Solid waste obtained from industrial tincture production from *Plantago major* L. leaves: Insight into chemical composition and bioactivity

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METHODS:
1- HPLC analysis
2- Microdilution assay
3- MTT assay

Introduction

Plantago major L. (Fig. 1) is through centuries used plant. It is known as a coolinary ingredient, but also as a medicine. This plant possesses secondary metabolites, which can have antibacterial effects and potentially help as a treatment during infections. Unfortinetly, during the production and the processing this medicinal plant, great amount of its residues is usually treated as a waste.

This work was based on examining P. major ethanol-water extract and solid waste of dried leaves after tincture production, their phenolic composition, antibacterial effect and cytotoxicity.

Figure 1. *Plantago major* plant (https://www.123rf.com/photo_96874310_plantago-major-plants.html)

For HPLC analysis four extracts were prepared. Two were ethanol-water mixture with concentration of 96% (v/v) (P96 and PW96) and the other two in concentration of 70% (v/v). This method was used to determine and quantified total phenolic compounds in every single one of the prepared extracts. According to the results, extracts were full of phenolic compounds. They could be separated into two groups, phenolic acids (chlorogenic acid, caffeic acid, p-coumaric acid, p-hydroxybenzoic acid) and flavonoids (rutin, quercetin 3-O-glucoside, isorhamnetin 3-O-glucoside, apigenin, quercetin, luteolin).

Table 1. The consistent of compounds from extracts. Data are presented as the means of triplicate measurements with standard deviation.

	P70 (mg/mL)	PW70 (mg/mL)	P96 (mg/mL)	PW96 (mg/mL)
Chlorogenic acid	1.601±0.045^b	1.323±0.056^c	0.606±0.016^a	0.443±0.005^d
Luteolin	0.590±0.009^a	0.667±0.004^b	0.443±0.007^c	0.680±0.001^b
Rutin	0.375±0.006^a	0.203±0.002^b	0.186±0.000^c	0.122±0.000^d
Caffeic acid	0.139±0.001^a	0.034±0.001^b	0.118±0.000^c	0.038±0.000^d
Quercetin 3-O-glucoside	0.083±0.001^a	0.070±0.000^b	0.054±0.001^c	0.061±0.000^d
Isorhamnetin 3-O-glucoside	0.053±0.001^a	0.083±0.001^b	0.036±0.000^c	0.082±0.002^d
Apigenin	0.049±0.000^a	0.043±0.001^b	0.036±0.001^c	0.041±0.001^d
Quercetin	0.035±0.001^a	0.023±0.001^b	0.036±0.000^c	0.026±0.000^d
p-Hydroxybenzoic acid	0.025±0.001^a	0.029±0.000^b	0.018±0.000^c	0.012±0.000^d
p-Coumaric acid	0.013±0.001^a	0.011±0.001^b	0.018±0.000^c	0.004±0.000^d
Total amount	2.964±0.049	2.486±0.061	1.563±0.010	1.488±0.003

*a,b,c,d – significant difference according to Tuckey's test ($p<0.05$)

Table 2. Minimal inhibitory concentration (MIC) of extracts on *Staphylococcus aureus* strains and *Acinetobacter baumannii* strain

STRAINS	P70 (mg/mL)	PW70 (mg/mL)
S. aureus ATCC 25923	1.25	1.25
S. aureus ATCC 43300	5	2.5
S. aureus Gp41	5	5
A. baumannii ATCC 19606	-	4

NOTE
After HPLC analysis it has been shown that better consistent,with greater amount of phenolic compound, have had extract and dried waste residues after tincture production, made in ethanol-water in the concentration of 70%. These mixtures were further investigated.

Table 3. Determined IC values of agents against two cell lines, HCT116 and Hs294T

	P70 (mg/mL)		PW70 (mg/mL)	
	HCT116	Hs294T	HCT116	Hs294T
IC_{25}	1	1.7	0.85	1.5
IC_{50}	1.5	>2	1.3	>2

MICRODILUTION ASSAY
Minimal inhibitory concentration (MIC) is the lowest concentration of the treatment which inhibits bacterial growth (Fig.2). Here, extracts were examined against *Staphylococcus aureus*, two ATCC strains (MSSA 25923 and MRSA 43300), one clinical isolate Gp41 and *Acinetobacter baumannii* ATCC 19606. Results are presented in Table 2. The lowest MIC value was determined for *S. aureus* ATCC 25923 at the concentration of 1.25 mg/mL for both P70 and PW70. The most resistant *S. aureus* strain was Gp41 with MIC values of 5 mg/mL. *A. baumanni* was resistant to P70 extract, but MIC value for PW70 was 4 mg/mL.

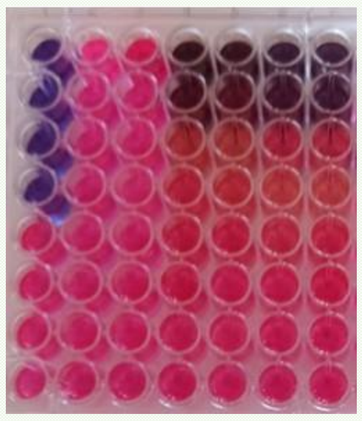

Figure 2. Microdiution assay

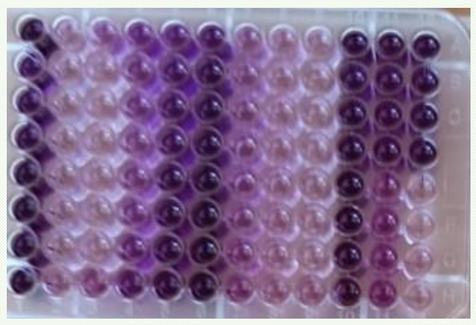

Figure 3. MTT assay

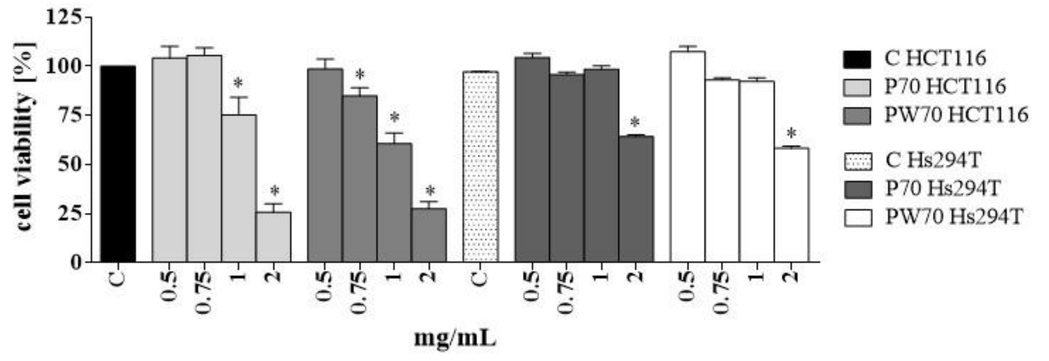

Fig 4. Cytotoxicity of ethanol-water *P. major* extract (P70) and solid waste of *P. major* dried leaves after tincture production (PW70)

MTT assay
Cytotoxicity of P70 and PW70 was investigated on two human cell lines, colon carcinoma (HCT116) and melanoma (Hs294T) (Fig 3). Cell viability was reduced in both ceases with tested agents in dose-dependent manner. Nevertheless, the effects on HCT116 cell line were stronger and this cell line was more susceptible on the treatments (Fig 4). PW70 had the lowest IC_{25} and IC_{50} value, which might be because of the different agent composition and bigger amount of luteolin in it.

CONCLUSION

Both extracts showed a great potential for further investgation. Extract made of solid waste *P. major* leaves had better antibacterial activity than P70. In addition, PW70 reduced cancer cell viability, which might suggest that this type of extract could be used, and not just discarded. Better understanding of mechanisms of actions and defining the most dominant compounds with strong therapeutic properties could help in further research of these extract and its application as a drug supplement.