

Supplementary

Biologically Active Preparations from the Leaves of Wild Plant Species of the Genus *Rubus*.

Łukasz Kucharski ^{1,*}, Krystyna Cybulska ², Edyta Kucharska ^{3,*}, Anna Nowak ¹, Robert Pelech ³ and Adam Klimowicz ¹

¹ Department of Cosmetic and Pharmaceutical Chemistry, Pomeranian Medical University in Szczecin, PL-70111 Szczecin, Poland; lukasz.kucharski@pum.edu.pl (Ł.K.); anowak@pum.edu.pl (A.N.); adam.klimowicz@pum.edu.pl (A.K.)

² Department of Microbiology and Environmental Chemistry, Faculty of Environmental Management and Agriculture, West Pomeranian University of Technology, Szczecin, PL-71434 Szczecin, Poland; Krystyna.Cybulska@zut.edu.pl (K.C.)

³ Faculty of Chemical Technology and Engineering, Department of Chemical Organic Technology and Polymeric Materials, West Pomeranian University of Technology, Szczecin, PL-70322 Szczecin, Poland; edyta.kucharska@zut.edu.pl (E.K.); rpelech@zut.edu.pl (R.P.)

* Correspondence: lukasz.kucharski@pum.edu.pl (Ł.K.); Tel.: +48-660-476-340, edyta.kucharska@zut.edu.pl (E.K.); Tel.: +48-888-615-273

Figure 1S presents the GC-MS chromatogram of the preparation obtained from leaves of the *Rubus idaeus* L.

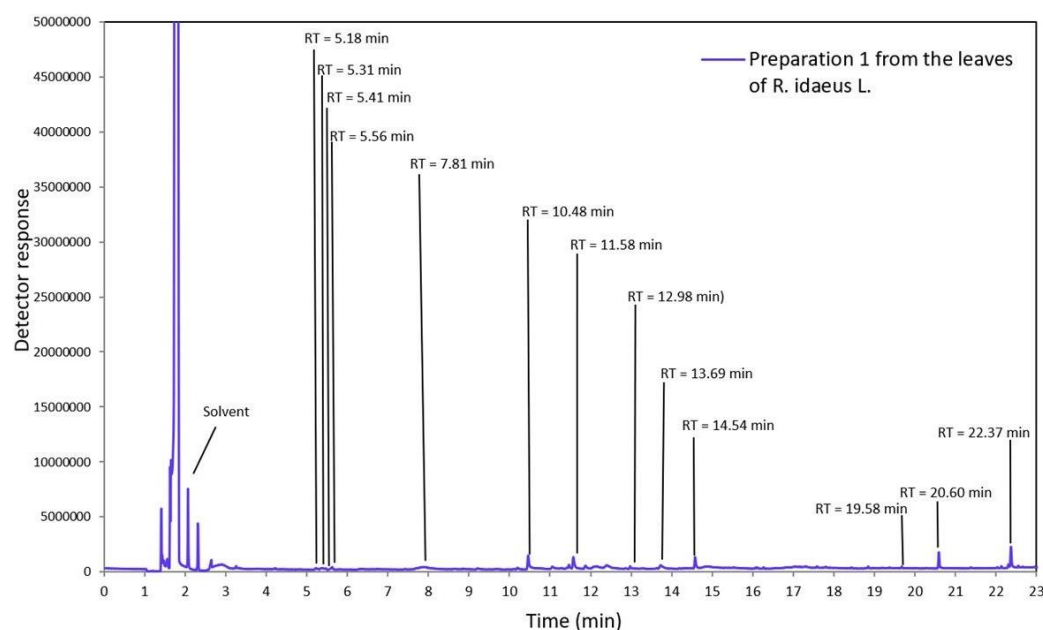


Figure 1S. GC-MS chromatogram of the preparation 1 (P1) obtained from leaves of the *Rubus idaeus* L. (2-Hexenal RT= 5.18 min, 2-Heptanone RT= 5.31 min, 2-Hexanol-3-methyl RT= 5.41 min, 4-Heptanol-3-ethyl RT= 5.56 min, 3-Hexanol-5-methyl RT= 7.81 min, 4-H-pyran-4-one RT= 10.48 min, 5-(Hydroxymethyl)furfural RT= 11.58 min, 2,4-Heptadienal RT= 12.98 min, 2-Nonanone RT= 13.69 min, Pyrogallol RT= 14.54 min, Dodecanoic acid RT= 19.58 min, Hexadecanoic acid RT= 20.60 min, Linoleic acid methyl ester RT= 22.37 min).

Figure 2S presents the GC-MS chromatogram of the preparation obtained from leaves of the *Rubus fruticosus* L.

Citation: Lastname, F.; Lastname, F.; Lastname, F. Title. *Molecules* **2022**, *11*, x. <https://doi.org/10.3390/xxxxx>

Academic Editor: Firstname Lastname

Received: date
Accepted: date
Published: date

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

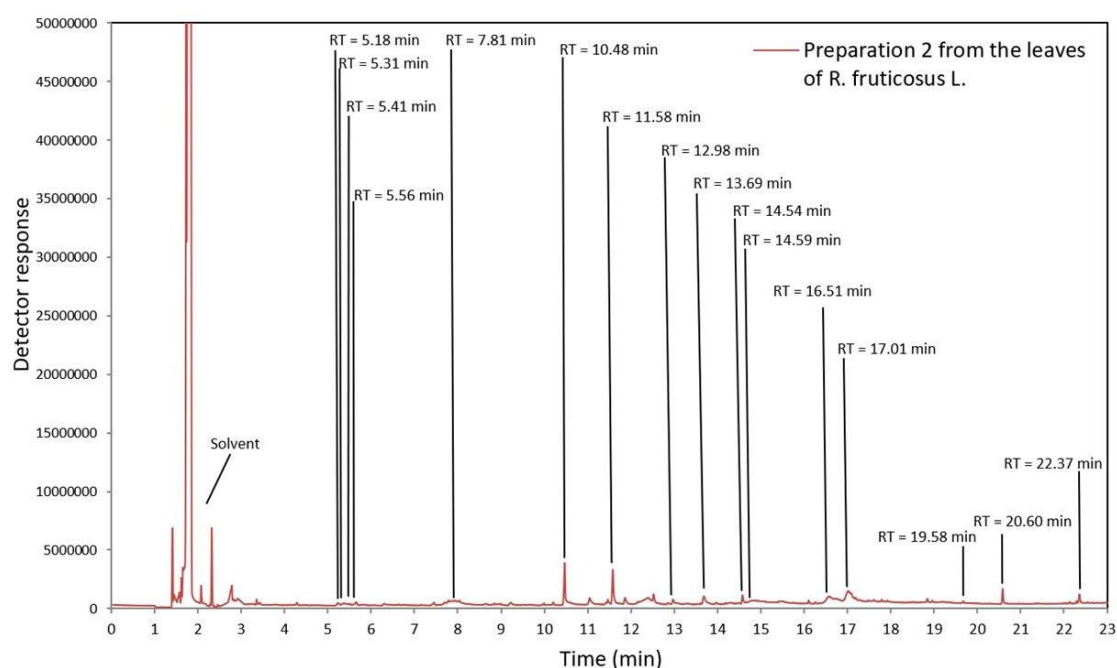
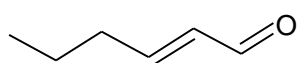
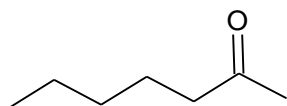


Figure 2S. GC-MS chromatogram of the preparation 2 (P2) obtained from leaves of the *Rubus fruticosus* L. (2-Hexenal RT= 5.18 min, 2-Heptanone RT= min 5.31, 2-Hexanol-3-methyl RT= 5.41 min, 4-Heptanol-3-ethyl RT= 5.56 min, 3-Hexanol-5-methyl RT= 7.81 min, 4-H-pyran-4-one RT= 10.48 min, 5-(Hydroxymethyl)furfural RT= 11.58 min, 2,4-Heptadienal RT= 12.98 min, 2-Nonanone RT= 13.69 min, Pyrogallol RT= 14.54 min, 2-Hydroxy-5-methylbenzaldehyde RT= 14.59 min, n-Decanoic acid RT= 16.51 min, Quinic acid RT= 17.01 min, Dodecanoic acid RT= 19.58 min, Hexadecanoic acid RT= 20.60 min, Linoleic acid methyl ester RT= 22.37 min).

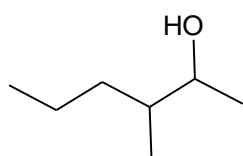
Figure 3S shows the structures of the compounds identified in the tested preparations obtained of the leaves of *R. idaeus* L. (P1) and *R. fruticosus* L. (P2).



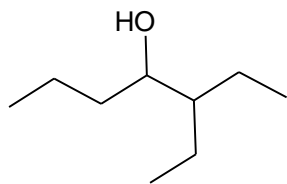
2-Hexenal RT= 5.18 min (present in preparations 1 and 2)



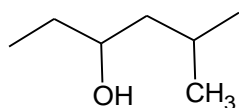
2-Heptanone RT= min 5.31 (present in preparations 1 and 2)



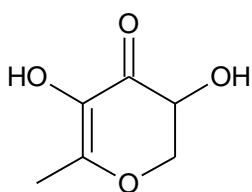
2-Hexanol-3-methyl RT= 5.41 min (present in preparations 1 and 2)



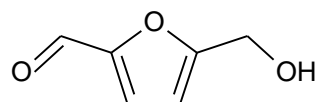
4-Heptanol-3-ethyl RT= 5.56 min (present in preparations 1 and 2)



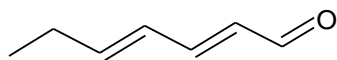
3-Hexanol-5-methyl RT= 7.81 min (present in preparations 1 and 2)



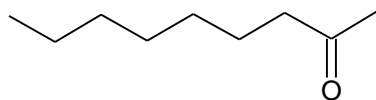
4-H-pyran-4-one RT= 10.48 min (present in preparations 1 and 2)



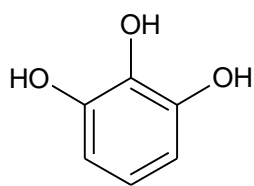
5-(Hydroxymethyl)furfural RT= 11.58 min (present in preparations 1 and 2)



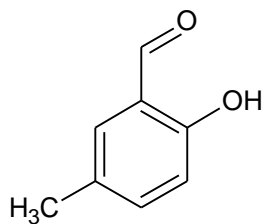
2,4-Heptadienal RT= 12.98 min (present in preparations 1 and 2)



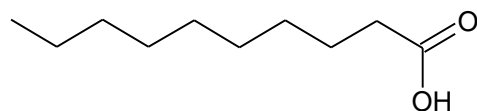
2-Nonanone RT= 13.69 min (present in preparations 1 and 2)



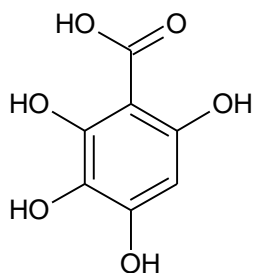
Pyrogallol RT= 14.54 min (present in preparations 1 and 2)



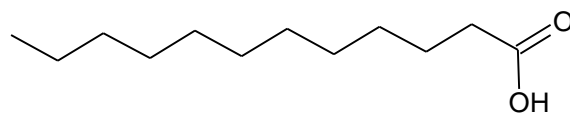
2-Hydroxy-5-methylbenzaldehyde RT= 14.59 min (present only in preparation 2)



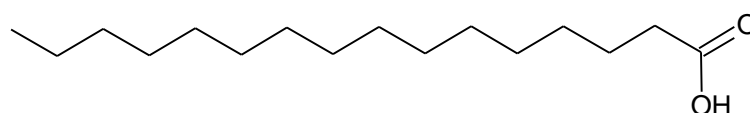
n-Decanoic acid RT= 16.51 min (present only in preparation 2)



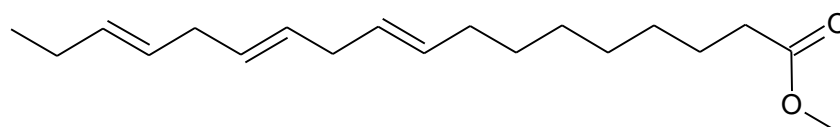
Quinic acid RT= 17.01 min (present only in preparation 2)



Dodecanoic acid RT = 19.58 min (present in preparations 1 and 2)



Hexadecanoic acid RT= 20.60 min (present in preparations 1 and 2)



Linoleic acid methyl ester RT= 22.37 min (present in preparations 1 and 2)

Figure 3S. The structures of the compounds identified in the tested preparations obtained of the leaves of *R. idaeus* L. and *R. fruticosus* L.

Table 1S present the sorted values for the statistical analysis of plant preparations P1 and P2, and the reference preparation (PR), according to EN 1500:2013 and against *Escherichia coli* K12 strain NCTC 10538.

Table 1S. Statistical analysis of plant preparations P1 and P2, and the reference preparation (PR), according to EN 1500:2013 and against *Escherichia coli* K12 strain NCTC 10538 - sorted results and calculation results for statistical test.

PR-P1	0.82	-0.04	-0.08	-0.12	-0.12	-0.13	-0.14	-0.30	-0.32	-0.32
0.82	0.82									
-0.04	0.39	-0.04								
-0.08	0.37	-0.06	-0.08							
-0.12	0.35	-0.08	-0.10	-0.12						
-0.12	0.35	-0.08	-0.10	-0.12	-0.12					
-0.13	0.35	-0.09	-0.11	-0.13	-0.13	-0.13				
-0.14	0.34	-0.09	-0.11	-0.13	-0.13	-0.14	-0.14			
-0.30	0.26	-0.17	-0.19	-0.21	-0.21	-0.22	-0.22	-0.30		
-0.32	0.25	-0.18	-0.20	-0.22	-0.22	-0.22	-0.23	-0.31	-0.32	
-0.32	0.25	-0.18	-0.20	-0.22	-0.22	-0.23	-0.23	-0.31	-0.32	-0.32
-0.37	0.23	-0.20	-0.22	-0.24	-0.24	-0.25	-0.25	-0.33	-0.34	-0.34
-0.38	0.22	-0.21	-0.23	-0.25	-0.25	-0.26	-0.26	-0.34		
-0.42	0.20	-0.23	-0.25	-0.27	-0.27	-0.28	-0.28			
-0.43	0.20	-0.23	-0.25	-0.27	-0.27	-0.28	-0.28			
-0.43	0.20	-0.24	-0.26	-0.27	-0.27	-0.28	-0.28			
-0.50	0.16	-0.27	-0.29	-0.31	-0.31	-0.32	-0.32			
-0.50	0.16	-0.27	-0.29	-0.31	-0.31	-0.32	-0.32			
-0.58	0.12	-0.31	-0.33							
-0.92	-0.05									
-1.01	-0.09									
PR-P2	0.27	0.24	0.20	0.15	0.14	0.11	0.08	0.08	0.07	0.05
0.27	0.27									
0.24	0.25	0.24								
0.20	0.23	0.22	0.20							
0.15	0.21	0.20	0.18	0.15						

Concentration of P2 0.6 g/100 mL	Nts:>330 R: <1.42	Nts:>330 R: <1.35	Nts:>330 R: <1.38	Nts:>330 R: <1.39	Nts:>330 R: <0.41	Nts:>165 R: <0.62
Concentration of P2 60 g/100 mL	10 ⁻⁰ :110±0.03a 10 ⁻¹ :<14 10 ⁻² :<14 Nts:>40 R: >3.90	10 ⁻⁰ :<14 10 ⁻¹ :<14 10 ⁻² :<14 Nts:>0 R: >4.72	10 ⁻⁰ :<14 10 ⁻¹ :<14 10 ⁻² :<14 Nts:>0 R: >4.75	10 ⁻⁰ :<14 10 ⁻¹ :<14 10 ⁻² :<14 Nts:>0 R: >4.76	10 ⁻⁰ :<14 10 ⁻¹ :<14 10 ⁻² :<14 Nts:>0 R: >3.78	10 ⁻⁰ :<14 10 ⁻¹ :<14 10 ⁻² :<14 Nts:>0 R: >3.69
Concentration of P2 60 g/100 mL contact time 300 ± 10 s	10 ⁻⁰ :<14 10 ⁻¹ :<14 10 ⁻² :<14 Nts:>0 R: >4.79	10 ⁻⁰ :<14 10 ⁻¹ :<14 10 ⁻² :<14 Nts:>0 R: >4.72	10 ⁻⁰ :<14 10 ⁻¹ :<14 10 ⁻² :<14 Nts:>0 R: >4.75	10 ⁻⁰ :<14 10 ⁻¹ :<14 10 ⁻² :<14 Nts:>0 R: >4.76	10 ⁻⁰ :<14 10 ⁻¹ :<14 10 ⁻² :<14 Nts:>0 R: >3.78	10 ⁻⁰ :<14 10 ⁻¹ :<14 10 ⁻² :<14 Nts:>0 R: >3.69

Each value is the mean of three replicates with the standard deviation in three independent experiments. Any two means in the same column followed by the same letter are not significantly ($P > 0.01$) different by Tukey's multiple range tests,

* medium used: Trypticasein Soy LAB-Agar (TSA), neutralizer used: solution of Polysorbate 80 (3.0 g/100 mL), sodium thiosulphate (1.0g/100 mL) and soy lecithin (0.3g/100 mL), incubation conditions: 24h at 37 ± 1 °C, loading substance: bovine serum albumin (0.03g/100 mL), the diluent used during the test: distilled water, test method and its validation: neutralization method for solutions, test temperature: 20 ± 1 °C, method of microbial counting: deepwell plate inoculation, stability of the preparation/diluent mixture: no precipitate formed during the test,

** medium used: Malt-extract Agar (MEA), neutralizer used: solution of Polysorbate 80 (3.0 g/100 mL), sodium thiosulphate (1.0 g/100 mL) and soy lecithin (0.3 g/100 mL), incubation conditions: 48h at 30 ± 1 °C for yeast and 120h at 30 ± 1 °C for fungal, loading substance: bovine serum albumin (0.03g/100 mL), the diluent used during the test: distilled water, test method and its validation: neutralization method for solutions, test temperature: 20 ± 1 °C, method of microbial counting: deepwell plate inoculation, stability of the preparation/diluent mixture: no precipitate formed during the test,

Nts - the number of units remaining after the test is performed,

R - reduction in the number of microorganisms during the test,

^a - different letters: values differ significantly between the analyzed preparations.

Table 3S. The results of disinfection tests of plant preparations by standard EN 13697:2015.

EN 13697:2015 (phase 2 stage 2)			
Test preparation	Treatment	* <i>Staphylococcus aureus</i> ATCC 6538	* <i>Pseudomonas aeruginosa</i> ATCC 15442
Preparation 1 (P1)			
Concentration of P1 14 g/100 mL	contact	10 ⁻⁰ :>330	10 ⁻⁰ :>330
		10 ⁻¹ :>330	10 ⁻¹ :>330
		10 ⁻² :289±0.09a	10 ⁻² :151±0.01a
		Nts :>100	Nts :100±0.01b
		R: 1.74±0.01ab	R: 1.73±0.02ab
Concentration of P1 21 g/100 mL	60 ± 10 s	10 ⁻⁰ :>330	10 ⁻⁰ :>330
		10 ⁻¹ :270±0.11ab	10 ⁻¹ :316.5±0.10ab
		10 ⁻² :166.5±0.03a	10 ⁻² :237.5±0.05a
		Nts :100±0.02a	Nts :100±0.06a
		R: 1.98±0.04a	R: 1.83±0.02a
		10 ⁻⁰ :254±0.03ab	10 ⁻⁰ :271.5±0.03ab

Concentration of P1 28 g/ 100 mL		10 ⁻¹ :163.5±0.01ab 10 ⁻² :94.5±0.04a Nts :>100 R: 2.25±0.01a	10 ⁻¹ :181±0.07ab 10 ⁻² :99.5±0.07a Nts :>100 R: 2.21±0.01a
Concentration of P1 35 g/100 mL		10 ⁻⁰ :191±0.03a 10 ⁻¹ :83.5±0.04a 10 ⁻² :31±0.02ab Nts :88±0.06ab R: 3.28±0.01ab	10 ⁻⁰ :187.5±0.05a 10 ⁻¹ :102.5±0.01a 10 ⁻² :55±0.01ab Nts :90±0.06ab R: 2.47±0.03ab
Concentration of P1 42 g/100 mL		10 ⁻⁰ :70±0.01a 10 ⁻¹ :25.5±0.03ab 10 ⁻² :1.5±0.04b Nts :22±0.09b R: 4.35±0.02b	10 ⁻⁰ :81±0.08a 10 ⁻¹ :32.5±0.04ab 10 ⁻² :7.5±0.09b Nts :30±0.01b R: 3.70±0.03b
Concentration of P1 49 g/100 mL		10 ⁻⁰ :21.5±0.03ab 10 ⁻¹ :2.5±0.02ab 10 ⁻² :0±0.00a Nts :0±0.00a R: 4.87±0.03	10 ⁻⁰ :28.5±0.04ab 10 ⁻¹ :10±0.01ab 10 ⁻² :0±0.00a Nts :0±0.00a R: 4.75±0.01
Concentration of P1 56 g/100 mL		10 ⁻⁰ :0±0.00a 10 ⁻¹ :0±0.00a 10 ⁻² :0±0.00a Nts :0±0.00a R: >7.10	10 ⁻⁰ :0±0.00a 10 ⁻¹ :0±0.00a 10 ⁻² :0±0.00a Nts :0±0.00a R: >7.11
Concentration of P1 63 g/100 mL		10 ⁻⁰ :0±0.00a 10 ⁻¹ :0±0.00a 10 ⁻² :0±0.00a Nts :0±0.00a R: >7.10	10 ⁻⁰ :0±0.00a 10 ⁻¹ :0±0.00a 10 ⁻² :0±0.00a Nts :0±0.00a R: >7.11
Concentration of P1 70 g/100 mL		10 ⁻⁰ :0±0.00a 10 ⁻¹ :0±0.00a 10 ⁻² :0±0.00a Nts :0±0.00a R: >7.10	10 ⁻⁰ :0±0.00a 10 ⁻¹ :0±0.00a 10 ⁻² :0±0.00a Nts :0±0.00a R: >7.11
Preparation 2 (P2)			
Concentration of P2 14 g/100 mL	contact time 60 ± 10 s	10 ⁻⁰ :>330 10 ⁻¹ :>330 10 ⁻² :241.5±0.06a Nts :>100 R: 1.84±0.02ab	10 ⁻⁰ :>330 10 ⁻¹ :>330 10 ⁻² :263.5±0.01ab Nts :100±0.07a R: 1.76±0.02ab
Concentration of P2 21 g/100 mL		10 ⁻⁰ :>330 10 ⁻¹ :219.5±0.11a 10 ⁻² :106.5±0.03ab	10 ⁻⁰ :>330 10 ⁻¹ :243.5±0.08b 10 ⁻² :180±0.09a

	Nts :100±0.02ab R: 2.19±0.01a	Nts :100±0.04ab R: 1.92±0.00a
Concentration of P2 28 g/100 mL	10 ⁻⁰ :226.5±0.01a 10 ⁻¹ :123±0.03ab 10 ⁻² :80.5±0.02sb Nts :100±0.01a R: 2.31±0.010b	10 ⁻⁰ :248.5±0.08a 10 ⁻¹ :140±0.04b 10 ⁻² :68±0.09b Nts :100±0.03b R: 2.35±0.00a
Concentration of P2 35 g/100 mL	10 ⁻⁰ :58±0.04a 10 ⁻¹ :5.5±0.09b 10 ⁻² :0±0.11a Nts :0±0.09b R: 4.46±0.02a	10 ⁻⁰ :74±0.08a 10 ⁻¹ :28.5±0.06ab 10 ⁻² :0±0.00ab Nts :30±0.09ab R: 4.31±0.03a
Concentration of P2 42 g/100 mL	10 ⁻⁰ :0±0.00a 10 ⁻¹ :0±0.00a 10 ⁻² :0±0.00a Nts :0±0.00a R: >7.12	10 ⁻⁰ :0±0.00a 10 ⁻¹ :0±0.00a 10 ⁻² :0±0.00a Nts :0±0.00a R: >7.08
Concentration of P2 49 g/100 mL	10 ⁻⁰ :0±0.00a 10 ⁻¹ :0±0.00a 10 ⁻² :0±0.00a Nts :0±0.00a R: >7.12	10 ⁻⁰ :0±0.00a 10 ⁻¹ :0±0.00a 10 ⁻² :0±0.00a Nts :0±0.00a R: >7.08
Concentration of P1 56 g/100 mL	10 ⁻⁰ :0±0.00a 10 ⁻¹ :0±0.00a 10 ⁻² :0±0.00a Nts :0±0.00a R: >7.10	10 ⁻⁰ :0±0.00a 10 ⁻¹ :0±0.00a 10 ⁻² :0±0.00a Nts :0±0.00a R: >7.08
Concentration of P2 63 g/100 mL	10 ⁻⁰ :0±0.00a 10 ⁻¹ :0±0.00a 10 ⁻² :0±0.00a Nts :0±0.00a R: >7.10	10 ⁻⁰ :0±0.00a 10 ⁻¹ :0±0.00a 10 ⁻² :0±0.00a Nts :0±0.00a R: >7.08
Concentration of P2 70 g/100 mL	10 ⁻⁰ :0±0.00a 10 ⁻¹ :0±0.00a 10 ⁻² :0±0.00a Nts :0±0.00a R: >7.10	10 ⁻⁰ :0±0.00a 10 ⁻¹ :0±0.00a 10 ⁻² :0±0.00a Nts :0±0.00a R: >7.08
Ethanol (E)		
Concentration of E 80 g/100 mL	10 ⁻⁰ :192±0.01a 10 ⁻¹ :95±0.01b 10 ⁻² :8.5±0.09ab Nts :>100 R: >3.93	10 ⁻⁰ :192±0.03a 10 ⁻¹ :80±0.08a 10 ⁻² :6±0.06ab Nts :>100 R: >3.93

Concentration of E 90 g/100 mL	10^{-0} :5.5±0.01a	10^{-0} :1.5±0.01a
	10^{-1} :0±0.00a	10^{-1} :0±0.00a
	10^{-2} :0±0.00a	10^{-2} :0±0.00a
	Nts :<0.1	Nts :0±0.00a
	R: >7.11	R: >7.11
Concentration of E 100 g/100 mL	10^{-0} :0±0.00a	10^{-0} :0±0.00a
	10^{-1} :0±0.00a	10^{-1} :0±0.00a
	10^{-2} :0±0.00a	10^{-2} :0±0.00a
	Nts :0±0.00a	Nts :0±0.00a
	R: >7.11	R: >7.11

Each value is the mean of three replicates with the standard deviation in three independent experiments. Any two means in the same column followed by the same letter are not significantly ($P > 0.01$) different by Tukey's multiple range tests,

* medium used: Trypticasein Soy LAB-Agar (TSA), neutralizer used: solution of Polysorbate 80 (3.0 g/100 mL), sodium thiosulphate (0.3 g/100 mL) and soy lecithin (0.3 g/100 mL), incubation conditions: 24h at 37 ± 1 °C, loading substance: bovine serum albumin (0.3 g/100 mL), the diluent used during the test: sterile hard water 30 mg/100g CaCO_3 , test method and its validation: neutralization method for solutions, test temperature: 20 ± 1 °C, method of microbial counting: deepwell plate inoculation, stability of the preparation/diluent mixture: no precipitate formed during the test,

Nts - the number of units remaining after the test is performed,

R - reduction in the number of microorganisms during the test,

^{a, b} - different letters: values differ significantly between the analyzed preparations.