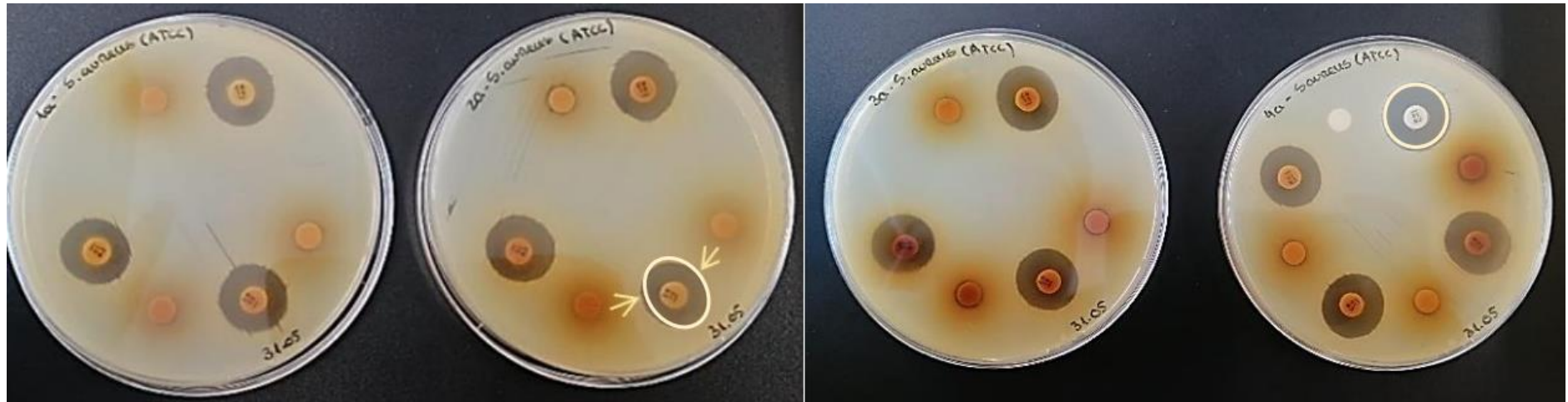**Figure S1.** Antibacterial assay *S. aureus* (ATCC 23235) example of the antagonistic effect of the extracts on the antibiotic used.

Table S1. Polyphenol Composition Analysis in Spent Mushroom Substrate Extracts: Impact of Solvent and Extraction Method

Samples	Total Phenols (mg GA g^{-1})	Ortho-diphenols (mg GA g^{-1})	Flavonoids (mg CAT g^{-1})
Solvent			
w	97.60 ± 2.46[b]	319.93 ± 4.26[b]	40.20 ± 1.35[b]
et	156.98 ± 2.13[a]	395.74 ± 4.41[a]	57.53 ± 1.35[a]
Extraction method			
SE	103.10 ± 4.12[cd]	242.80 ± 7.63[e]	242.80 ± 7.63[c]
LE	98.52 ± 3.69[d]	260.89 ± 6.83[e]	260.89 ± 6.83[c]
LE-HT	190.61 ± 4.12[a]	517.07 ± 7.63[a]	517.07 ± 7.63[a]
UE	111.37 ± 3.69[c]	347.17 ± 8.36[c]	347.17 ± 8.36[b]
COMB	103.52 ± 4.12[cd]	323.16 ± 6.83[d]	323.16 ± 6.83[b]
COMB-HT	156.63 ± 4.12[b]	455.91 ± 7.63[b]	455.91 ± 7.63[a]

w, distilled water; et, ethanol 50% (v/v) solvent; SE, short extraction; LE, 24 h extraction; LE-HT, 24 h extraction with high temperature; UE, ultrasound extraction; COMB, combined methods (LE+UE); COMB-HT, combined methods (LE-HT+UE). The presented values are means ± standard deviation of triplicate measurements. Means in the same column with different letters were significantly different ($P<0.05$, ANOVA, Tukey-HSD).

Table S2. Results of the evaluation of the antioxidant capacity of the spent mushroom substrate extracts, by solvent and by extraction method.

Samples	ABTS$^{\bullet+}$ (mmol Trolox g^{-1})	DPPH$^{\bullet}$ (mmol Trolox g^{-1})	FRAP (mmol Trolox g^{-1})
Solvent			
w	0.487 ± 0.010[b]	0.327 ± 0.008[b]	0.675 ± 0.012[a]
et	0.665 ± 0.011[a]	0.777 ± 0.007[a]	0.696 ± 0.012[a]
Extraction method			
SE	0.409 ± 0.017[c]	0.525 ± 0.013[c]	0.570 ± 0.020[c]
LE	0.510 ± 0.017[b]	0.463 ± 0.013[d]	0.573 ± 0.022[c]
LE-HT	0.762 ± 0.021[a]	0.855 ± 0.014[a]	1.031 ± 0.020[a]
UE	0.533 ± 0.019[b]	0.411 ± 0.014[e]	0.563 ± 0.025[c]
COMB	0.538 ± 0.017[b]	0.475 ± 0.014[d]	0.521 ± 0.020[c]
COMB-HT	0.703 ± 0.019[a]	0.584 ± 0.014[b]	0.857 ± 0.020[b]

w, distilled water; et, ethanol 50% (v/v) solvent; SE, short extraction; LE, 24 h extraction; LE-HT, 24 h extraction with high temperature; UE, ultrasound extraction; COMB, combined methods (LE+UE); COMB-HT, combined methods (LE-HT+UE). The presented values are means ± standard deviation of triplicate measurements. Means in the same column with different letters were significantly different ($P<0.05$, ANOVA, Tukey-HSD).

Table S3. Diameter measurements for each extract alone compared with the positive control (gentamicin).

	S. aureus (ATCC 23235)	*A. hydrophila* (C2GSPA1)	*P. aeruginosa* (C3GSPR1)	*V. fluvialis* (RimA1TCBS)
Control (+)	18.0[a]	18.0[a]	12.0[a]	23.0[a]
w-SE	0.0[c]	8.0[e]	8.0[d]	7.0[c]
et-SE	7.0[b]	0.0[j]	9.0[c]	0.0[d]
w-LE	0.0[c]	9.0[d]	9.0[c]	7.0[c]
et-LE	7.0[b]	8.0[e]	8.0[d]	0.0[d]
w-LE-HT	0.0[c]	9.0[c]	9.0[c]	8.0[b]
et-LE-HT	7.0[b]	10.0[b]	10.0[b]	7.0[c]
w-UE	7.0[b]	8.0[f]	8.0[d]	0.0[d]
et-UE	0.0[c]	0.0[j]	9.0[c]	0.0[d]
w-COMB	0.0[c]	7.0[g]	8.0[d]	0.0[d]
et-COMB	0.0[c]	0.0[i]	7.0[f]	0.0[d]
w-COMB-HT	0.0[c]	8.0[f]	8.0[d]	0.0[d]
et-COMB-HT	7.0[b]	7.0[h]	8.0[e]	7.0[c]

Means (n=2) in the same column with different letters were significantly different (P<0.05, ANOVA, t-student). Caption: "w" distilled water, "et" ethanol 50% (v/v) solvent, "SE" short extraction, "LE" 24h extraction, "LE-HT" 24h extraction with high temperature, "UE" ultrasound extraction, "COMB" combined methods (LE+UE), "COMB-HT" combined methods (LE-HT+UE).

Table S4. Halo measurements for the extracts according to solvent and extraction method used.

	S. aureus (ATCC 23235)	A. hydrophila (C2GSPA1)	P. aeruginosa (C3GSPR1)	V. fluvialis (RimA1TCBS)
Solvent				
w	1.17[b]	8.17[a]	8,17[b]	3.67[a]
Et:H$_2$0	4.67[a]	4.17[b]	8.67[a]	2.33[b]
Method				
SE	3.5[a]	4.0[d]	8.5[b]	3.5[b]
LE	3.5[a]	8.5[b]	8.5[b]	3.5[b]
LE-HT	3.5[a]	9.5[a]	9.5[a]	7.5[a]
UE	3.5[a]	4.0[d]	8.5[b]	0.0[c]
COMB	0.0[b]	3.5[e]	7.5[d]	0.0[c]
COMB-HT	3.5[a]	7.5[c]	8.0[c]	3.5[b]

Means (n=2) in the same column with different letters were significantly different (P<0.05, ANOVA, t-student). Caption: "w" distilled water, "et" ethanol 50% (v/v) solvent, "SE" short extraction, "LE" 24h extraction, "LE-HT" 24h extraction with high temperature, "UE" ultrasound extraction, "COMB" combined methods (LE+UE), "COMB-HT" combined methods (LE-HT+UE).

Table S5. Halo measurements for the extracts combined with gentamicin (CN10) compared with the positive control (gentamicin).

	S. aureus (ATCC 23235)	*S. aureus* (C511)	*S. aureus* (C612)	*E. faecium* (C1)	*E. faecium* (C14)	*E. coli* (ATCC 25922)	*A. hydrophila* (C2GSPA1)	*P. aeruginosa* (C3GSPR1)	*V. fluvialis* (RimA1TCBS)
Control (+)	18.0^b	15.0^b	17.0^a	17.0^{ab}	16.0^c	16.0^a	18.0^a	12.0^c	23.0^a
w-SE	16.0^d	13.0^d	16.5^{ab}	15.0^d	15.0^d	0.0^j	16.0^c	9.0^e	20.0^c
et-SE	17.0^c	13.0^d	15.5^b	15.0^d	15.0^d	14.0^d	16.0^c	10.0^d	20.0^d
w-LE	16.0^d	14.0^c	16.5^{ab}	16.0^c	16.0^c	0.0^j	17.0^b	9.0^e	20.0^d
et-LE	17.0^c	13.0^d	16.5^{ab}	16.5^{bc}	16.5^{bc}	14.0^c	15.0^d	10.0^d	21.0^b
w-LE-HT	17.0^c	15.0^b	17.0^a	16.0^c	16.0^c	7.0^i	18.0^a	13.0^a	20.0^d
et-LE-HT	16.0^d	14.0^c	16.5^{ab}	16.0^c	16.0^c	12.0^f	14.0^e	9.0^e	20.0^d
w-UE	17.0^c	14.0^c	16.5^{ab}	16.0^c	16.0^c	16.0^a	16.0^c	9.0^e	20.0^d
et-UE	17.0^c	13.0^d	16.0^{ab}	17.0^{ab}	17.0^{ab}	15.0^b	15.0^d	9.0^e	20.0^d
w-COMB	16.0^d	14.0^c	16.5^{ab}	16.5^{bc}	16.5^{bc}	13.0^e	16.0^c	9.0^e	20.0^d
et-COMB	17.0^c	13.0^d	16.0^{ab}	16.0^c	16.0^c	12.0^g	15.0^d	9.0^e	19.0^e
w-COMB-HT	17.0^c	14.0^c	16.5^{ab}	16.5^{bc}	16.5^{bc}	13.0^e	16.0^c	9.0^e	20.0^d
et-COMB-HT	18.0^a	15.0^a	16.5^{ab}	17.5^a	17.5^a	9.0^h	16.0^c	12.0^b	20.0^d

Means (n=2) in the same column with different letters were significantly different (P<0.05, ANOVA, t-student). Caption: "w" distilled water, "et" ethanol 50% (v/v) solvent, "SE" short extraction, "LE" 24h extraction, "LE-HT" 24h extraction with high temperature, "UE" ultrasound extraction, "COMB" combined methods (LE+UE), "COMB-HT" combined methods (LE-HT+UE).

Table S6. Halo measurements for the extracts combined with gentamicin according to solvent and extraction method used.

	S. aureus (ATCC 23235)	*S. aureus* (C511)	*S. aureus* (C612)	*E. faecium* (C1)	*E. faecium* (C14)	*E. coli* (ATCC 25922)	*A. hydrophila* (C2GSP$_{A1}$)	*P. aeruginosa* (C3GSP$_{R1}$)	*V. fluvialis* (RimA1TCBS)
Solvent									
H_2O	16.50[b]	14.00[a]	16.58[a]	16.00[a]	16.00[a]	8.17[b]	16.50[a]	9.67[b]	20.08[a]
Et:H_2O	17.00[a]	13.50[b]	16,17[a]	16.33[a]	16.33[a]	12.67[a]	15.17[b]	9.83[a]	20.00[a]
Method									
SE	16.50[c]	13.00[c]	16.00[a]	15.00[c]	15.00[c]	7.00[e]	16.00[a]	9.50[c]	20.25[ab]
LE	16.50[c]	13.50[b]	16.50[a]	16.25[b]	16.25[b]	7.00[e]	16.00[a]	9.50[c]	20.50[a]
LE-HT	16.50[c]	14.50[a]	16.75[a]	16.00[b]	16.00[b]	9.50[d]	16.00[a]	11.00[a]	20.00[b]
UE	17.00[b]	13.50[b]	16.25[a]	16.50[ab]	16.50[ab]	15.50[a]	15.50[b]	9.00[d]	20.00[b]
COMB	16.50[c]	13.50[b]	16.25[a]	16.25[b]	16.25[b]	12.50[b]	15.50[b]	9.00[d]	19.50[c]
COMB-HT	17.50[a]	14.50[a]	16.50[a]	17.00[a]	17.00[a]	11.00[c]	16.00[a]	10.50[b]	20.00[b]

Means (n=2) in the same column with different letters were significantly different ($P<0.05$, ANOVA, t-student). Caption: "w" distilled water, "et" ethanol 50% (v/v) solvent, "SE" short extraction, "LE" 24h extraction, "LE-HT" 24h extraction with high temperature, "UE" ultrasound extraction, "COMB" combined methods (LE+UE), "COMB-HT" combined methods (LE-HT+UE).