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STUDIES REGARDING THE CYTOTOXICITY OF A BIOPRODUCTS BASED ON POLYSACCHARIDES DERIVED FROM *PLANTAGO* SP.

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Abstract

Cytotoxicity studies performed on HUVEC and CaCo-2 cell lines with a polysaccharides-based bioproduct isolated from Plantago lanceolata as well with inulin (for comparison) revealed moderate cytotoxicity after 24-hour exposure to inulin and polysaccharides from Plantago lanceolata. Inulin showed a high selectivity index (SI = 11) for the CaCo-2 tumour cell line, indicating specificity for this cell line. After 48 hours, the polysaccharides from Plantago lanceolata showed no cytotoxicity on either studied cell line. Inulin showed no cytotoxicity to HUVEC cells but exhibited cytotoxicity to CaCo-2 cells, giving a higher selectivity index (SI = 25.3) after 48 hours of exposure. Overall, the polysaccharides from Plantago lanceolata showed similar physicochemical properties to inulin, without cytotoxicity on HUVEC cells and moderate cytotoxicity on CaCo-2 cells after 48 hours of exposure.

Key words*: cytotoxicity, Plantago lanceolata, selectivity index.*

INTRODUCTION

Polysaccharides from plants represent potential sources of fibre for functional foods. From this perspective, an interesting species is *Plantago lanceolata*, an indigenous plant from spontaneous flora, from which bioproducts with therapeutic properties can be obtained (Pol et al., 2021; Zaharie et al., 2022; Radu et al., 2010). At present, this plant are distributed entire the worldwide (Figure 1) (Ianovici et al., 2010; Rojas Sandoval, 2023). According to data published by Bräutigam & Franz (Bräutigam & Franz, 1985), the crude polysaccharides extract obtained from *Plantago lanceolata* contains as a major compounds D-galactose, L-arabinose, and D-galacturonic acid. The leaves of this species contain polysaccharides with units consisting of galactose (44%), arabinose (32.4%), rhamnose (6.9%), mannan (4%), glucose (9.2%), and xylose (1.9%) (Pol et al., 2021; Clifford et al., 2002). Recent studies performed on three species of *Plantago* have shown that, overall, the plant may contain 12% fibre and 11.64% carbohydrates (Alghamadi, 2018). Polysaccharides from *Plantago,* obtained through acid hydrolysis, contain sorbitol, glucitol, fructose, glucose, sucrose, and raffinose (Jiang et al., 2019). Guil-Guerrero (Guil-Guerrero, 2001) determined the mineral, fatty acid, vitamin, nitrate, and oxalic acid content for three *Plantago* species from south-eastern Spain, and the results obtained showed that these plants had a low carbohydrate content, ranging between 1.99-2.81 g/100 g fresh plant.

Polysaccharides isolated from indigenous plants can represent potential sources of fiber for new functional foods. From this perspective, an interesting species is *Plantago lanceolata*, an indigenous plant from the spontaneous flora, from which bioproducts with therapeutic properties can be obtained. Our previous studies (Zaharie et al., 2022) revealed that the polysaccharide fraction isolated from *Plantago lanceolata* exhibits prebiotic activities for probiotic microorganisms such as *Lactobacillus* sp.

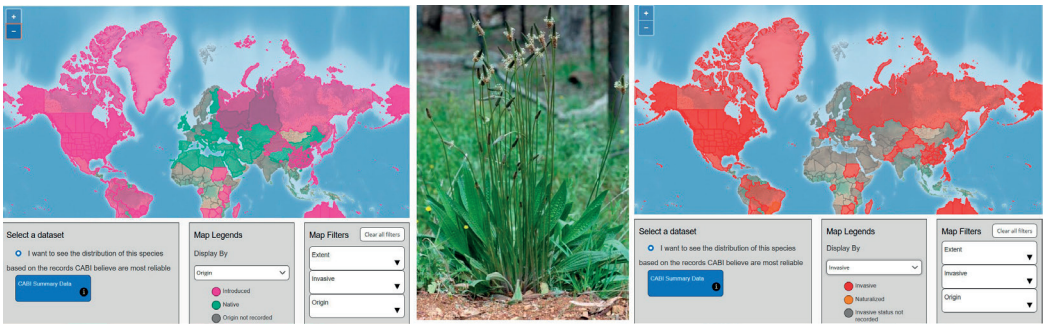


Figure1. The global distribution of the plant *Plantago lanceolata* (Sources: https://www.cabidigitallibrary.org/doi/full/10.1079/cabicompendium.41813#sec-9; https://tropical.theferns.info/image.php?id=Plantago+lanceolata

In order to assess its potential for developing functional foods, the present study aimed to: 1) characterise physical chemical this fraction; and 2) to evaluate the effect of this biomaterial on normal and/or tumoral cell lines, by studies performed *in vitro*.

MATERIALS AND METHODS

Electrospray ionisation mass spectrometry (ESI-MS) analysis of the macromolecule sizes in the polysaccharide fraction isolated from Plantago sp. was performed using high-performance liquid chromatography (HPLC) with LC-MS/TOF 6224 system (Agilent Technology, Houston, TX, USA) (Băbeanu et al., 2022; Ho et al., 2003). The polysaccharides isolated from *Plantago* sp. were injected into the ionisation chamber as 0.1% solution made in 99% DMSO (dimethyl sulfoxide, Merck, Bucharest, Romania).

Infrared spectra were recorded in the 400-4000 cm^{-1} range using a Perkin Elmer FT-IR GX spectrophotometer (Perkin Elmer Ltd., Beaconsfield, UK), equipped with an ATR device, Dynascan interferometer, and DTGS (deuterated triglycine sulfate) detector. The infrared spectra were analysed comparatively, for a sample containing polysaccharides isolated from *Plantago lanceolata*, and for an inulin sample (inulin from chicory, with average molecular mass = 3.5-5.5 kDa, Merck, Bucharest, Romania). Cell proliferation tests were conducted according to the methodology described by Zaharie et al. (2022), using two standardised human cell lines: HUVEC ATCC PCS 100-010 and CaCo-2 ATCC HTB-37, The cell lines were provided by S.C. BioZyme SRL, Cluj-Napoca, Romania. The proliferation index (PI) was calculated using the following formula:

PI = (%viability of treated cells)/% viability of untreated cells (1)

where:

Viability % of treated cells = % of viable cells in each tested experimental variant;

Viability % of untreated cells = % of viable cells in the control sample (considered as a reference)

The analysed polysaccharides were isolated from the aerial part of the *Plantago lanceolata,* collected from the spontaneous flora of Sibiu, Romania (voucher specimen deposited at INCDCF Bucharest, under the number Plal19) according to methodology of Zaharie and colaborators (Zaharie et al, 2022).

RESULTS AND DISCUSSIONS

The results obtained from ESI-MS analysis (presented in Figure 2 and Table 1) show that the polysaccharides isolated from *Plantago lanceolata* contain species with molecular mass ranging between 0.2-1.94 kDa (Figure 2).

Infrared spectroscopy analysis (Figure 3 a, b; Table 1) conducted on the studied polysaccharide fraction, in comparison with inulin, revealed the following:

a) an intense band due to the carbon-carbon double bond (νC=C) appears at 1575 cm^{-1} in the polysaccharides isolated from *Plantago* sp. The same bands it is also present in inulin but shifted to 1617 cm^{-1};

b) a high-intensity band at 1398 cm^{-1}, which appears in the polysaccharide fraction isolated

from Plantago sp., is also observed in inulin, though as a weaker band which appears at 1417 cm^{-1} and is attributed to the C-H stretching vibration (νC-H); c) an intense band appears at 1084 cm^{-1} in the sample of Plantago's polysaccharides which also was found in inulin at 1099 cm^{-1}, but as a weaker band. These bands are mainly due to the stretching of the glycosidic bond (C-O-C) and C-C or C-O bond stretching from the pyranosic ring; d)the medium-intensity band from 878 cm^{-1} which appears in the in the polysaccharides from *Plantago* sp., also appears as a medium-intensity band in inulin at 873 cm^{-1} and is attributed to the vibration of the 2-keto pyranosic ring. The both biomaterials (polysaccharides from *Plantago* sp. and inulin) exhibit: a1) C-H stretching vibrations detected in the IR spectrum at 2922 cm^{-1} (*Plantago* sp.) and 2902 cm^{-1} (inulin); a2) bands attributed to intermolecular hydrogen bonding vibrations, appearing at 3238 cm^{-1} for polysaccharides from Plantago sp. and 3257 cm^{-1} for inulin (Balan et al., 2018).

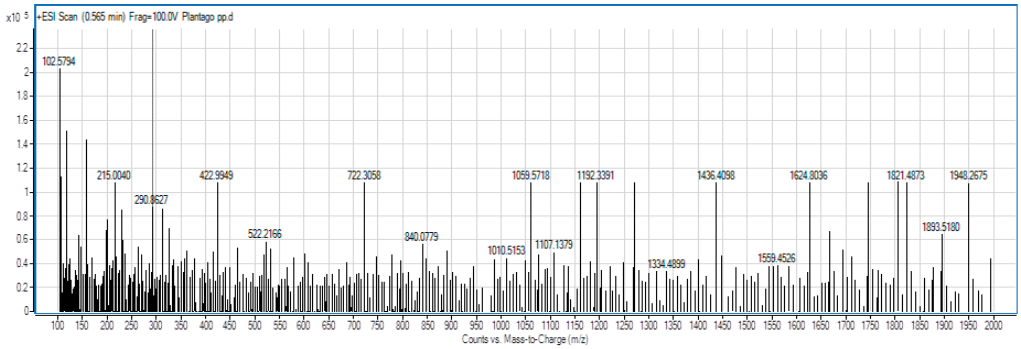


Figure 2. ESI-MS analysis for polysaccharides fraction isolated from *Plantago lanceolata*

Table 1 The infrared bands appearing in the FT-IR spectra of inulin and the polysaccharide fraction isolated from *Plantago lanceolata*

Domain	Inulin	*Plantago lanceolata* polisaccharides	Attribution	Bibliographic references
<900 cm^{-1}	817 cm^{-1} (m)	835 cm^{-1}	C-C-C; O-C-O;C-H -bending vibration of 2-cetose	Balan et al., 2018 Akram & Garud, 2020
	873 cm^{-1} (m)	878 cm^{-1} (m)	-bending vibration δ(C1-H) in the ring (2-ketofuranosic)	Akram & Garud, 2020
900-1500 cm^{-1}	1028 cm^{-1} (i)	1054 cm^{-1}	stretching vibration of the C=O; νC-O (C-O); νC-O-C	Balan et al., 2018 Akram & Garud, 2020 Melanie et al., 2015
	1099 cm^{-1} (s)	1084 cm^{-1} (i)	- stretching vibration of the C-C bond in the pyranose ring -stretching vibration of the C-O bond in the pyranosic ring -stretching vibration of the glycosidic C-O-C bond	Redondo-Cuenca et al., 2021
	1213 cm^{-1}	1194 cm^{-1}	- stretching vibration of the C-C bond from pyranosic ring -stretching vibration of the C-O bond from pyranosic ring - stretching vibration of the C-O-C from glycosidic bond	Redondo-Cuenca et al., 2021
	1319 cm^{-1}	1334 cm^{-1}	βO-H (OH) ν C-H, νO-H	Akram & Garud, 2020 Melanie et al., 2015
	1417 cm^{-1} (s)	1398 cm^{-1} (i)	δC–Hs (CH2)	Akram & Garud, 2020 Melanie et al., 2015
1550-2500 cm^{-1}	1617 cm^{-1} (s)	1575 cm^{-1} (i)	C=C	Balan si col., 2018
2500-3600 cm^{-1}	2902 cm^{-1}	2922 cm^{-1}	ν C–Hs (CH2)	Akram & Garud, 2020; Melanie et al., 2015 Redondo-Cuenca et al., 2021
	3257 cm^{-1}	3238 cm^{-1}	νO-H (OH) intermolecular hydrogen bonds	Akram & Garud, 2020 Melanie et al., 2015
(i) = intense band; (m) = medium band; (s) = weak band				

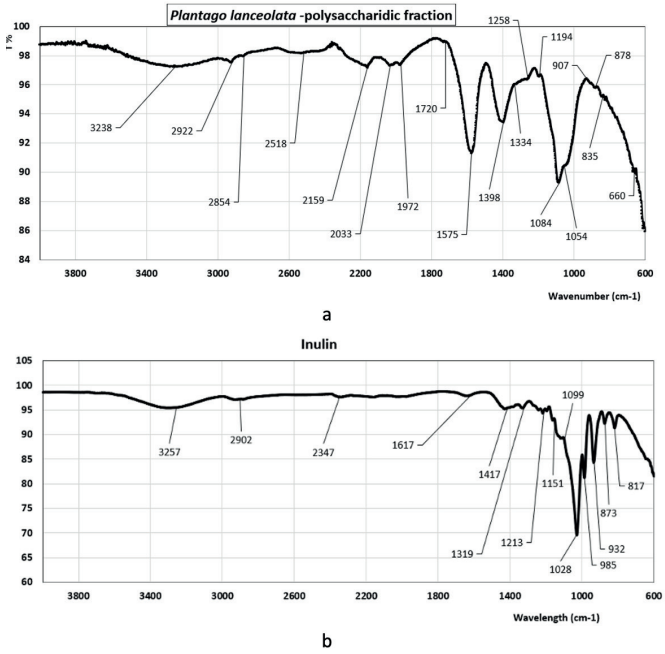
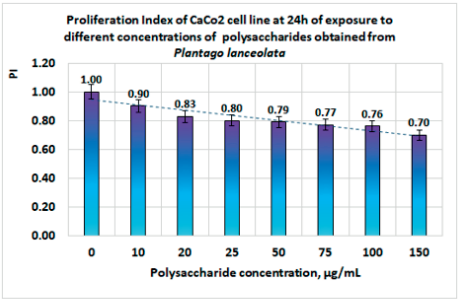


Figure 3. FT-IR Spectra for**:** a) polysaccharides fraction isolated from *Plantago lanceolata;* b) inulin

The infrared spectra confirm that both molecules contain C=C bonds and glycosidic C-O-C bonds. In inulin, the C-O-C band appears at 1028 cm^{-1}, whereas in the polysaccharides isolated from Plantago lanceolata, it appears at 1054 cm^{-1}. For biomaterial derived from *Plantago lanceolata*, this band is structured as a shoulder, which appears at 1054 cm^{-1} and as a well-defined intense band with a maximum at 1084 cm^{-1} (Balan et al., 2018).

The exposure for 24 hours of the studied cell lines to inulin and/or polysaccharides isolated from *Plantago lanceolata* (Figure 4 a, b; Figure 5 a, b) leads to the following results: a1) in the case of exposing the CaCo-2 cell line to polysaccharides from *Plantago* sp., the PI values ranging from 0.7- 1 µg/mL are obtained for polysaccharide concentrations between 0-150 µg/mL (Figure 4 a); a2) exposure of the CaCo-2 cell line to inulin concentrations between 45-0 µg/mL results in PI values ranging from 0.49-1, with inulin being cytotoxic to CaCo-2 cells starting at concentrations of 30 µg/mL (Figure 4 b); a3) when exposing the HUVEC cell line to polysaccharides from *Plantago* sp., the proliferative index (PI) obtained ranges from 0.86-1 for a concentration range between 0-150 µg/mL (Figure 5 a); a4) exposure of the HUVEC cell line to inulin results in the values of PI ranging between 0.8-1 for inulin concentrations between 45-0 µg/mL (Figure 5 b).

Exposure for 48 hours of the studied cell lines to inulin and/or polysaccharides isolated from *Plantago lanceolata* (Figure 6 a, b; Figure 7 a, b) leads to the following results: b1) in the case of exposing the CaCo-2 cell line to polysaccharides from *Plantago lanceolata* for 48 hours, PI values ranging from 0.94-1 are obtained for polysaccharide concentrations in the culture medium between 150-0 µg/mL (Figure 6 a); b2) at the exposure of the CaCo-2 cell line to inulin concentrations between 0-45 µg/mL are obtain PI values situated between 0.15-1, with inulin being cytotoxic for the CaCo-2 cell line at concentrations greater than 1.5 µg/mL (Figure 6 b); b3) the exposure of the HUVEC cell line to polysaccharides isolated from *Plantago lanceolata* generate PI values between 1-1.08 for polysaccharide concentrations sitiated between 0-150 µg/mL (Figure 7 a);



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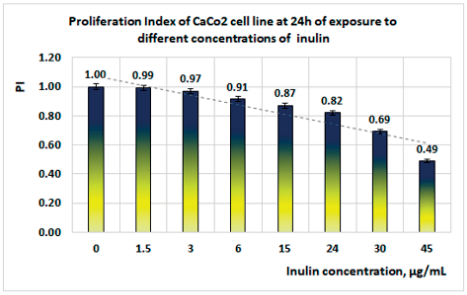

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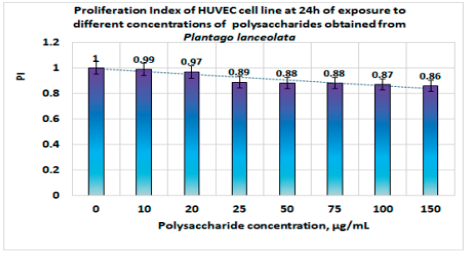
Figure 4. The effect of exposing the CaCo-2 cell line for 24 hours to polysaccharides isolated from: a) *Plantago lanceolata*; b) inulin



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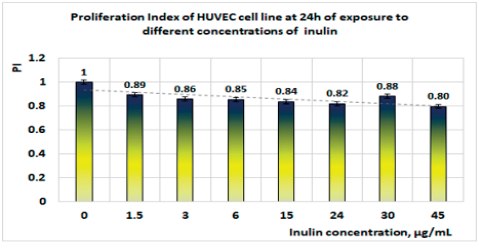
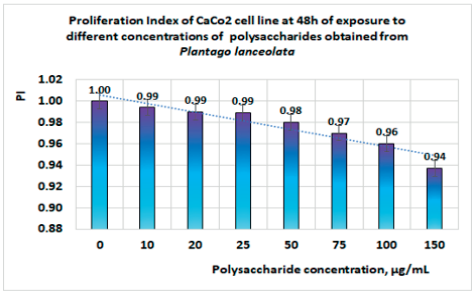


b

Figure 5. The effect of exposing the HUVEC cell line for 24 hours to polysaccharides isolated from: a) *Plantago lanceolata*; b) inulin (Source: own studies)

b4) exposure of the same normal cell line to inulin for 48 hours, for inulin concentrations ranging between 0-45 µg/mL, generates the PI values situated between 1-0.82 (Figure 7 b).



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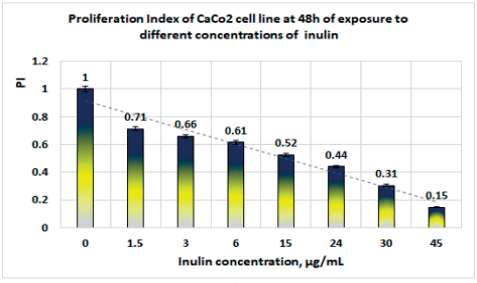

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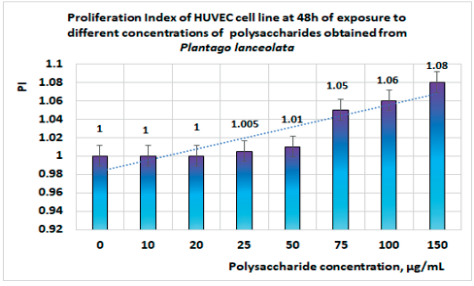
Figure 6. The effect of exposing the CaCo-2 cell line for 48 hours to polysaccharides isolated from: a) *Plantago lanceolata*; b) inulin



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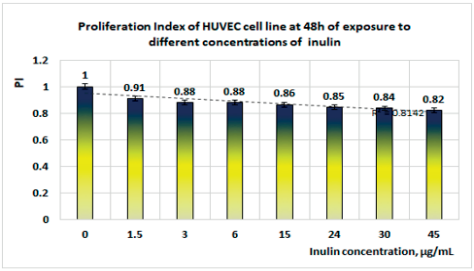

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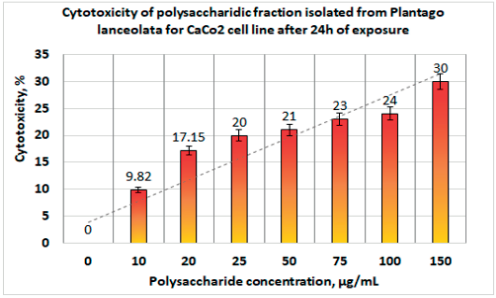
Figure 7. The effect of exposing the HUVEC cell line for 48 hours to polysaccharides isolated from: a) *Plantago lanceolata*; b) inulin

As a general observation, it is noted that, for the normal HUVEC cell line, polysaccharides isolated from *Plantago lanceolata* have a lower

cytotoxic effect than inulin, for which PI values smaller than 1 are obtained.

Regarding the cytotoxicity calculated for the CaCo-2 and HUVEC cell lines, in the case of the two biopreparations (polysaccharides from *Plantago* sp. and inulin), the results showed that for concentrations of polysaccharides isolated from *Plantago* sp. between 0-150 µg/mL, a dose-effect relationship is obtained (Figure 8 a), with the maximum cytotoxicity reaching 30%. In the case of exposure to inulin, the cytotoxicity generated on the CaCo-2 cell line is higher.

A dose-effect relationship is also evident (Figure 8 b). The maximum cytotoxicity obtained in this case is 51.13% for 45 µg inulin/mL



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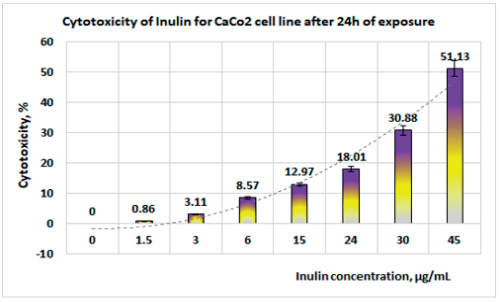
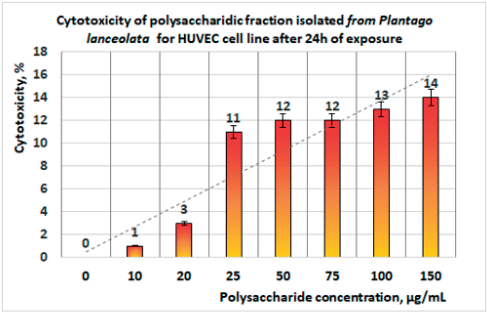


a

Figure 8. The cytotoxic effect generated by exposing the CaCo-2 cell line for 24 hours to: a) Polysaccharides isolated from *Plantago lanceolata*; b) inulin

In the case of the normal HUVEC cell line, the maximum cytotoxicity obtained at exposure to polysaccharides isolated from *Plantago lanceolata* is 14% (Figure 9 a). It corresponds to a concentration of 150 µg polysaccharides/mL. In the case of inulin, at a concentration of 45 µg/mL in the culture medium, the cytotoxicity obtained is 20.4% (Figure 9 b). In general, after 24 hours of exposure, inulin exhibits a more pronounced cytotoxic effect on the CaCo-2 and HUVEC cell lines.



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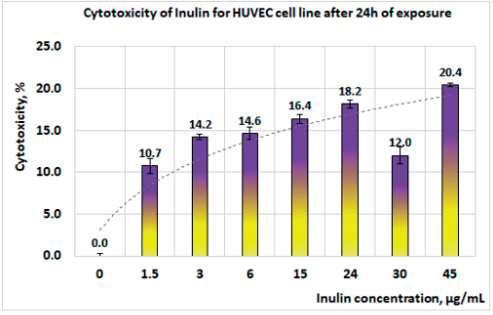

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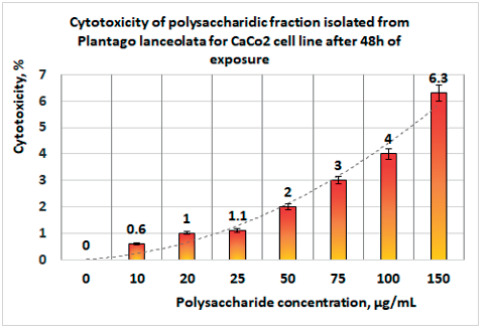
Figure 9. The cytotoxic effect generated by exposing the HUVEC cell line for 24 hours to: a) polysaccharides isolated from *Plantago lanceolata*; b) inulin (Source: own studies)

Cytotoxicity studies performed at 48 hours showed that, in the case of the CaCo-2 cell line, exposure to polysaccharides isolated from *Plantago lanceolata* resulted in a medium cytotoxicity of 6.3% for a polysaccharide concentration of 150 µg/mL in the culture medium (Figure 10 a).

The other hand, exposure of the same cell line to 45 µg/mL of inulin in the culture medium resulted in a cytotoxicity of approximately 85.33% (Figure 10 b).

In the case of the normal HUVEC cell line, it can be observed that after 48 hours of exposure to polysaccharides derived from *Plantago lanceolata*, no cytotoxic effects are observed (Figure 11 a).

However, in the case of inulin, a cytotoxic effect of 17.9% is observed at a concentration of 45 µg/mL (Figure 11 b).



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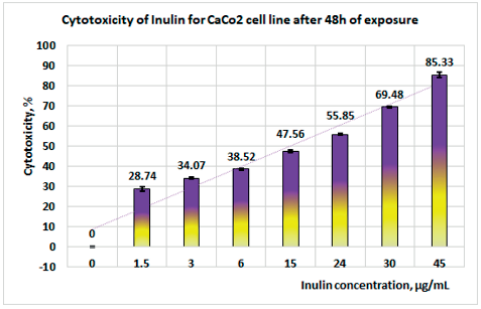

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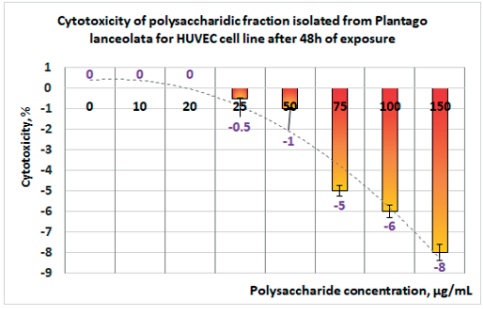
Figure 10. The cytotoxic effect generated by exposing the CaCo-2 cell line for 48 hours to: a) Polysaccharides extracted from *Plantago lanceolata*; b) inulin (Source: own studies)



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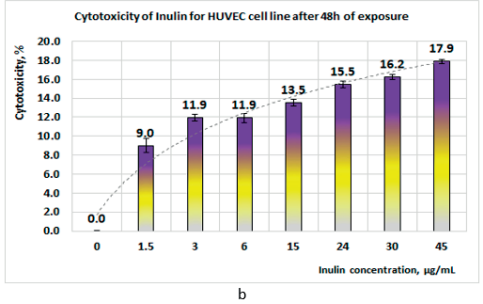

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Figure 11. The cytotoxic effect generated by exposing the HUVEC cell line for 48 hours to: a) polysaccharides extracted from *Plantago lanceolata*; b) inulin (Source: own studies)

The evaluation of the IC50 *in vitro* for inulin and polysaccharides isolated from *Plantago lanceolata* for two standardized cell lines, HUVEC and CaCo-2, at 24-hour and 48-hour exposure times showed that, generally, after 24 hours of exposure, moderate cytotoxicity was observed for both cell lines.

However, after 48 hours of exposure, inulin exhibited high cytotoxicity for the CaCo-2 cell line (Table 2). The evaluation of the selectivity index for the two studied biomaterials (Table 2) showed that the polysaccharides isolated from *Plantago lanceolata* do not exhibit selectivity *in vitro* for the CaCo-2 cell line, whereas inulin, both at 24 hours and 48 hours of exposure, shows significant *in vitro* selectivity for the CaCo-2 cell line (Selectivity - S = 11 for CaCo-2 at 24 hours and 25.36 at 48 hours of exposure, Table 2).

Table 2. Evaluation of the selectivity of the studied biomaterials on the CaCo-2 cell line at 24-hour and 48-hour exposure times. Source: own studies

Polysaccharide sources	Cells line	Exposure time, h	IC50 µg/mL±StDev	S.I.
Plantago lanceolata	CaCo-2	24	196.62±1.97	2
Chickory	CaCo-2	24	44.43±0.44	11
Plantago lanceolata	HUVEC	24	400.00±4.00	-
Chickory	HUVEC	24	427.52±4.28	-
Plantago lanceolata	CaCo-2	48	1210.65±12.11	-
Chickory	CaCo-2	48	19.83±0.20	25.36
Plantago lanceolata	HUVEC	48	-809.31±8.09	-
Chickory	HUVEC	48	502.78±5.03	-
(S.I.=Selectivity Index; S.I.=(IC50 HUVEC cell line/IC50 CaCo-2 cell line)				

The results obtained are consistent with those reported in the specialised literature for the studies carried out *in vitro*, or on lab animals. Thus, studies conducted by Lafontaine and collaborators regarding the effects of galactooligosaccharides and fructo-oligosaccharides on colon epithelial cells cultured in monolayer indicated that the polysaccharides studied have a positive effect on the integrity of the epithelial barrier (Lafontaine et al., 2020). Other studies have shown that the use of inulin as an adjunct during chemotherapy with doxorubicin results obtaining a cytotoxic response at lower concentrations of doxorubicin when used concomitantly with inulin (Schoener et al., 2013). Rahamooz-Haghighi et al. obtained

polysaccharides from *Plantago* sp. through alcoholic extraction and tested their cytotoxicity *in vitro* on standardised cell lines of colorectal adenocarcinoma HCT-116, colon cancer type SW-480, and a normal HEK-293 cell line (Rahamooz-Haghighi et al., 2021).

The tested concentration range used was ranged 25-200 µg/mL of crude extract/mL of culture medium, and the exposure times to the polysaccharides were 24 hours and 72 hours. The results obtained after 24 hours showed that the crude polysaccharide extract has cytotoxic effects on the HCT-116 cell line at concentrations higher than 400 µg/mL, at which cell viability decreases to 80%. In the case of the SW-480 and HEK-293 cell lines, no cytotoxic effects were observed after 24 hours of exposure (Rahamooz-Haghighi et al., , 2021). After 48 hours of exposure to the crude polysaccharide extract, the authors found that for the HCT-116 cell line, viability decreased to 75% at 400 µg/mL. For the HEK-293 and SW-480 cell lines, cell viability decreased slightly but remained above 80%. (Rahamooz-Haghighi et al., 2021). After 72 hours of exposure, the reduction in viability for the HCT-116 cell line was more pronounced, with cell viability reaching 85% at 25 µg/mL of crude extract in the culture medium. At 400 µg/mL of crude extract in the culture medium, after 72 hours, cell viability decreased to 60%, and the biopreparation became cytotoxic for this cell line. The SW-480 cell line ranked second in terms of cytotoxicity after 72 hours of exposure, with cell viability of 65% at 400 µg/mL in the culture medium. In the case of the HEK-293 cell line, a dose-effect relationship was observed, but the cell viability did not decrease below 80% (Rahamooz-Haghighi et al., 2021).

Regarding the cytotoxicity of the crude polysaccharides towards *Artemia salina*, the results obtained indicated no cytotoxic effects, with values of 100% achieved for all the concentration ranges studied (from 7.81 to 1000 µg/mL crude polysaccharide extract) (Rahamooz-Haghighi et al., 2021).

The tests performed on the lab animals did not indicate cytotoxic effects leading to mortality. In the case of laboratory animals, the concentrations tested were 175, 1750, and 5000 µg/kg body weight (Rahamooz-Haghighi et al., 2021). *In vitro* and *in vivo* studies conducted by Gao and collaborators (Gao et al, 2021) on the total polysaccharide fraction isolated from a Plantago species showed that:

a) the tests performed on the CD 11CT cells, derived from bone marrow exposed to polysaccharides concentrations ranging from 12.5-200 µg/mL, and exposure times of 24 h and 48 h, the measured viability was around of 90% regardless of the polysaccharide concentration in the culture medium;

b) the tests performed on the canine mammary adenocarcinoma cell lines (CIPp) with polysaccharides obtained from *Plantago* sp. at exposure times of 24 h and 48 h for polysaccharides concentrations ranging from 8-250 µg/mL, showed that the cell viability remained unaffected (viability = 100%);

c) the tests made on the murine 4T1 tumour cell lines, demonstrated that if laboratory animals with induced tumour pathology by 4T1, were treated with the polysaccharide extract isolated from *Plantago* sp., tumour growth was slightly inhibited;

d) if the crude polysaccharide extract was administered concomitantly with the chemotherapy reagent (taxol), it was found that after 20 days of treatment, the tumour volume decreased from 2000 mm^3 to 1000 mm^3, with tumour progression slowed by approximately 50%. Zhang and collaborators showed that the mechanism of action of crude polysaccharides isolated from *Plantago* sp. is based on inhibiting the migration processes of 4T1 cells, improving phagocytosis processes, and the release of reactive oxygen species by macrophage cells (Zhang et al., 2023).

The polysaccharide extract from *Plantago* sp. mediates the polarization of macrophages to the M1 phenotype, which suppresses the proliferation, migration, and invasion of 4T1 tumour cells (Zhang et al., 2023). Regarding the probiotic effect, studies by Zhang and collaborators (Zhang et al., 2021) on polysaccharides isolated from various *Plantago* species (*P. asiatica, P. ciliata, P. depressa, P. major, P. lanceolata, P. media*) led to the following conclusions:

a) in general, the polysaccharides isolated from *Plantago* sp., are resistant to the environment from digestive system;

b) under the action of digestive enzymes, the bonds between the elementary units from the polysaccharides are partially broken, forming monosaccharides such as fructose, sucrose, and lactose, which, in turn, become nutrients for the microbiota;
c) regarding biological properties, the same authors showed that these polysaccharides have immunomodulatory effects in infections and inflammatory processes, inhibiting carcinogenesis processes that can occur in the digestive system.
Considering that the polysaccharides derived from *Plantago lanceolata* do not show cytotoxicity towards the HUVEC cell line and taking into consideration a value of IC50 = 0, it results that in this case, the cytotoxicity generated *in vitro* by polysaccharides isolated from *Plantago lanceolata* on the CaCo-2 tumour cell line is practically non-existent.
The limitations of this study lie in the fact that the results are obtained solely from cell proliferation studies, where experimental methods used may generate errors. Future developments of these studies include the purification of the polysaccharide fraction obtained from *Plantago lanceolata* and conducting cytotoxicity studies in the presence or absence of an antitumour reagent to identify possible synergistic effects (Ioan et al., 2020).

CONCLUSIONS

The physicochemical tests performed on the polysaccharides obtained from Plantago lanceolata showed that they contain species with an average molecular weight ranging from 0.1-1.94 kDa. The FTIR spectra, made in comparison with inulin, demonstrated that the structure of the two biomaterials is similar, with characteristic bands of the C-O-C glycosidic bond specific to plant polysaccharides being identified, as well as bands corresponding to the stretching vibration of the C-C bond and the C-O bond in the pyranosic ring of polysaccharides.
Cytotoxicity tests performed *in vitro* for polysaccharides obtained from *Plantago lanceolata* and inulin indicated that exposure to these biomaterials for 24 hours of HUVEC or CaCo-2 cell lines, may cause moderate cytotoxicity for both cell lines.
After 24h of exposure, the selectivity index obtained for inulin for the CaCo-2 cell line was 11. This value indicates the selectivity of inulin for the CaCo-2 tumour cell line. Increasing the exposure time to 48 hours for both biomaterials resulted in high IC50 values, indicating the absence of cytotoxicity of the bioproduct derived from *Plantago lanceolata* for both HUVEC and CaCo-2 cell lines. The results obtained *in vitro* for inulin, after 48 hours of exposure, showed that it does not exhibit cytotoxicity for the HUVEC cell line, but it does present cytotoxicity for the CaCo-2 tumour cell line, with the selectivity index obtained in this case being 25.3. The cytotoxicity tests revealed that polysaccharides from *Plantago lanceolata* exhibit low cytotoxicity toward both HUVEC and CaCo-2 cell lines, especially after 48 hours of exposure, suggesting good biocompatibility. In contrast, inulin showed selective cytotoxicity toward the CaCo-2 tumor cell line, with a selectivity index of 25.3 after 48 hours, indicating its potential as a selective antitumor agent. These findings support the potential use of *Plantago lanceolata* polysaccharides in functional foods

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