarus* against *E. coli* 9, at the studied test concentrations, revealed that the difference between the effect of different concentrations of *F. fomentarus* on *E. coli* 9 growth was significantly different.

DISCUSSION

Keepers et al. [22] proposed that the antibacterial agent is accepted to be bacteriostatic when the MBC/MIC ratio is equal to and greater than 4 and accepted to be bactericidal when this ratio is less than 4.

The MBC/MIC ratio of *F. fomentarus* extracts, *P. hartigii* extracts, and *F. pinicola* extracts are given in Table 8, 9, and 10 respectively.

Table 8 presents that the dH_2O extract of *F. fomentarus* showed bactericidal activity against all bacteria, while petroleum ether extract against *E. coli* 1, *E. coli* 3, *E. coli* 7, *E. coli* 11, and *E. coli* 12, n-hexane extract against *E. coli* 1, *E. coli* 7, *E. coli* 8, and *E. coli* 12, acetone extract *E. coli* 1, *E. coli* 3, *E. coli* 5, *E. coli* 6, *E. coli* 7, *E. coli* 8, *E. coli* 9, *E. coli* 10 and *E. coli* 12, and methanol extract against all bacteria except *E. coli* 4. Other *F. fomentarus* extracts against other bacteria presented bacteriostatic activity.

According to Table 9, the dH_2O extract of *P hartigii* has bactericidal activity against all bacteria, whereas petroleum ether extract against *E. coli* 6, *E. coli* 7, and *E. coli* 11, n-hexane extract against *E. coli* 1, *E. coli* 5, and *E. coli* 7, chloroform extract against *E. coli* 1, *E. coli* 4, *E. coli* 6, *E. coli* 7, *E. coli* 9, *E. coli* 10 and *E. coli* 12, acetone extract against *E. coli* 1, *E. coli* 2, *E. coli* 9 and *E. coli* 12, and methanol against *E. coli* 1, *E. coli* 3, *E. coli* 7, *E. coli* 8, *E. coli* 9, *E. coli* 10, *E. coli* 11, and *E. coli* 12. Other extract solution combinations exhibited bacteriostatic activities.

TABLE 8
MBC/MIC ratio of *F. fomentarus* extracts against *E. coli* strains

	Petroleum Ether	n-Hexane	Chloroform	Acetone	Methanol	dH_2O
E. coli 1*	2	2	4	2	1	1
E. coli 2	4	4	8	8	1	1
E. coli 3	2	4	8	2	1	1
E. coli 4	4	4	8	4	4	1
E. coli 5	4	4	8	2	2	1
E. coli 6	4	4	8	2	2	1
E. coli 7	2	1	8	1	1	1
E. coli 8	4	1	8	2	1	2
E. coli 9	4	4	4	2	1	1
E. coli 10	4	4	8	2	2	1
E. coli 11	2	8	8	4	2	1
E. coli 12	1	2	4	1	2	1

* *E. coli* ATCC 25922

TABLE 9
MBC/MIC ratio of *P. hartigii* extracts against *E. coli* strains

	Petroleum Ether	n-Hexane	Chloroform	Acetone	Methanol	dH_2O
E. coli 1*	4	1	2	2	2	1
E. coli 2	4	4	4	2	4	1
E. coli 3	4	4	4	4	1	2
E. coli 4	4	4	2	4	4	2
E. coli 5	4	2	4	4	4	2
E. coli 6	2	4	2	4	4	2
E. coli 7	2	2	2	4	2	1
E. coli 8	4	4	4	4	2	1
E. coli 9	4	4	2	1	2	2
E. coli 10	4	4	2	4	1	2
E. coli 11	2	4	4	4	1	2
E. coli 12	8	4	1	2	2	1

* *E. coli* ATCC 25922

TABLE 10
MBC/MIC ratio of *F. pinicola* extracts against *E. coli* strains

	Petroleum Ether	n-Hexane	Chloroform	Acetone	Methanol	dH_2O
E. coli 1*	2	1	1	4	4	1
E. coli 2	4	4	2	4	8	2
E. coli 3	4	2	2	4	2	1
E. coli 4	4	4	4	8	8	2
E. coli 5	2	2	4	4	4	2
E. coli 6	4	4	4	8	16	4
E. coli 7	4	2	2	8	4	2
E. coli 8	4	4	2	4	2	1
E. coli 9	4	2	4	8	4	1
E. coli 10	4	4	4	8	2	1
E. coli 11	4	4	16	8	8	2
E. coli 12	2	2	4	4	8	1

* *E. coli* ATCC 25922

In the same way, Table 10 shows that the distilled water extract of *F. pinicola* has bactericidal activity against all bacteria except *E. coli* 6, while the petroleum ether extract against *E. coli* 1, *E. coli* 5, and *E. coli* 12, n-hexane extract against *E. coli* 1, *E. coli* 3, *E. coli* 5, *E. coli* 7, *E. coli* 9, and *E. coli* 12, chloroform extract against *E. coli* 1, *E. coli* 2, *E. coli* 3, *E. coli* 7, *E. coli* 8, and *E. coli* 12, and the methanol extract showed bactericidal activity against *E. coli* 3, *E. coli* 8, and *E. coli* 10 bacteria. Other extract solution combinations exhibited bacteriostatic activities.

In the present study, all three macrofungi extracts were found to have changing degrees of antibacterial activity against the *E. coli* strains. The results presented that MIC and MBC values differ from extract to extract against different *E. coli* strains. The n-hexane extract of *F. fomentarius*, which has MIC and MBC values of 0.19 mg/mL against *E. coli* 8, presented the most significant antibacterial activity as compared to other extracts.

The MIC values of *F. fomentarus* showed that all extracts against all bacteria presented good antibacterial activity, but especially the n-hexane extract presented the best activity against all strains. On other hand, the MIC values for *F. fomentarus* showed that methanol extract against *E. coli* 2, *E. coli* 3, and *E. coli* 7 had low antibacterial activity.

In this study, the MIC values for the *F. fomentarius* water extract were observed to be either 4.70 or 9.40 mg/mL. On the other hand, the MIC value for the methanol extract was found to be between 3.13 and 12.54 mg/mL.

According to the results, it can be proposed that the antibacterial activity of *F. fomentarius* water extract and *F. fomentarius* methanol extract presented equal or better antibacterial activity against *E. coli* when compared to the report published by Kolundžić et al. [23], Jiang et al. [24], and Dundar et al. [25].

Altuner and Akata [26] found that the extract of *P. hartigii* was inactive against *E. coli*. The main reason for the difference in results is probably based on the *E. coli* strains used in these two studies, which were different from each other. On the other hand, secondary metabolites and their concentrations in the fungi, the location of collection, and collection time, which could affect the secondary metabolite composition would also be important in the activity they present.

In this study, the MIC value for the n-hexane extract of *F. pinicola* was observed to be either 26.27 or 52.55 mg/mL. On the other hand, the MIC value for the methanol extract was found to be between 6.63 and 26.51 mg/mL, while the MBC values for the n-hexane extract were either 26.27 or 105.09 mg/mL, and the methanol extract was between 26.51 and 106.02 mg/mL.

The results in this recent study are similar to the report published by Bala et al. [15] previously, which showed that n-hexane extracts presented better antibacterial results against *E. coli* only at higher concentrations. In addition, methanol extract had the second-lowest MIC value compared to others, which is similar to the results reported by Khadhri et al. [16].

Pala et al. [27] tested the antimicrobial potential of some mushroom extracts against some commonly found pathogenic bacteria and fungi strains. When the results obtained in this present study are compared to the results proposed by Pala et al, it can be concluded that the results regarding *F. pinicola* extracts obtained in this present study showed better antibacterial activity. The main reason for this difference in these results is the *E. coli* strains used in these two studies were different. In addition, secondary metabolites and their concentrations in the macrofungi samples, the location of collection, and collection time could also affect the activity.

On the other hand, the time-kill kinetics test shows that the antibacterial activity of *F. fomentarus* methanol extract was variable against *E. coli* 9 depending on the concentration, as seen in Figure 1.

This study revealed that the extract leads to a decrease in bacterial growth at 2x MIC and 4x MIC in the first 6 to 7 hours of incubation. To the best of our knowledge, this is the first study that has been

conducted on *F. fomentarus* by using time-kill kinetics.

CONCLUSION

Medicinal mushrooms are accepted as one of the alternatives to the treatment of infectious diseases. They are also a good source for new anti-infective agents against many pathogenic microorganisms. This recent study has the potential to contribute to the literature about the pharmaceutical properties of macrofungi.

All macrofungi extracts were found to be active against microorganisms, but *F. fomentarius* and *P. hartigii* n-hexane extracts were the most active extracts according to MIC and MBC values. According to the results, it is possible to propose that *F. fomentarius* and *F. pinicola* methanol extracts could contain bioactive compounds, which may serve as potential antibacterial agents. But further detailed studies are required to isolate and identify the bioactive compounds from these macrofungi.

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Received: **10.05.2021**
Accepted: **25.03.2022**

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