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ANTIBACTERIAL AND ANTIOXIDATIVE EFFECTS OF ORANGE PEEL (*Citrus sinensis* Osbeck cv. Washington navel) EXTRACTS OBTAINED ULTRASOUND ASSISTED EXTRACTION

ByProExtract

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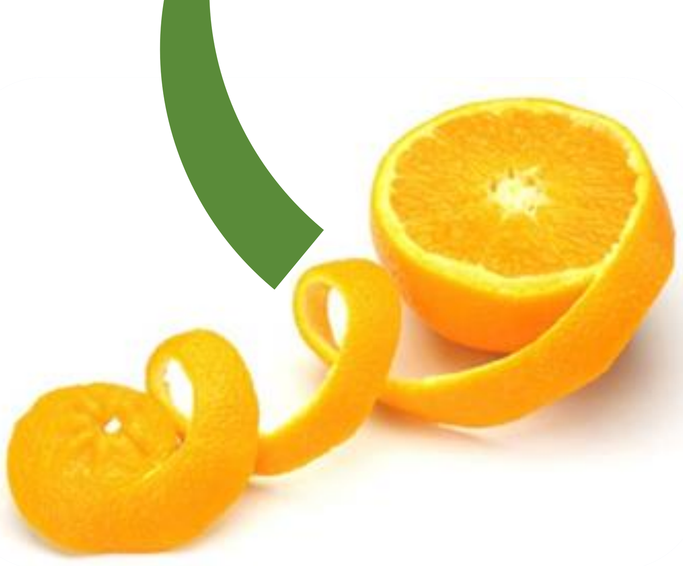
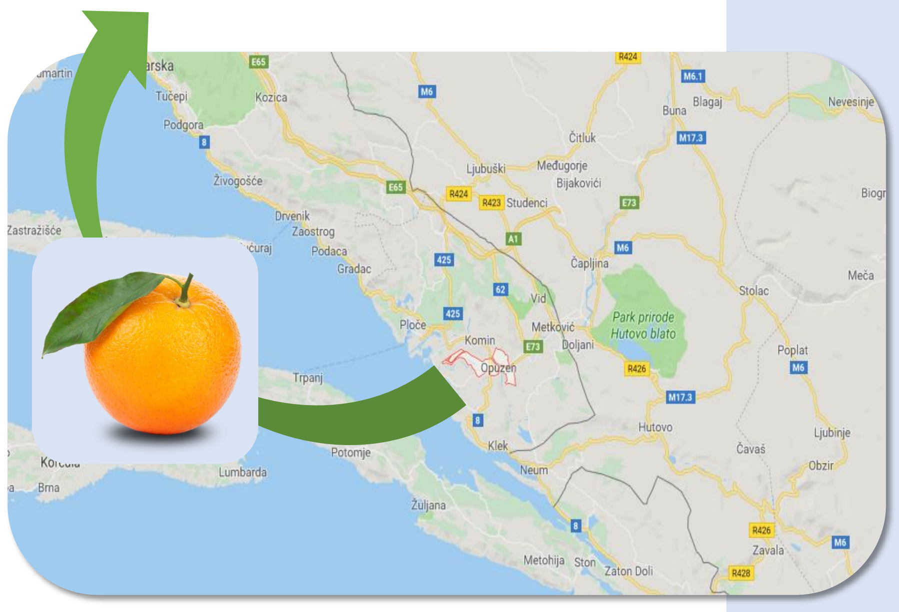
Introduction

Sweet orange is an evergreen citrus plant that belongs to the Rutaceae family. The great importance of oranges is the abundance of secondary plant metabolites, of which triterpenes, glycosides and flavonoids are the most represented in the peel. Previous research has shown that citrus peel contains essential oils used for antibacterial and antioxidant purposes. The isolation of antioxidant and antibacterial components is done by using different types of extraction, and the ultrasonic method is one of the newest method that is being used. Ultrasonic extraction technology can increase the extraction of polyphenols, anthocyanins, aromatic substances, oils and polysaccharides. The mechanical effects of ultrasound provide greater penetration of solvents into the materials to be extracted and thus increase mass transfer. The aim of this study was to determine how different conditions of ultrasonic extraction of orange peel (*Citrus sinensis* Osbeck cv. Washington navel) affect total antibacterial and antioxidative activity by determining the total concentration of phenolic compounds, total antioxidative and antibacterial activity on Gram-positive and Gram-negative human pathogens.

Materials and methods





This study was performed on a peel obtained from orange *Citrus sinensis* Osbeck cv. Washington navel. The plants were grown under the same climatic and cultural conditions in Opuzen (southern Croatia). The fruits were harvested during the November 2017 and freshly treated by removing the peel, which was further dried and milled using laboratory mill (IKA M 20 Universal mill) and sieved applying a vertical vibratory sieve shaker (Retsch AS 200, Germany) for 20 min. Ultrasound-assisted extraction of orange peel samples was done at 3 different temperatures (30, 50 and 70 °C) with different extraction times (15, 30 and 45 min), with different solvent-plant ratio (10, 30 and 50 mL/g) and with different ethanol/water ratio (20, 50 80 % v/v).

The extraction was carried out in an Elma ultrasonic bath Elmasonic P 70H at a frequency of 37 kHZ at 50 W. After ultrasonic extraction, the samples were filtered through filter paper and stored at +4°C until further analysis. The Folin-Ciocalteu method was used to determine the total phenolic compounds (TPC), and the modified DPPH method was used to test the total antioxidant activity.

The antibacterial activity of orange peel extracts was examined by the broth microdilution method in the terms of minimum inhibitory concentration (MIC) against two Gram-positive *Bacillus subtilis* and *Staphylococcus aureus*, and two Gram-negative *Escherichia coli* and *Pseudomonas aeruginosa.*

Results

Sample	Temp (°C)	Time (min)	Solvent/solid ratio (ml/g)	Ethanol/water ratio (v/v) (%)	TPC ($mg_{GAE}\ g^{-1}$)	DPPH (%)	MIC ($mg\ mL^{-1}$) *E. coli*	*P. aeruginosa*	*B. subtilis*	*S. aureus*
1	70	45	30	50	33.65 + 2.34	82.07 ± 2.14	2.08	1.04	2.08	1.04
2	70	30	30	20	33.44 + 2.29	66.67 ± 6.13	1.04	1.04	2.08	1.04
3	30	30	30	20	32.83 + 3.13	60.82±5.19	1.04	1.04	2.08	1.04
4	30	45	30	50	34.58 + 2.34	62.56 ± 1.82	1.04	1.04	1.04	1.04
5	30	30	10	50	ND	ND	ND	ND	ND	ND
6	50	15	10	50	ND	ND	ND	ND	ND	ND
7	50	30	50	20	37.08 + 3.55	36.62 ±2.94	1.25	0.63	1.25	0.63
8	50	45	30	20	22.28 + 3.55	57.93 ± 1.00	1.04	1.04	2.08	1.04
9	50	30	30	50	32.80 + 2.02	57.93 ± 2.15	1.04	1.04	1.04	2.08
10	70	15	30	50	32.79 + 2.75	73.14 ± 0.76	1.04	0.52	1.04	1.04
11	30	30	30	80	25.22 + 2.31	51.39 ± 1.58	1.04	0.52	0.52	1.04
12	70	30	30	80	33.04 + 1.40	65.35 ± 1.15	0.52	0.52	0.52	0.52
13	50	30	10	20	ND	ND	ND	ND	ND	ND
14	30	15	30	50	29.33 + 0.69	59.04 ±9.78	0.52	0.52	0.52	0.52
15	50	30	30	50	31.15 + 1.86	56.03 ± 5.30	0.52	0.52	0.52	0.52
16	50	30	10	80	ND	ND	ND	ND	ND	ND
17	70	30	10	50	ND	ND	ND	ND	ND	ND
18	50	30	30	50	27.87 + 1.91	57.09 ± 13.61	0.52	1.04	0.52	0.52
19	50	45	30	80	24.50 + 1.14	54.81 ± 3.51	0.52	1.04	0.52	1.04
20	50	30	30	50	29.27 + 1.14	63.24 ± 7.08	1.04	1.04	1.04	1.04
21	50	15	50	50	30.02 + 1.08	38.80 ± 8.27	1.04	1.04	1.04	0.63
22	70	30	50	50	41.37 + 2.28	48.61 ± 1.46	0.063	0.63	0.31	0.63
23	30	30	50	50	37.45 + 4.01	34.13 ± 4.68	0.063	0.313	0.31	0.32
24	50	15	30	80	25.65 + 1.66	52.15 ± 6.83	1.04	0.52	0.52	0.52
25	50	30	50	80	30.42 + 2.34	38.48 ±1.29	1.04	0.31	0.31	0.63
26	50	45	50	50	38.83 + 3.29	47.39 ± 1.03	0.625	0.31	0.31	0.63
27	50	45	10	50	ND	ND	ND	ND	ND	ND
28	50	30	30	50	34.33 + 2.56	48.64 ± 3.90	1.04	1.04	0.52	1.04
29	50	15	30	20	38.55 + 0.38	52.44 ± 0.42	1.04	1.04	2.08	1.04
						AMIKACIN	0,00016	0,0003	0,00002	0,00002

ND- not determined

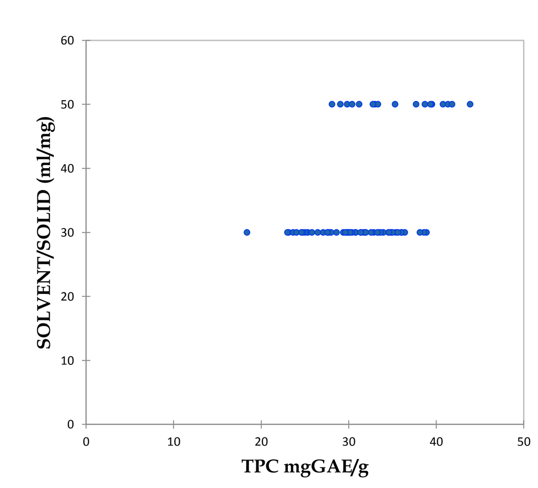


Figure 1. Correlation between solvent/solid ratio(ml/g) and total phenolic compounds (mgGAE/g) (r=0,409; p<0,05)

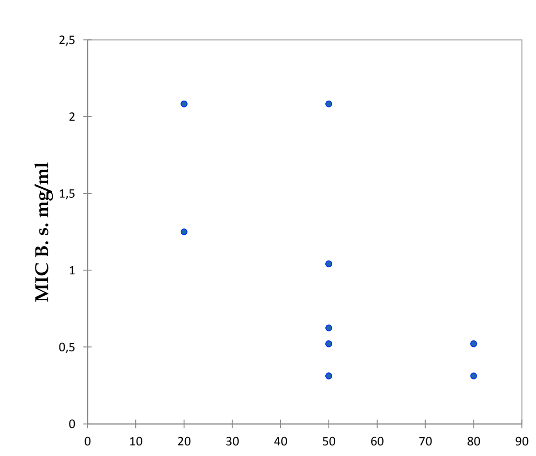


Figure 2. Correlation between MIC for *B. subtilis* (mg/ml) and ethanol (%) (r=-0,67; p<0,05)

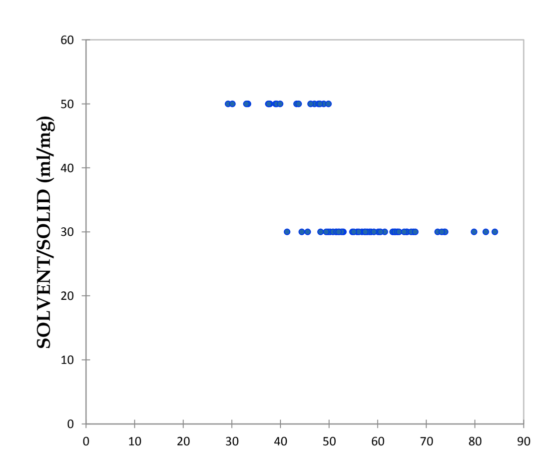


Figure 3. Correlation between solvent/solid ratio(ml/g) and antioxidative activity (%) (r=-0,72; p<0,05)

Conclusions

- Higher solvent/plant ratio resulted in a slightly higher yield of total phenolic compounds upon ultrasonic extraction
- Higher ultrasound extraction temperature resulted in an increased total antioxidant activity
- Higher solvent/plant ratio resulted in significant reduced antioxidant activity
- Higher ethanol/water ratio resulted in inreased antibacterial activity

Aknowledgment

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