SUPPLEMENTARY MATERIAL

Water-soluble extracts from edible mushrooms *(Agaricus bisporus)* as inhibitors of the hepatitis C viral replication

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Table 1S. Reagents, antibodies and primers used in this study

Product	Cat.no	Company

ß-actin antibody	#4967	Cell Signaling
Core	ALX-804-277	Enzo Life Science
NS3 antibody	Anti-NS3(JFH-1) Monoclonal Clone 2E3	Bioffrontech
Goat Anti-Mouse IgG (H+L)-HRP	#172-1011	BioRad
Goat Anti-Rabbit IgG-HRP	#170-6515	BioRad
Hs_RRN18S_1_SG QuantiTect Primer Assay	QT00199367	QIAGEN
JFH-2 fwd/rev	Fwd-CTGTGAGGAACTACTGTCT Rev-CGCCCTATCAGGCAGTACCA	NON-COMMERCIAL
SensoLyte520 HCV Protease Assay Kit	#71145	AnaSpec, Inc.
HCV NS3/4A protease genotype 1b, recombinant	AS-61017-5	AnaSpec, Inc.
HCV NS3/4A protease genotype 2a, recombinant	AS-72167-5	AnaSpec, Inc.
HCV NS3/4A protease genotype 3a, recombinant	AS-72168-5	AnaSpec, Inc.
QuantiTect Rev. Transcription Kit	205311	QIAGEN
M-PER® Mammalian Protein Extraction Reagent,	78501	Thermo Scientific
WesternBright™ ECL,	K-12045	Advansta
Quercetin dihydrate	HWI00580	HWI ANALYTIK GmbH

Table 2S. Viability and toxicity in Huh7.5 cells using different concentrations of mushroom extract (*Ab*AEE extract)

*Ab*AEE concentration (mg/ml)	Total cell count	Viable cell count	Death cell Count	Viability	Toxicity
10	13.6 x 10^6	1.3 x 10^6	12.3 `x 10^6	9.55 %	90.44 %
5	16.3 x 10^6	3.1 x 10^6	13.2 x 10^6	19.01 %	80.98 %
2	18.8 x 10^6	9.1 x 10^6	9.7 x 10^6	48.40 %	51.59 %
1	23.6 x 10^6	20.2 x 10^6	3.4 x 10^6	85.59 %	14.40 %
0.2	25.2 x 10^6	23.8 x 10^6	1.4 x 10^6	94.44 %	5.55 %
0.1	25.9 x 10^6	25 x 10^6	0.9 x 10^6	96.52 %	3.47 %

Table 3S. Viability and toxicity in LX2 cells using different concentrations of mushroom extract (*Ab*AEE extract)

AB concentration (mg/mL)	Total cell count	Viable cell count	Death cell Count	Viability (%)	Cell death (%)
10	14.8×10^6	1.8×10^6	13.0×10^6	12.16	87.83
5	18.2×10^6	3.6×10^6	14.6×10^6	19.78	80.21
2	23.8×10^6	13.6×10^6	10.2×10^6	57.14	42.85
1	25.6×10^6	23.2×10^6	2.4×10^6	90.62	9.37
0.2	27.6×10^6	26.3×10^6	1.3×10^6	95.28	4.71

Table 4S. List of metabolites identified in the different fractions of *Ab*AEE.

Metabolite	MS spectra (m/z)	Product ions (m/z)	UF-3 kDa	AqF-3 kDa	LLE-OrgF_s	Delgado-Povedano et

						al., 2016
Alkaloid derivative						
2-Methylpyrrolidine	86.0969	43.0539 41.0390	+	+	-	+
Alkylamines						
Pantothenic acid	220.1178	90.0556 72.0443	+	+	-	+
Spermidine	146.1651	84.0811 72.0809	+	+	-	+
Pyrroline						
1-Pyrroline	70.0652	43.0542 41.0385	+	+	-	+
Amino acids and derivatives						
2,5-Dihydrophenyl-alanine	168.1019	126.0551 81.0707	-	-	+	+
2-Aminobutyric acid	102.0562	102.0562	+	+	-	+
3-Amino-2-naphthoic acid	188.0703	170.0594 142.0651	+	+	-	+
3-Amino-3-(4-hydroxyphenyl) propanoate	180.0665	119.0495 93.0340	+	+	-	+
5-Aminopentanoate	118.0864	74.0588 58.0625	+	+	-	+
Acetylcarnitine	204.1231	85.0288 60.0809	+	+	-	+
Citrulline	176.1031	113.0708 70.0650	+	+	-	+
Cycloleucine	130.0863	84.0809	+	+	-	+

		67.0540				
Cystathionine	223.0746	134.0267 88.0212	+	+	-	+
Ergothioneine	230.0957	127.0322 60.0809	+	+	+	+
L-2-Aminoadipic acid	160.0616	116.0720 98.0609	+	+	-	+
L-Agaritine	268.1292	84.0439 77.0389	+	+	-	+
L-Arginine	173.1040	131.0823 112.0868	+	+	-	+
L-Asparagine	133.0610	74.0233 46.0283	+	+	-	+
L-Aspartic acid	132.0302	88.0405 71.0139	+	+	-	+
L-Carnitine	162.1124	103.0389 60.0808	+	+	-	+
L-Glutamate	148.0602	85.0284 56.0494	+	+	-	+
L-Glutamine	147.0761	84.0442 56.0493	+	+	-	+
L-Isoleucine	132.1019	86.0967 69.0699	+	+	-	+
LL-2,6-Diamino heptanedioate	189.0886	84.0444 74.0237	+	+	-	+

L-Leucine	132.1020	86.0967 44.0492	+	+	-	+
L-Lysine	147.1129	84.0809 56.0492	-	+	-	+
L-Methionine	150.0585	61.0105 56.0496	+	-	-	+
L-Phenylalanine	166.0860	120.0804 103.0544	+	+	-	+
L-Proline	116.0703	70.0652 43.0539	+	+	-	+
L-Serine	104.0353	74.0243 42.0356	+	+	-	+
L-Threonine	120.0651	74.0599 56.0495	+	+	-	+
L-Tryptophan	205.0972	146.0600 118.0649	+	+	-	+
L-Tyrosine	182.0806	136.0753 91.0542	+	+	-	+
L-Valine	118.0863	72.0809 55.0543	+	+	-	+
N2-Acetyl-L-ornithine	175.1078	116.0692 70.0653	+	+	-	+
N6-Acetyl-L-2,6-diamino heptanedioate	233.1134	87.0433 84.0465	+	+	-	+

N-Carbamylglutamate	189.0518	128.0347 102.0560	+	+	-	+
Nε-Acetyl-L-lysine	189.1230	126.0909 84.0811	+	+	-	+
Ornithine	133.0973	116.0708 70.0651	-	+	-	+
Pipecolic acid	130.0864	84.0810 56.0493	+	+	-	+
Pyroglutamic acid	128.0353	128.0352 82.0294	+	+	-	+
Saccharopine	277.1393	130.0864 84.0809	+	+	-	+
Peptides						
Cyclo(L-phe-L-pro)	245.1286	120.0802 70.0654	-	-	+	+
Oxidized glutathione	611.1446	306.0762 272.0886	+	+	-	+
γ-D-Glutamylglycine	205.0822	142.0494 84.0442	+	+	-	+
Quinoline derivative						
5,6-Dihydroxy-3-methyl-2-oxo-1,2,5,6-tetrahydroquinoline	194.0812	120.0455 57.0332	-	-	+	+
Indoles						
Formyl índole	146.0596	118.0649 91.0543	-	-	+	+

Indole-3-acetate	174.0559	144.0439 130.0656	-	-	+	+
Benzaldehyde						
4-Hydroxy benzaldehyde	123.0444	95.0484 77.0381	+	+	-	+
Carboxylic acids and derivatives						
2,2-Dymethyl succinic acid	145.0505	101.0602 83.0504	+	+	-	+
3-Hydroxy-3-methyl-glutaric acid	161.0454	59.0143 57.0349	+	+	-	+
Citric acid	191.0195	111.0093 87.0089	+	+	-	+
Lactic acid	89.0246	43.0188 41.0032	+	+	-	+
Malic acid	133.0142	72.9928 71.0135	+	+	-	+
Succinic acid	117.0198	73.0297 55.0194	-	+	-	+
Cinnamic acid derivative						
p-Coumaric acid	165.0542	147.0442 119.0493	+	+	-	+
Disaccharides						
Cellobionic acid	357.1043	113.0233 89.0233	+	+	-	+
***α*-1,5-L-Arabinobiose**	281.0876	89.0244 71.0132	+	+	-	+
Monosaccharides						
Sedoheptulose-7-	289.0332	96.9693	+	+	-	+

phosphate		78.9592				
D-Glucosamine 6-phosphate	258.0385	171.0060 78.9615	-	+	-	+
D-Glucose 6-phosphate	259.0226	96.9693 78.9592	+	+	-	+
D-Mannitol	183.0862	69.0337 57.0330	+	+	-	+
D-Mannitol 1-phosphate	261.0379	96.9694 78.9591	+	+	-	+
Sedoheptulose	209.0664	59.0128 57.0335	+	+	-	+
***α*-D-Glucose**	179.0556	71.0141 59.0134	+	+	-	+
Sugar acids and derivatives						
Galactonic acid	195.0511	75.0090 59.0139	+	+	-	+
Glucuronic acid	193.0349	75.0088 59.0139	+	+	-	+
Glyceric acid	105.0189	59.0143 56.9850	+	+	-	+
2-Dehydro-3-deoxy-L-arabinonate	147.0300	85.0284 57.0335	+	+	-	+
Glycerol-2-phosphate	171.0066	96.9688 78.9594	+	+	-	+
Xylonate	165.0402	75.0089 59.0140	+	+	-	+

Fatty acids and conjugates						
2-Hydroxyisocaproic acid	131.0715	85.0660 69.0340	+	+	-	+
3-Hydroxycapric acid	187.1341	59.0138 41.0031	-	-	+	+
6,7-Epoxystearic acid	297.2438	297.2427 185.1178	-	-	+	+
9,10-Dihydroxystearic acid	315.2541	298.2502 44.9971	-	-	+	+
9,10-Epoxy-18-hydroxystereate	313.2382	113.0961 99.0804	-	-	+	+
Epsilcapramine	132.1020	59.0724 132.1018	+	+	-	+
Stearic acid	283.2646	283.2638	-	-	+	+
***α*-Licanic acid**	293.2089	116.0505 45.0336	-	-	+	+
Fatty amides						
13-Docosenamide	338.3418	83.0855 57.0697	-	-	+	+
Oleamide	282.2793	71.0861 57.0693	-	-	+	+
Sphingolipids						
C16 Sphinganine	274.2739	256.2622 106.0853	-	-	+	+
Sphinganine	302.3058	284.2943 57.0692	-	-	+	+
Phospholipid and derivative						

Phosphocholine	184.0731	124.9997 86.0967	+	+	-	+
Glycero phosphocholine	258.1099	124.9996 104.1070	+	+	-	+
No metal oxoanionic compounds						
Phosphate	96.9694	96.9690 78.9594	+	+	-	+
Phosphite	78.9588	78.9599 62.9646	+	+	-	+
Phenol derivative						
p-Aminophenol	110.0601	93.0336 65.0387	-	+	+	+
Purine nucleoside derivative						
Xanthosine	283.0650	151.0260	+	+	-	+
Purine nucleotides and derivatives						
3´-AMP	346.0559	211.0014 78.9590	+	+	-	+
AMP	348.0702	136.0619 97.0285	+	+	-	+
ADP	426.0220	158.9250 78.9593	+	+	-	+
cGMP	344.0405	150.0421 108.0203	+	+	-	+
GDP	442.0173	344.0402 150.0421	+	+	-	+
GDP-mannose	604.0701	442.0177 424.0057	+	+	-	+

GMP	364.0654	152.0567 97.0289	+	+	-	+
N6-(1,2-Dicarboxyethyl)-AMP	464.0814	252.0724 234.0620	+	+	-	+
Purines and purine derivatives						
Adenine	134.0469	107.0360 92.0248	+	+	+	+
Guanine	152.0568	135.0302 110.0351	+	+	+	+
Hypoxanthine	137.0454	119.0351 110.0349	+	+	+	+
Uric acid	167.0210	124.0154 41.9985	+	+	-	+
Xanthine	151.0260	108.0196 41.9987	+	+	+	+
Pyrimidine nucleotides and derivatives						
UMP	323.0291	96.9691 78.9592	+	+	-	+
cUMP	305.0183	111.0197 41.9985	+	+	-	+
UDP-D-xylose	535.0374	323.0286 78.9591	+	+	-	+
UDP-glucose	565.0479	384.9842 323.0289	+	+	-	+
UDP-glucuronic acid	579.0271	402.9954	+	+	-	+

		323.0289				
UDP-N-acetylglucosamine	606.0744	384.9850 282.0392	+	+	-	+
Pyrimidine derivative						
Uracil	111.0199	41.9988	+	-	+	+
Pyridine derivative						
Nicotinic acid	124.0394	80.0497 78.0335	+	+	-	+

MS: Mass spectrometry

UF-3 kDa: mushroom fraction (≤3 kDa) obtained by ultrafiltration

LLE-AqF: Liquid-liquid extraction aqueous fraction

LLE-OrgFs: Liquid-liquid extraction organic fractions

Table 5S. Toxicity in rats using a high (750 mg/ml), medium (500 mg/ml) and low (250 mg/ml) *AbAEE* extract dose in water for 15 days.

Groups	**(mg/ml)**	**Ni**	**Nf**	**Deaths**

Tox1	750	5	5	0
Tox2	500	5	5	0
Tox3	250	5	5	0

No death or toxicity has been observed after pathological examination of the liver, lungs, heart and intestines. Ni= initial number of animals in the experiment. Nf= final number of animals in the experiment.