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Pectic polysaccharides extracted from unprocessed and steam-distilled lavender biomass

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The present research is focused on the extraction of polysaccharides from lavender biomass. One sample of unprocessed lavender: L_UNTR_22_Z (GALEN–N, Zelenikovo, Bulgaria; 2022) and two by-products of lavender industrially processed by steam distillation: L_SD_22_M (ECOMAAT distillery, Mirkovo, Bulgaria; 2022 crop) and L_SD_22_Z (GALEN–N distillery, Zelenikovo, Bulgaria; 2022 crop) were investigated. The biomass was pretreated with 70% ethanol aiming at removal of low-molecular substances and secondary metabolites (pigments, sugars, polyphenols, etc.) which could interfere with further extraction process of polysaccharides. The obtained alcohol-insoluble parts were subjected to acid extraction and acid-soluble pectic polysaccharides were obtained. The highest yield was achieved for sample L_SD_22_M (7.54±0.11%). Furthermore, different pectic polysaccharides building the cell walls of lavender biomass, were extracted from the alcohol-insoluble parts by successive fractional extraction with hot water, 0.05 $(NH_4)_2C_2O_4$, 0.1 M HCl and cold 0.05 M NaOH. The oxalate extractable pectic polysaccharide predominated in all investigated samples suggesting that most of the pectins were ionically bound by Ca^{2+} bridges in the lavender cell walls. The highest total yield of pectic polysaccharides was obtained for L_SD_22_M (14.98±0.16%). The present research suggests that lavender biomass could be successfully valorized and pectic polysaccharides with total yield ranging from 10.55±0.12% to 14.98±0.16% could be obtained.

Keywords: *Lavandula angustifolia,* by-products, valorization, pectic polysaccharides, fractional extraction

INTRODUCTION

Lavender originates from the mountainous Mediterranean regions of Europe and North Africa. Today it is grown in many European, South American, North African, Southwest Asian countries, and Australia. Despite the fact that 32 species and numerous subspecies and varieties are known, only three of them: the ordinary lavender (*Lavandula angustifolia*), lavender aspic (*Lavandula spica* L.) and lavandin (*Lavandula hybrida* Rev.) play a major role in essential oil production [1, 2]. The essential oil obtained from *L. angustifolia* is distinguished from the others by its high quality and therefore, common lavender is the main type grown in Bulgaria [3]. What is more, during the last few years the lavender became among the most exploited species. Lavender is a plant that is used as an anti-erosion plantation, its inflorescences, as well as the essential oil are included in the composition of many preparations with application in medicine [4]. The concentration of essential oil in the fresh plant is low and large amounts of by-products remain after steam distillation. Disposal or composting is among the common practices for the treatment of the industrially processed lavender biomass. However, it can also serve as a raw material for the extraction of valuable biologically active substances that can be used in the food, cosmetic and perfume industries [5].

Pectins or pectic polysaccharides are quite common multifunctional components in the cell walls of almost all higher plants. Pectin macromolecules are made up of more than 15 monosaccharides. Among these, α-D-galacturonic acid is the major building block. Pectin has a wide range of applications in the food and cosmetic industries as gelling agent, stabilizer and thickener [6]. Due to the scarce information in the literature on pectic polysaccharides extracted from lavender by-products the aim of the present work was to investigate two lavender by-products and one untreated lavender for obtaining of polysaccharides, as a possible method for by-products valorization.

MATERIALS AND METHODS

The solid by-products of lavender essential oil industry were kindly provided by: Galen-N Ltd. (Zelenikovo, Brezovo, Bulgaria; 2022 harvest); from steam distillation of *Lavandula angustifolia*, abbreviated as L_SD_22_Z; EKOMAAT Ltd.

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(Mirkovo, Sofia, Bulgaria; 2022 harvest); from steam distillation of *Lavandula angustifolia*, abbreviated as L_SD_22_M. The raw untreated lavender was provided by Galen-N Ltd. (Zelenikovo, Brezovo, Bulgaria; 2022 harvest) and further referred to as L_UNTR_22_Z.

Preparation of alcohol-insoluble residues (AIR)

Lavender biomass was treated with 70% ethanol (v/v) and after filtration, AIRs of the initial raw materials were prepared as described in [7].

Acid extraction (0.1 M hydrochloric acid) of AIRs

The acid extraction was performed twice. The first extraction was performed with 1000 mL of 0.1 M aqueous hydrochloric acid (HCl). The extraction took place for 1 h at 85°C, pH 1.5 and continuous stirring. The slurry was filtered through a nylon cloth (250 mesh). The second extraction was carried out on the solid residue from the first extraction using 1000 mL of 0.1 M aqueous HCl under the same conditions. The two filtrates were precipitated with 96% ethanol (1:2 parts by volume) to obtain the acid-soluble pectic polysaccharides.

Determination of ash content

Ash is the total inorganic residue produced after combustion or complete oxidation of the organic matter in the various samples. 1 g of the sample (m_1) was placed in a pre-weighed porcelain crucible (m_C). The crucible was placed in an oven heated to 600°C and held for 4 h. The crucible was removed and after tempering in a desiccator, ws weighed until a constant weight (m_2) was established.

The ash content (A, %) was calculated according to the formula:

$$A, \% = \frac{(m_C+m_1)-m_2}{m_C+m_1} \times 100 \qquad (1)$$

Determination of protein content in the lavender raw materials

The determination of the protein content in the lavender raw materials was performed according to the AOAC Method 976.06 using automated Kjeldahl system MultiKjel K-365 with potentiometric titrator Metrohm ECO (Büchi Labortechnik, Switzerland) and conversion factor 6.25 for converting the amount of nitrogen to protein. The polyuronide content PU and degree of esterification DE were determined as described in [8]. The protein content of the polysaccharides, the amounts of neutral sugars and the anhydrogalacturonic acid content were determined spectrophotometrically using the Bradford method with AMRESCO E535-KIT (AMRESCO, Solon, Ohio, USA) with bovine gammaglobuline as standard, the phenol-sulfuric acid method with D-galactose as standard and the m-hydroxydiphenyl method with D-galacturonic acid as standard, respectively, as described in details in [9]. The molecular mass of the isolated polysaccharides was determined using an ELITE LaChrome HPLC system (Hitachi) with a VWR Hitachi Chromaster 5450 light refraction detector and an OHpak SB-806M column (Shodex®). Samples and standards were eluted with 0.1 M $NaNO_3$ at an elution rate of 0.8 mL/min, column temperature of 30°C and detector temperature of 35°C. The column was equilibrated with standards of Shodex® pullulans (Showa DENKO, Japan) (2 mg/mL) with molecular weights of 6.2×10^3, 10.0×10^3, 21.7×10^3, 48.8×10^3, 113.0×10^3, 200.0×10^3, 366.0×10^3, and 805.8×10^3 Da.

RESULTS AND DISCUSSION

The general characteristics of the by-products and untreated lavender are presented in Table 1.

Analyses of the main physicochemical parameters of the lavender biomass showed that L_SD_22_Z had the highest mineral content (7.45±0.20%) and the highest polyuronide content (8.0±0.2%) compared to the other two materials.

In the next experiments, the AIRs were subjected to an extraction with 0.1 M HCl in order to obtain pectic polysaccharides. This extraction is mimicking the industrial processing of the by-products from the fruit juice industry and obtaining of apple and citrus pectins [10]. The yield and the general physico-chemical parameters of the isolated polysaccharides are presented in Table 2.

Table 1. General characteristics of lavender by-products and raw untreated lavender

Parameter \ By-product	L_SD_22_M	L_SD_22_Z	L_UNTR_22_Z
Ash, %	5.41±0.07[b]	7.45±0.20[a]	4.10±0.33[c]
Protein, %	8.15 ± 1.6[a]	6.72 ± 0.28[b]	5.94 ± 0.12[c]
PU, %	7.5±0.1[b]	8.0±0.2[a]	7.1±0.1[c]
DE, %	83.9±0.5[a]	78.9±0.5[c]	81.6±0.3[b]

PU - polyuronide content; DE – degree of esterification. The results are expressed as mean ± SD (n = 3). [a,b,c] Different letters in a row mean statistical difference (Tuckey's test, $p < 0.05$).

Table 2. Yield and physico-chemical parameters of pectic polysaccharides extracted by 0.1 M HCl from lavender AIRs

	Yield, %	Crude protein, %	Neutral sugars, μg/mg	Molecular weight, $\times 10^4$ Da	Proteins, μg/mg
L_SD_22_M	7.54±0.15[a]	5.32±0.24[a]	599.71±24.26[b]	2.47	nd
L_SD_22_Z	6.62±0.16[b]	4.65±0.19[b]	713.55±19.34[a]	2.34	1.37±0.07
L_UNTR_22_Z	6.92±0.21[b]	4.85±0.17[b]	585.87±21.04[b]	3.28	nd

The results are expressed as mean ± SD (n = 3). [a,b] Different letters in a column mean statistical difference (Tuckey's test, $p < 0.05$).

Table 3. Yield and physico-chemical parameters of pectic polysaccharides obtained by sequential fractional extraction with deionized water, 0.05 M $(NH_4)_2C_2O_4$, 0.1 M HCl and 0.05 M NaOH of L_SD_22_M, L_SD_22_Z and L_UNTR_22_Z AIRs

	Extractor	Yield, %	Neutral sugars, μg/mg	Molecular weight, $\times 10^4$, Da	Proteins, μg/mg
L_SD_22_M	Deionized water	4.60	503.10	3.07	5.20
	0.05 M $(NH_4)_2C_2O_4$	7.59	391.52	8.31	0.85
	0.1 M HCl	2.28	551.13	2.23	nd
	0.05 M NaOH	0.59	446.04	2.01	nd
L_SD_22_Z	Deionized water	4.05	428.81	2.08	8.37
	0.05 M $(NH_4)_2C_2O_4$	7.12	523.16	7.26	nd
	0.1 M HCl	2.38	474.29	2.92	2.15
	0.05 M NaOH	0.62	431.35	1.91	5.65
L_UNTR_22_Z	Deionized water	0.99	252.25	3.51 1.19	4.92
	0.05 M $(NH_4)_2C_2O_4$	4.69	432.76	26.17	0.41
	0.1 M HCl	3.87	384.74	3.07	nd
	0.05 M NaOH	1.01	467.51	2.38	5.05

nd – not determined.

The highest yield of acid-soluble pectic polysaccharides was observed for alcohol-insoluble parts of lavender by-product L_SD_22_M: 7.54%. The highest amount of neutral sugars was found for L_SD_22_Z: 713.55 μg/mg polysaccharide. For all three polysaccharides the amount of proteins was very low.

The experiments employing sequential extraction with different extractants suggested that the highest pectin yield was observed for the ammonium oxalate as extractant and the percentage of pectin was around 7%, except for the L_UNTR_22_Z, where the yield was 4.69% (Table 3). These results could be explained by the ionic bonding by Ca^{2+} ions of the pectic polysaccharides present in the lavender cell walls. The yield of water-soluble pectic polysaccharides for L_UNTR_22_Z was quite low: 0.99%, compared to the two by-products (4.60 and 4.05% for the L_SD_22_M and L_SD_22_Z, respectively). This might suggest that the steam distillation serves as initial pretreatment, which disrupts the cell walls of lavender biomass and facilitates further extraction of pectic polysaccharides from the by-products.

The evaluation of the monosaccharide composition of the extracted polysaccharides (Table 4) showed that the major building monomer of the extracted polysaccharides from L_SD_22_M (with 0.1 M HCl): 717.62 μg/mg; L_SD_22_Z (with deionized water): 312.42 μg/mg; L_UNTR_22_Z (with 0.1 M HCl): 448.78 μg/mg, was galacturonic acid. Small amounts of glucuronic acid were also detected. The second most abundant monosaccharide was galactose. The other monosaccharides detected that are characteristic of pectic polysaccharides were arabinose and xylose.

Table 4. Uronic acids and monosaccharide composition of pectic polysaccharides extracted by deionized water, 0.05 M $(NH_4)_2C_2O_4$, 0.1 M HCl and 0.05 M NaOH of L_SD_22_M, L_SD_22_Z and L_UNTR_22_Z AIRs

	Extractor	GlcA	GalA	Gal	Ara	Fuc	Xyl	Man
		(Polysaccharide, μg/mg)						
L_SD_22_M	Deionized water	30.63	517.67	88.59	32.17	1.95	13.03	12.73
	0.05 M $(NH_4)_2C_2O_4$	12.38	331.90	54.23	33.77	nd	22.11	7.12
	0.1 M HCl	8.31	717.62	91.79	19.40	nd	20.45	5.26
	0.05 M NaOH	6.83	444.44	134.27	16.80	nd	16.08	9.03
L_SD_22_Z	Deionized water	25.51	312.42	56.41	20.04	1.10	15.99	19.59
	0.05 M $(NH_4)_2C_2O_4$	10.44	233.26	54.78	52.02	nd	17.02	4.07
	0.1 M HCl	4.81	272.23	52.00	12.15	nd	10.12	17.98
	0.05 M NaOH	5.22	165.77	69.74	14.59	nd	12.85	9.12
L_UNTR_22_Z	Deionized water	43.14	189.43	49.94	10.13	nd	9.58	25.71
	0.05 M $(NH_4)_2C_2O_4$	10.40	243.89	32.76	27.36	nd	10.17	15.60
	0.1 M HCl	11.16	448.78	76.22	26.58	nd	55.33	10.63
	0.05 M NaOH	7.48	221.73	69.11	10.88	nd	14.32	3.40

GlcA – D-Glucuronic acid; GalA – D-Galacturonic acid; Gal – D-Galactose; Ara – D-Arabinose; Fuc – L-Fucose; Xyl – D-Xylose; Man – D-Manose; nd – not determined.

CONCLUSION

The hypothesis of the present work was to investigate the lavender biomass as a potential source of pectic polysaccharides – a possible pathway for lavender by-products valorization. Two solid residues obtained after steam distillation (the traditional way of lavender processing) and one untreated lavender pulp were investigated. Composition analysis showed that lavender by-products were rich in pectic polysaccharides, proteins, neutral sugars, etc. The highest yield of acid-soluble pectic polysaccharides was observed for the alcohol-insoluble parts of lavender by-product L_SD_22_M: 7.54%. The highest yield from the sequential fractional extraction of polysaccharides was observed using ammonium oxalate as extractant. This could suggest that a large part of the pectic polysaccharides were present in the lavender cell wall biomass, ionically bonded by divalent cations (mostly Ca^{2+}). We conclude that lavender residues after steam distillation could serve as a potential source of pectic polysaccharides, which combined with other approaches will allow valorization of the lavender by-products from essential oil industry.

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