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ULTRASOUND-ASSISTED EXTRACTION OF PHENOLIC COMPONENTS FROM TOBACCO WASTE

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INTRODUCTION

Tobacco is the most widely grown plant in the world which is not used for food. Leaves are most valuable part of tobacco plant and other parts like stem, root, rib are consider as a waste.
Tobacco waste is a by-products produced in large quantities during processing of tobacco and it contain different classes of useful bioactive components such as phenolic components.
In this study phenolic components were successfully extracted from 3 types of tobacco waste and leaves using ultrasound-assisted extraction (UAE) at different extraction parameters.
The content of phenolic components was determined using reversed-phase High Performance Liquid Chromatography (HPLC).
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Fig.1 Tobacco plant

PLANT MATERIAL

Tobacco leaves and 3 types of tobacco waste (machine crumbs, dust and rib) were obtained from tobacco factory „Fabrika Duhana Sarajevo" Bosnia and Herzegovina in 2018. Before the extraction, the plant material was grounded using laboratory mill.

a) b) c) d)

Fig.2 a) tobacco leaves b) dust c) rib d) machine crumbs

UTRASOUND-ASSISTED EXTRACTION

Ultrasound-assisted extraction (UAE) from different fractions of tobacco waste and leaves was performed. The influence of extraction temperatures (30, 50, 70 °C), time (15, 30, 45 min), ethanol : water ratio (40%, 60%, 80% v/v) and solvent-solid ratio (10, 30 and 50 mL/g) on the phenolic content in obtained extracts was determined.
Extraction was performed in ultrasound-bath Elma, Elmasonic P 70 H, with frequency 37 kHz and power 50W. Afterwards, obtained extracts were filtered through filter paper and stored at 4°C until HPLC analysis.

DETERMINATION OF PHENOLIC COMPONENTS BY HPLC

Before HPLC analysis, all sample extracts were filtered trough 0.2 µm PTFE filter. The content of polyphenols was determined using reversed-phase High Performance Liquid Chromatography (Agilent technologies 1260 Infinity II) using modified method described in Wang et al. (2008)., under following condition: mobile phase: 0.1% phosphoric acid-methanol (55:45, v/v), injection volume 20µl, flow rate 0.5 ml/min and pressure 76.2 bar.
Used column was zorbax Eclipse Plus C18 (particle size 250 mm x 4.6mm, 5 µm) on room temperature (25°C). Analysis was monitored at 210 nm on DAD detector.

RESULTS

Table 1. Phenolic content in obtained extracts at different UAE parameters

RUN	UAE CONDITIONS				LEAVES			MACHINE CRUMBS			DUST			RIB		
	Temperature (°C)	Time (min)	Ethanol (%)	Solid: Solvent ratio (v/v)	Chlorogenic acid (ng/µl)	Caffeic acid (ng/µl)	Rutin (ng/µl)	Chlorogenic acid (ng/µl)	Caffeic acid (ng/µl)	Rutin (ng/µl)	Chlorogenic acid (ng/µl)	Caffeic acid (ng/µl)	Rutin (ng/µl)	Chlorogenic acid (ng/µl)	Caffeic acid (ng/µl)	Rutin (ng/µl)
1	70	30	30	80	43.32	3.21	36.09	7.57	2.42	11.46	6.98	-	7.67	3.44	-	2.84
2	50	30	30	60	76.31	3.35	38.30	34.98	1.04	11.72	31.47	-	8.99	5.88	-	1.45
3	50	30	30	60	107.09	2.64	27.14	33.9	2.16	11.69	32.39	-	8.72	21.44	-	1.76
4	70	45	30	60	89.99	3.87	40.71	35.24	1.73	10.91	34.9	-	9.87	6.27	-	1.95
5	30	30	30	40	131.62	2.99	25.82	31.39	1.87	9.05	29.38	-	5.66	18.16	-	-
6	50	15	10	60	222.15	3.15	28.16	55.64	-	8.19	42.9	-	4.31	14.12	-	-
7	70	30	50	60	37.47	2.30	58.05	7.75	2.44	23.86	7.62	1.72	17.34	-	2.02	4.15
8	50	30	30	60	108.29	2.68	30.16	33.96	1.51	10.86	30.57	-	9.04	7.31	-	1.28
9	30	30	30	80	21.13	2.64	25.83	6.59	-	4.51	5.99	-	3.87	2.51	-	1.42
10	50	30	30	60	85.15	3.30	36.79	34.02	1.94	13.02	30.69	-	6.98	5.42	-	-
11	50	30	10	40	272.09	3.15	31.10	80.42	-	6.88	50.84	-	4.15	12.87	-	-
12	50	30	50	80	22.14	-	61.73	13.13	1.65	17.22	4.02	2.6	16.89	-	6.66	4.77
13	50	30	50	40	51.39	4.43	49.80	4.17	3.71	21.79	11.15	-	16.7	5.93	2.85	3.21
14	50	45	50	60	37.48	3.27	69.06	5.46	5.39	21.37	6.8	2.74	17.38	2.53	1.84	4.44
15	30	15	30	60	58.49	1.94	27.73	36.88	-	10.12	32.09	-	5.94	12.47	-	-
16	50	15	50	60	38.16	2.40	59.96	8.12	1.49	21.2	5.92	1.17	15.06	-	1.6	3.94
17	50	45	30	80	49.99	3.67	52.00	30.2	2.1	13.24	25.88	-	9.7	2.66	2.39	2.02
18	50	45	30	40	140.54	2.76	26.90	32.29	1.95	8.75	15.32	-	6.97	5.98	-	-
19	50	45	10	60	200.89	2.55	29.90	55.77	-	4.85	45.8	-	5.82	16.45	-	-
20	30	30	50	60	27.44	1.82	58.36	8.92	1.6	23.82	6.83	1.83	16.9	1.82	-	5.78
21	50	15	30	80	23.42	3.44	23.52	6.94	-	6.83	7.03	-	7.77	2.5	-	-
22	70	30	30	40	119.15	3.23	29.08	34.53	2.35	8.66	14.98	-	6.14	6.94	-	-
23	70	30	10	60	194.43	1.69	32.79	60.5	-	6.51	46.27	-	4.27	13.71	-	-
24	50	30	10	80	171.30	2.49	26.65	42.48	-	5.4	29.03	-	4.14	9.69	-	-
25	30	45	30	60	94.95	2.54	28.39	37.65	1.88	9.11	31.27	-	7.55	20.54	-	-
26	50	15	30	40	142.19	2.87	24.96	32.21	2	7.55	14.09	-	4.44	5.93	-	-
27	50	30	30	60	91.85	2.92	31.12	38.12	2.54	10.9	31.08	-	8	6.63	-	1.84
28	30	30	10	60	231.85	3.07	42.76	65.35	-	9.37	39.74	-	3.28	6.16	-	2.02
29	70	15	30	60	69.22	3.45	38.95	35.19	2.24	14.49	31.9	-	7.96	13.44	-	-

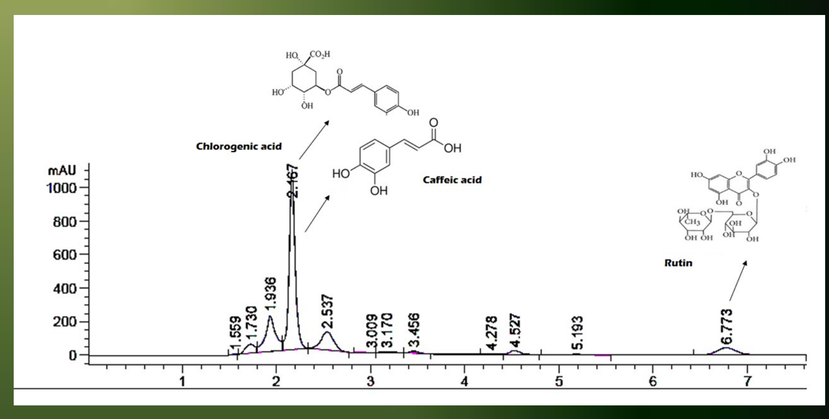

Fig.3 Chromatogram of detected phenolic components (machine crumbs, run 29)

CONCLUSION

Phenolic components were successfully extracted from tobacco leaves and waste using ultrasound-assisted extraction. Mayor phenolic components in tobacco leaves, namely, chlorogenic acid, caffeic acid and rutin, were detected in all waste samples.
In compariation with leaves, all tobacco waste extracts contained the same phenols but in lower concentration. Ultrasound extraction conditions had statistically significant influence on the content of phenolic components.

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