HEALTH PROMOTERS FROM POTATO AND PUMPKIN INSTANT PURÉE

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Introduction

Vegetable based foods are easy to assimilate and provide high amount of nutrients and different bioactive compounds. In early nutrition, an important role is played by vegetable purées obtained from potatoes, carrots or parsnips. Potatoes are considered a nourishing food that is rich in calories and biologically active compounds like *β*-carotene, polyphenols, ascorbic acid, tocopherol, α – lipoic acid, selenium and dietary fiber. The aim of this research was to examine the effects of conventional drying method on the quality parameters of a non-dairy solid product based on potato (*Solanum tuberosum* L.), pumpkin (*Cucurbita moschata* L.) and *Lactobacillus delbrueckii* subsp. *bulgaricus* Lb12 strain.

Materials and method

Fresh potatoes (*Solanum tuberosum* L.) of the Cumidava variety, packed in 2.5 kg pouches (packer Agrico–M, Covasna, Romania) and pumpkin (*Cucurbita moschata* L.) were bought from a local supermarket (Galati, Romania) to serve as raw material for the vegetable purée. The lyophilized culture of lactic bacteria *Lactobacillus delbrueckii* subsp. *bulgaricus* Lb12 was provided by the Chr. Hansen (Hoersholm, Denmark) and it contained *Lactobacillus delbrueckii* subsp. *bulgaricus* Lb12.

All the purée samples were evaluated by the microbiological, microstructural, flow, thixotropy, oscillatory rheological measurement, Texture Profile Analysis and phytochemical properties point of view.

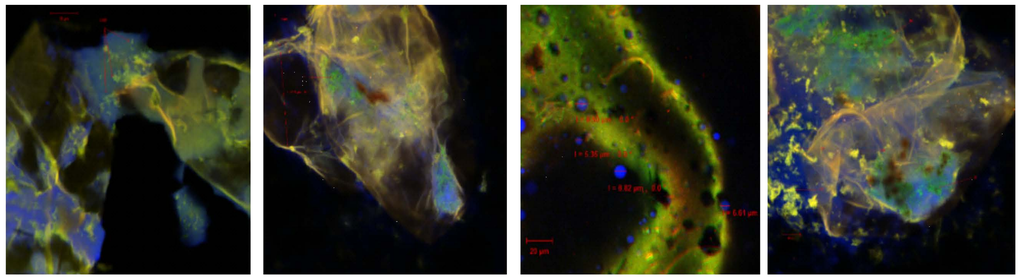
Results and discussions

All the purées presented a high content of total carotenoids, β – carotene and total antioxidant activity, as it can be seen in Table 1. The textural parameters (firmness, cohesiveness, adhesiveness) of the reconstituted samples did not present significant differences compared to fresh samples.

Table 1. Phyto-chemical characteristics of fresh and reconstituted vegetable purée

Purée sample	Total carotenoids, mg/g	β-carotene, mg/g	Antioxidant activity, µg Trolox/mL
Fresh vegetable purée			
Potato purée	4.10 ± 0.48	3.63 ± 0.37	0.070 ± 0.003
Potato and pumpkin purée	23.74 ± 1.25	20.47 ± 0.65	0.135 ± 0.018
Reconstituted vegetable purée			
Potato purée	21.82 ± 1.37	22.48 ± 0.62	0.133 ± 0.016
Potato and pumpkin purée	278.63 ± 0.74	247.20 ± 0.85	0.671 ± 0.025

The initial Lb12 population for both fresh and dried samples was approximately 8.9 log CFU/g and it varied between 7.47 and 7.09 log CFU/g during the storage period. Confocal analysis of vegetable purée for fresh and dried samples (Figure 1), even after reconstitution, shows the presence of bioactive compounds in the category of carotenoids in the form of clusters with green fluorescence (505 – 530 nm) and probiotic bacteria in yellow (550 nm). The presence of these elements in the complex matrix of the product generates functionality and enhances the nutritional value.



a) b) c) d)

Figure 1. Confocal laser scanning microscopy images of fresh and reconstituted vegetable purée with potatoes and pumpkin: a) fresh potato purée, b) fresh potato and pumpkin purée, c) reconstituted potato purée, d) reconstituted potato and pumpkin purée

Conclusions

This study is of high impact and novelty for the new goup of non-dairy based probiotic product. In general, the drying of vegetable purée at low temperature (e.g. 50°C) can better preserve the quantity of bioactive compounds (total carotenoids, beta-carotene and lycopene)

Lactobacillus delbrueckii subsp. *bulgaricus* Lb12 possessed a good viability in the dried samples (7.2 log CFU·g^{-1}) after 28 days of storage, due to the fibres and phenolic compounds present in the vegetables.

Further studies are needed to investigate the biodisponibility of the samples.

Acknowledgments

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Bioactive Compounds from Gonads Using Different Extraction Methods

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Introduction

The gonads, roes and fish skin are the main fish by-products, but still they could be source of valuable active compounds such as fatty acids, proteins, peptides, vitamins, etc., which could be used in various applications. Lipids from these wastes are mainly used as sources of polyunsaturated fatty acids (PUFAs) and vitamins.

Materials and method

Gonads purchased from fish farms, was collected in plastic bags and stored at temperatures of -20 °C, ethanol grade were supplied by Scharlau (Spain).

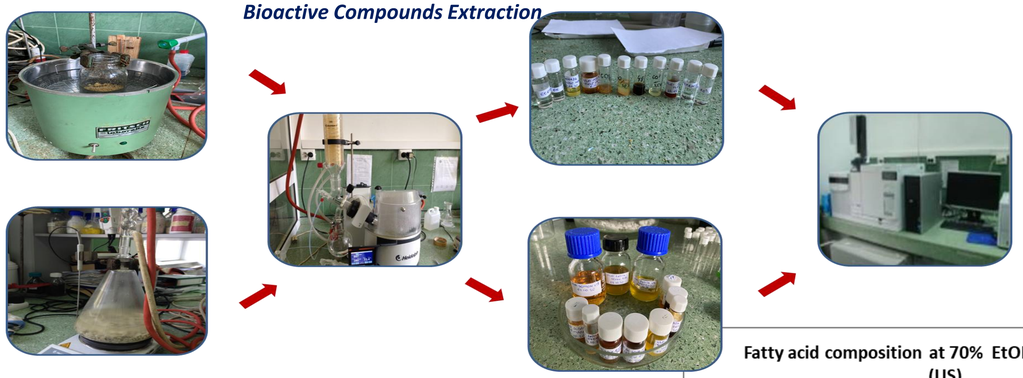
The analysis of active compounds was performed using GC-MS/MS TRIPLE QUAD (Agilent 7890 A).

Ultrasound assisted extraction and reflux condenser was used for the bioactive compounds extraction.

Reflux extraction									
EtOH concentration, %	25			50			70		
Time, min	15	30	60	15	30	60	15	30	60
The amount extracted, g	3.19	2.33	3.25	3.8	2.36	5.46	1.89	2.88	4.14

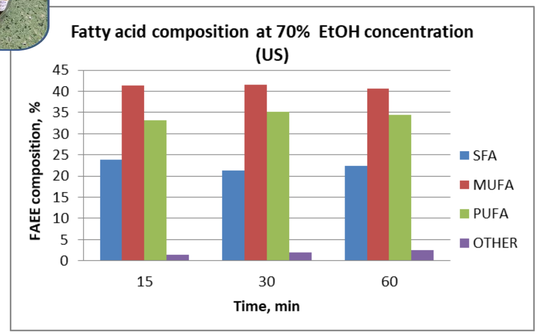
Results and discussions

Bioactive Compounds Extraction



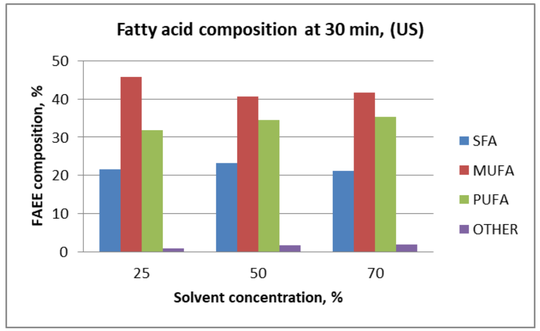
Ultrasound assisted extraction									
EtOH concentration, %	25			50			70		
Time, min	15	30	60	15	30	60	15	30	60
The amount extracted, g	3.19	2.33	3.25	3.8	2.36	5.46	1.89	2.88	4.14

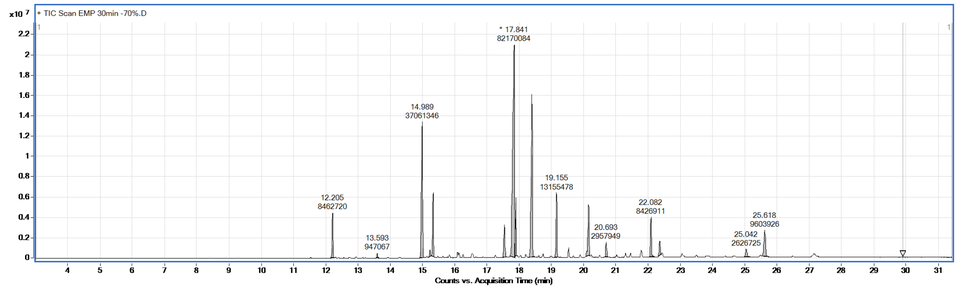
Classic extraction									
EtOH concentration, %	25			50			70		
Time, h	24	48	72	24	48	72	24	48	72
The amount extracted, g	2.02	1.57	1.27	0.5	2.93	5.03	1.63	1.73	0.9



Conclusions

Fatty acid composition, % (US)									
	25%			50%			70%		
Time, min	15	30	60	15	30	60	15	30	60
SFA	25.18	21.55	22.36	23.05	23.18	18.42	23.9	21.25	22.43
MUFA	46.32	45.69	47.07	45.01	40.7	46.08	41.45	41.59	40.63
PUFA	28.5	31.9	27.2	31.94	34.47	25.85	33.21	35.24	34.4
OTHER	0	0.86	3.37	0	1.65	9.65	1.44	1.92	2.54





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Separation of 2-ketogluconic acid by synergic reactive extraction

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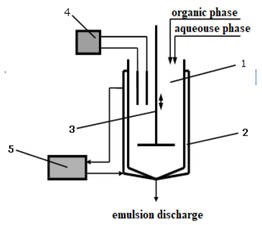
Introduction

- 2-Keto-D-gluconic acid is a compound produced over 40,000 tons/year due to its wide use in food industry: as food antioxidant, as additive to maintain food color, flavors and aroma, and its ability to block the formation of ammonium nitrite (carcinogenic) during food processing.
- Its biotechnological production has been improved significantly, but separation needs constant attention, mainly due to involvement of multiple downstream steps that generates high costs.
- Taking into account that limited research has been carried out on the reactive extraction of 2-ketogluconic acid, this study was focused on analyzing the pH dependent extraction performance and the molar ratios of acid and extractant (Amberlite LA-2) dissolved in three solvents with 1-octanol as phase modifier.

Materials and method

Liquid-liquid extraction experiments for 2-KGA separation were carried out in an extraction column with vibratory mixing that offers a high interfacial area, using:

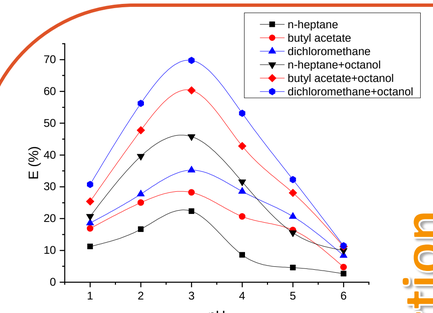
- Aqueous solution of 2-ketogluconic acid, 1g/l
- Dichloromethane, butyl acetate, n-heptane, 1-octanol.
- Amberlite LA-2

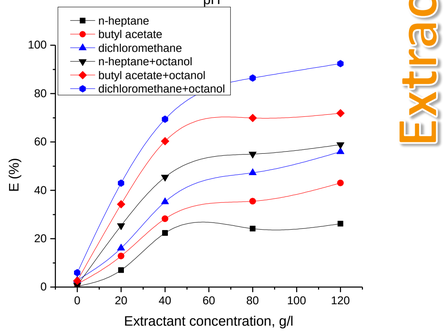


1. Glass column
2. Thermostat jacket
3. Stirrer
4. Digital pH meter
5. Thermostat

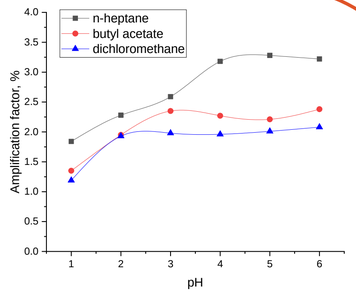
Results and discussions

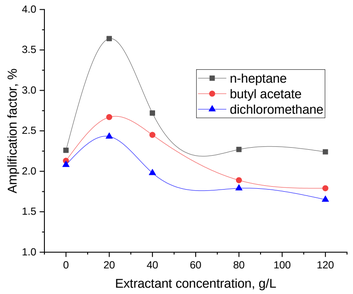
The pH-value of aqueous phase exhibits an important influence on reactive extraction efficiency, as it controls the form in which the acid exists in aqueous solutions: dissociated at pH value superior to pKa (2.66), and un-dissociated at pH value lower than pKa. To quantify the effect of 1-octanol addition on the extraction yield, the amplification factor was used (calculated as the ratio between the extraction yield in the presence and in the absence of the phase modifier).





Extraction



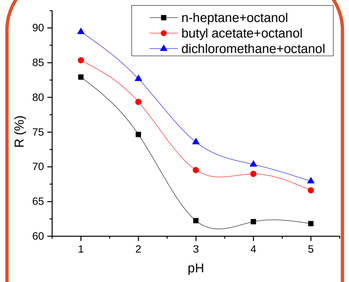


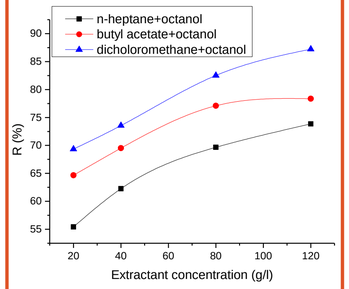
Influence of pH and extractant conc. on reactive extraction with and without 1-octanol

The amplification factors values vs pH and extractant conc. corresponding to 1-octanol addition

The reactive extraction mechanism

Solvent	n	Ke, (L/mol)
n-heptane/octanol	0.788	11.94
Butyl acetate/octanol	0.86	65.95
Dichloromethane/ octanol	1.17	311.6



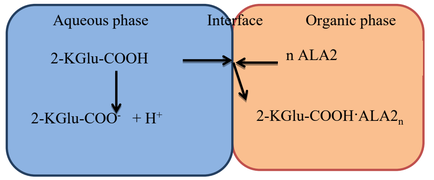


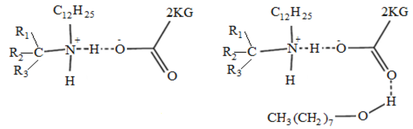
Influence of Amberlite LA-2 concentration and initial phase pH on back extraction efficiency

Stripping

Conclusions

- The highest extraction yield and distribution coefficient are achieved for pH of 3 and 120 g/l extractant, for all the studied solvents.
- The reactive extraction is based on H bond formation between the 2-ketogluconic acid and Amberlite LA-2.





- The addition of 1-octanol as polar modifier strongly increased the extraction efficiency for all solvents, with bigger values for the inactive organic solvent with the lowest dielectric constant (n-heptane)

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Microencapsulation of extract of European plants, rich in phenolic compounds, with health benefits and food interest

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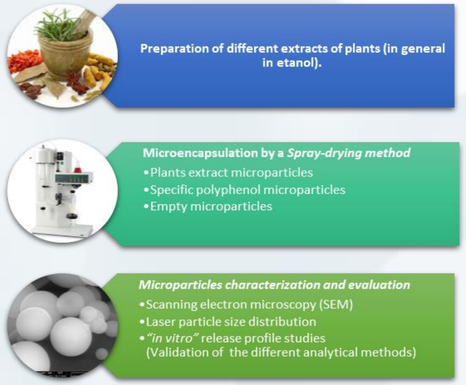
Introduction

- Several European native plants were used in the traditional medicine during centuries.
- Phenolic compounds, extracted from plants, exhibit several therapeutic properties (e.g. antioxidant, anticancer, antiallergenic, anti-inflammatory and antiviral), allowing to reduce the oxidative stress and to prevent some health conditions like cancer, arteriosclerosis and ageing processes.
- However, in general, phenolic compounds are very sensitive and with low bioavailability in human body.
- Microencapsulation is a promising alternative to improve their stability and bioavailability, to protect and to improve sensitive compounds with a controlled release, enabling their incorporation in active food products, nutraceuticals and in therapeutic formulations.
- So, the purpose of the present work is to prepare controlled release microparticles using the extract of different plants, by a spray-drying technique.

Materials and method

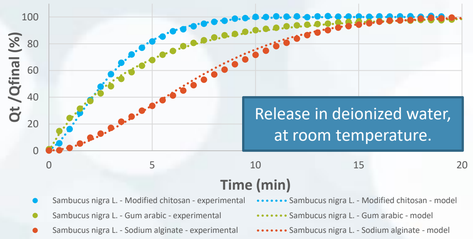
- Different European native plants (*Sambucus nigra L.*, *Laurus nobilis L.* and *Salvia officinalis L.*) were selected considering their health potential associated to their composition.

- Phenolic extracts were prepared and different microparticles were obtained by a spray drying process using different biopolymers (modified chitosan, gum arabic, sodium alginate).

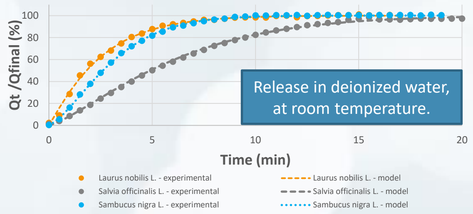


Results and discussions

- Characterization of the microparticles *Sambucus nigra L.* extract prepared with different encapsulating agent.



- Characterization of the microparticles with different extracts prepared with modified chitosan.



Weibull model

$$M_t = M_\infty \left[1 - e^{-\left(\frac{t-t_0}{\tau_d}\right)^{\beta}}\right]$$

- Microparticles with rough or smooth surface and with fast or slow release were obtained depending on the encapsulating agent used.
- All microcapsules exhibited spherical form.
- The encapsulation efficiency was around 90-100%.
- Microparticles in general with good quality were prepared by a spray-drying technique.
- The Weibull model fits the experimental results.

Conclusions

- This work shows that it is possible to encapsulate different formulations and different biopolymers, through a spray drying process.
- These microparticles can be easily incorporated in commercial instantaneous powder food products like gelatin or even cookies that can be fortified with bioactive compounds.

Acknowledgments

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Anti-inflammatory potential of new complexes of diclofenac hydrazones using in vitro methods

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Introduction

AIM

Anti-inflammatory activity of new complexes with hydrazone structure using 2 in vitro methods: inhibition of serum albumin denaturation and erythrocyte membrane stability test.

Materials and method

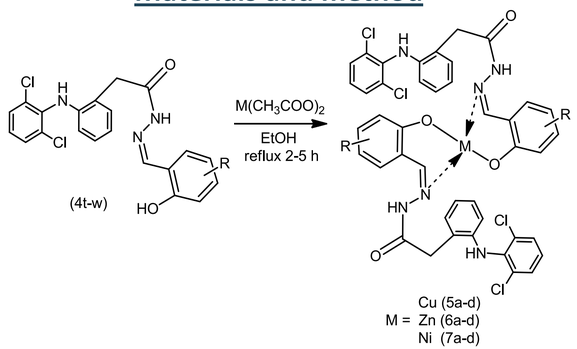

Figure 1. General scheme of synthesis of complexes with hydrazone structure

For the evaluation of the anti-inflammatory effect, 10 mg/mL stock solutions were obtained by dissolving the compounds studied in DMSO, from which different dilutions were subsequently made.

For the serum albumin denaturation inhibition assay the samples were treated with 3 mL of 1% bovine albumin (aqueous solution), then incubated for 20 min at 37^{0}C, then 5 min at 72^{0}C, cooled for 10 min and then add 1 mL of saline phosphate buffer (pH = 7.2). The turbidity of the samples was read at 416 nm against distilled water.

For the erythrocyte membrane stability assay, the samples were treated with 1 mL phosphate buffer (pH = 7.4), 2 mL hyposaline solution (0.36% NaCl solution) and 0.5 mL HRBC solution (10% v/v). Samples were incubated at 37^{0}C for 30 min, then centrifuged at 3000 rpm for 20 min. The absorbance of the supernatant was read at the wavelength of 560 nm compared to the sample blank.

Results and discussions

Table 1. Physico-chemical characteristics of complexes with hydrazone structure (**5a-d**, **6a-d**, **7a-d**)

Compound	M	R	η (%)	m.p. (^{0}C)
5a	Cu^{2+}	2-OH	45	252
5b		2,3-diOH	52	255
5c		2,4-diOH	65	250
5d		2,5-diOH	60	258
6a	Zn^{2+}	2-OH	68	285
6b		2,3-diOH	54	280
6c		2,4-diOH	70	270
6d		2,5-diOH	59	273
7a	Ni^{2+}	2-OH	66	310
7b		2,3-diOH	72	305
7c		2,4-diOH	63	306
7d		2,5-diOH	68	303

Table 2. EC_{50} values (mg / mL) of inhibition of serum albumin denaturation for 2-hydroxy-substituted hydrazones (**4t-w**) and corresponding complexes (**5a-d**, **6a-d**, **7a-d**)

Compound	EC_{50} mg/mL	Compound	EC_{50} mg/mL	Compusul	EC_{50} mg/mL
Diclofenac	38.776 ± 0.022	5b	37.886 ± 0.196	6d	26.348 ± 0.138
4t	52.437 ± 0.106	5c	37.998 ± 0.205	7a	28.387 ± 0.189
4u	44.378 ± 0.162	5d	18.382 ± 0.096	7b	53.072 ± 0.238
4v	27.325 ± 0.180	6a	18.776 ± 0.127	7c	13.178 ± 0.045
4w	32.076 ± 0.098	6b	49.988 ± 0.255	7d	30.125 ± 0.099
5a	41.167 ± 0.123	6c	18.108 ± 0.087		

Table 3. EC_{50} values (mg / mL) of erythrocyte membrane stability for 2-hydroxy-substituted hydrazones (**4t-w**) and corresponding complexes (**5a-d**, **6a-d**, **7a-d**)

Compound	EC_{50} mg/mL	Compound	EC_{50} mg/mL	Compound	EC_{50} mg/mL
Diclofenac	109.684 ± 0.019	5b	68.237 ± 0.281	6d	52.276 ± 0.208
4t	43.871 ± 0.287	5c	97.887 ± 0.343	7a	22.478 ± 0.161
4u	38,675 ± 0.205	5d	41.265 ± 0.289	7b	35.190 ± 0.242
4v	61.369 ± 0.309	6a	24.282 ± 0.200	7c	31.268 ± 0.188
4w	109.342 ± 0.297	6b	46.316 ± 0.321	7d	40.620 ± 0.179
5a	46.549 ± 0.162	6c	32.118 ± 0.192		

For the inhibition of serum albumin denaturation the most active compound was **7c** (M = Ni, R = 2,4-diOH, EC_{50} = 13.178 ± 0.045) which was found to be 3 times more active than diclofenac (EC_{50} = 38.766 ± 0.022).
For the erythrocyte membrane stability the most active compound was **7a** (M = Ni, R = 2-OH, EC_{50} = 22.478 ± 0.161) which was found to be 4.9 times more active than diclofenac (EC_{50} = 109.684 ± 0.019).

Conclusions

The most intense in vitro anti-inflammatory effect was observed for compounds **6c** (M = Zn, R = 2,4-diOH) and **7c** (M = Ni, R = 2,4-diOH), obtained following the complexation reaction between hydrazone 2,4-dihydroxy-substituted diclofenac and $Zn(CH_3COO)_2 \cdot 2\ H_2O$ (**6c**) and $Ni(CH_3COO)_2 \cdot 4\ H_2O$ (**7c**), respectively. In vitro anti-inflammatory tests demonstrating that the substitution of the aromatic nucleus in the 2,4 position with hydroxyl radicals is the most effective.

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Acknowledgment or Contact

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PEGylated phenothiazine as water soluble building blocks for biomaterials

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Introduction

Phenothiazine **(PTZ)** is a fused ring heterocyclic compound with high potential to be used in a wide range of applications. Though, due to its poor solubility in ordinary solvents[1], its applicability in the biomedical field is limited. In this context the researchers attention went to finding new ways to increase its solubility. Therefore, in this study we used phenothiazine PEGylation with the final aim to obtain water soluble compounds, proper to be used in biomedical purposes[2].

Materials and methods

Three **PTZ** derivatives were obtained using three different synthetic routes. The first derivative was obtained by direct alkylation of a tosylated poly(ethylene glycol)PEG chain resulting the **(PP)** compound. The other two were synthesized by grafting the PEG chain *via* an ester function **(PPO)**, and an amide function **(PPN),** respectively.

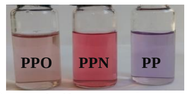

Figure 1. Compounds water solution under natural light

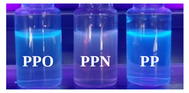

Figure 2. Compounds water solution under UV light

Scheme 1. Synthesis of the PEGylated derivatives.

All three compounds were characterized by spectroscopic, optical and morphological methods. Their biological activity was evaluated *in vitro* on **NHDF** and **HeLa** cell lines.

Results and discussions

Structural characterization

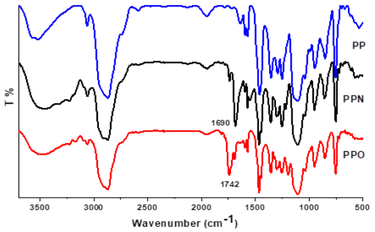

Figure 3. FTIR spectra of PEGylated compounds

The successful synthesis of the compounds was confirmed by FTIR and NMR spectroscopy. The FTIR spectra **(Fig. 3)** displayed the characteristic vibrations of the main groups present in the final compounds. The NMR spectra **(Fig. 4)** showed the disappearance of the chemical shifting characteristic to the hydrogen linked to the nitrogen atom of phenothiazine, and chemical shifting characteristic to the new synthesized compounds in the right ratio of their integrals.

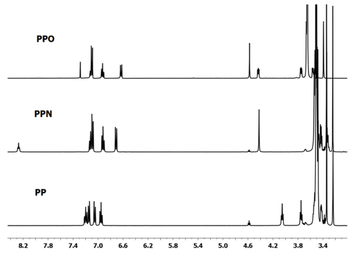

Figure 4. ^{1}H-NMR spectra of PEGylated compounds

Photophysical behavior

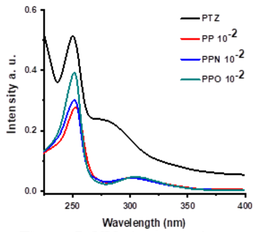

Figure 5. UV-vis absorption spectra of PEGylated compounds and PTZ in water

The photophysical behavior of the compounds was investigated by UV-vis spectroscopy in comparison with the pristine **PTZ**. The compounds absorption spectra **(Fig. 5)** showed the two absorption bands from phenothiazine, with the difference that the second one is bathochromic shifted with 25 nm. This is a consequence of aggregate formation, due to the amphiphilic nature of the compounds.

On the other side, the samples were able to emit blue light under UV lamp illumination **(Fig. 2).** The recorded emission spectra **(Fig. 6)** confirmed the visual observations by the presence in the spectra of a band with a maximum in the blue region at 450 nm.

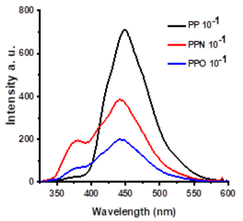

Figure 6. Photoluminescence spectra of PEGylated compounds in water

Self-assembling behavior

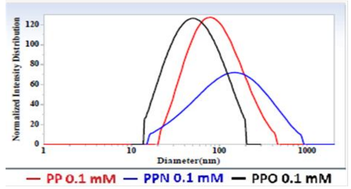

Figure 7. DLS graphs of the studied compounds

The UV-vis findings, according to which the compounds are able to self assemble into aggregates, were confirmed by DLS **(Fig. 7)** measurements.

In all cases the aggregates were nanometric with a mean diameter of 200 nm and a quite low dimensional polydispersity. The morphological investigations by SEM, AFM and POM techniques **(Fig. 8),** demonstrated the spherical shape of the aggregates and their uniformity.

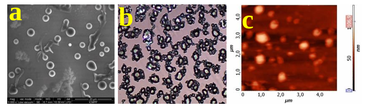

Figure 8. a) SEM **b)** POM images of the PP embedded into a solid PVAB matrix and **c)**AFM images of the PP in pure form

In vitro biocompatibility

All three compounds presented a good biocompatibility on Normal Human Dermal Fibroblast **(NHDF)** cells for concentrations up to 0.1 mM, while for **PPN** the concentration increased up to 1 mM.

The **PP** and **PPO** presented a good antitumor activity on Human Cervical Cancer **(HeLa)** cells at concentration 0.1 mM, with a relative cell viability of 58 % for **PP** and 34 % for **PPO**.

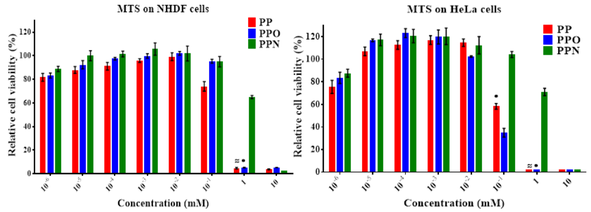

Figure 9. Cell viability on **NHDF** and **HeLa** cells.

Conclusions

- Three PEGylated phenothiazines were synthesized and their structure was confirmed by FTIR and ^{1}H-NMR spectroscopy.
- They presented slight luminescence.
- Because of the **PEG** content the compounds were water soluble, and due to their amphiphilic nature they formed aggregates through self assembling.
- The new compounds were biocompatible and two of them presented good antitumor activity.

References

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[2] S. Cibotaru, A. I. Sandu, D. Belei, L. Marin, Mater. Sci. Eng. C. **116** 111216 (2020)

Acknowledgment

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Cannabidiol-rich hemp oil induces apoptosis in cancer cell lines

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Introduction

Cannabidiol (CBD) is one of the major cannabinoids found in *Cannabis sativa* L., known as industrial hemp. CBD is a non-toxic and non-psychoactive cannabinoid that has been shown to exhibit a versatile spectrum of pharmacological effects, including antitumor activity in multiple cancer types [1]. Hemp oil can be extracted and enriched in bioactive compounds by different methods. This study aims to evaluate the *in vitro* anti-cancer effect of CBD-rich hemp oil.

Materials and method

Hemp oil was extracted from whole plant in ethanol and decarboxylated at 90ºC for 1h (soft conditions) in order to obtain maximum CBD yield (97.58%).

Cytotoxicity and the half maximal inhibitory concentration (IC50) of CBD-rich hemp oil on malignant melanoma (MeWo), adenocarcinoma (HeLa), hepatocellular carcinoma (HepG2) and osteosarcoma (HOS) cells vs. normal fibroblasts (NHDF) was determined by MTS assay.

Apoptosis induced by hemp oil in cancer cells was demonstrated using acridine orange/ethidium bromide (AO/EB) staining and real-time quantitative PCR for B-cell lymphoma protein 2 (BCL-2)-associated X (BAX) and BCL-2 gene expression.

References

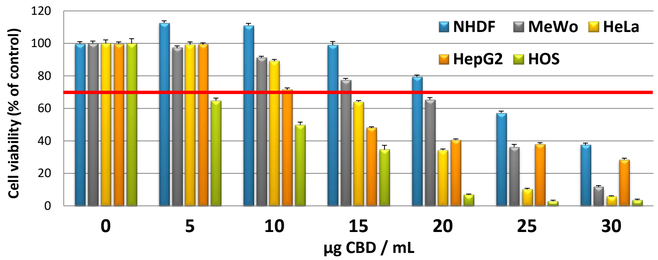
1. Massi P. et al., Br. J. Clin. Pharmacol. 2012.

Acknowledgements

This work was funded by PCCDI – UEFISCDI project no. PN-III-P1-1.2-PCCDI-2017-0697/13PCCDI/2018 and InoMatPol, ID P_36_570, Contract no. 142/10.10.2016).

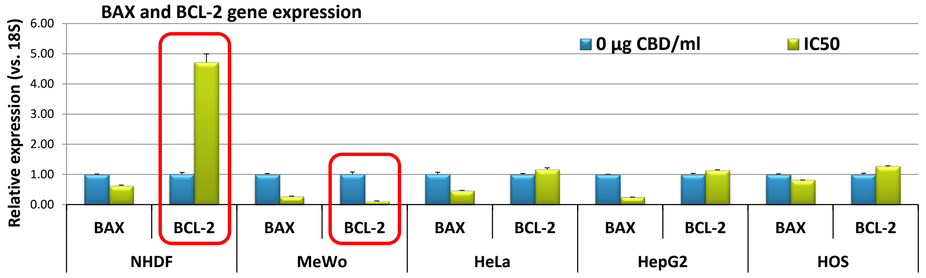
Results and discussions

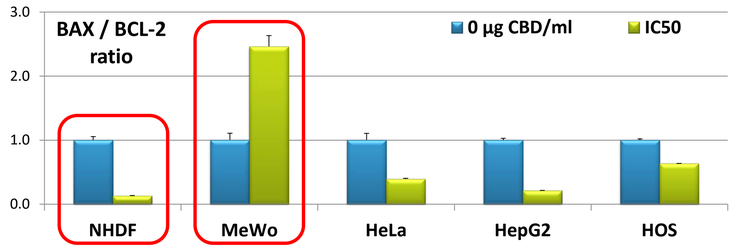
CBD-rich hemp oil promotes proliferation of NHDF at 0 - 15 µg/mL, followed by cytotoxic effects at higher concentrations. CBD-rich hemp oil is cytotoxic in a dose dependent manner for cancer cell lines at lower CBD concentrations than for NHDF cells. HeLa, HepG2, HOS cells are more sensitive to CBD compared to MeWo cells.



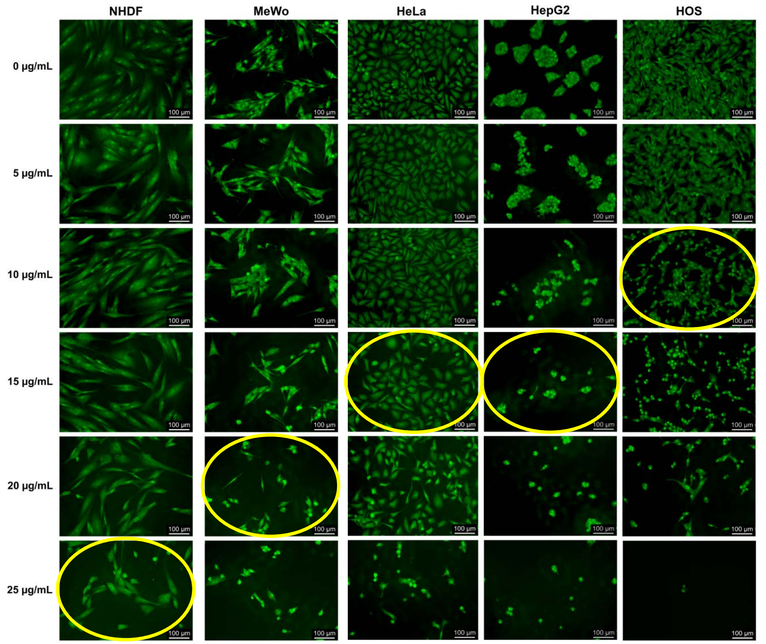
Cell line	IC50 (µg CBD/ml)
NHDF	26,65
MeWo	21,59
HeLa	16,89
HepG2	16,85
HOS	8,42

IC50 of CBD-rich hemp oil induces down-regulation of BAX gene expression in all cell lines and extreme up-regulation of BCL-2 in NHDF cells. Bax promotes cell death, while Bcl-2 prevents apoptosis by inhibiting the activity of Bax. The **BAX/BCL-2 ratio > 1** in MeWo cells indicates that they are sensitive to apoptosis, but **BAX/BCL-2 < 1** for the other cell lines indicates resistance to apoptotic stimuli.





CBD-rich hemp oil induces dose-dependent morphological changes in treated cancer cells (cell shrinkage and detachment, cytoplasmic condensation, vesicle formation, spheroid disaggregation of HepG2 cells). AO/EB staining shows that CBD-rich hemp oil induces apoptosis hallmarks (chromatin condensation, nuclear fragmentation, apoptotic bodies' formation, cytoplasmic shrinkage, cell detachment, spheroid disaggregation of HepG2 cells) in treated cancer cells at lower doses than in normal fibroblasts. Fibroblasts exhibit the first signs of apoptosis at 20 µg CBD/mL, while in cancer cells the apoptotic features appear at 5-10 µg CBD/mL and increase in a dose-dependent manner.



Conclusions

CBD-rich hemp oil showed dose-dependent cytotoxic effects and induced apoptosis at low doses in cancer cells, but not in dermal fibroblasts, suggesting that CBD oil could be used as complementary anti-cancer treatment.

Novel salt tolerant bacterial strains with plant growth promoting (PGPR) potential isolated from saline soils

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Introduction

Salinity is widespread stress factors, which reduces plant productivity due to their effects on plant physiological and metabolic processes. Soil salinity is known to repress plant growth in the form of osmotic stress, followed by ion toxicity. Osmotic stress causes various physiological changes, such as membrane damage, nutrient imbalance, impairing of reactive oxygen species (ROS) detoxification, decreased photosynthetic activity, and decrease in stomatal aperture (Numan et al., 2018; Zhang et al., 2018). Plant growth promoting rhizobacteria (PGPR) can affect and diminish the effect salt stress. Salt tolerant PGP bacterial strains can influence the survival and adoptability of plants using different mechanisms: production of plant growth promoting substances (siderophore, indole acetic acid) and phytohormones, nutrient fixation, changing the physicochemical properties of saline soil (Gupta and Huang, 2014).

Objectives

Isolation of salt tolerant bacterial strains → Selection of salt tolerant bacterial strains based on PGP properties → *In vivo* plant tests with selected bacterial strains

Materials and method

ISOLATED BACTERIAL STRAINS

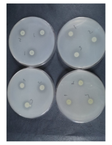
INORGANIC PHOSPHORUS MOBILIZATION ASSAY:

Pikovskaya's medium

Overnight grown bacterial cultures were point inoculated (10 µL, OD=0.3) in three replicates

The plates were incubated at 28 °C, for 3 days

The detection and measurement of a clear (halo) zone around the bacterial colony.



SIDEROPHORE PRODUCTION:

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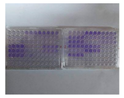
The diameter of the halo orange zone formed around the bacterial colonies indicating the siderophore production was measured



EXOPOLISACHARIDE PRODUCTION:

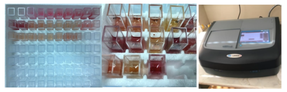
The production was determined using microtiter plate assay

Bacterial strains were cultured in Nutrient broth, for 24 hours at 28°C

Determine the growth rate of bacterial strains by the absorbance reading at 595 nm

The medium was removed, and the biofilms were stained with 0.01% crystal violet for 20 minutes

After three washing steps with distilled water, the dye was solubilized with 96% ethanol (10 minutes), and quantified measuring the absorbance at 570 nm



PHYTOHORMONE (INDOLE-3-ACETIC ACID) PRODUCTION:

Production was realized by spectrophotometric measurement

Bacterial strains were grown in 5 mL TSB, at 28 °C and 150 rpm for 72 hours

1 mL of culture supernatant was mixed with2 mL of Salkowsky reagent, than incubated for 30 minutes in the dark at room temperature

The absorbance of the samples was measured at 570 nm, and the IAA concentration calculated using a standard curve

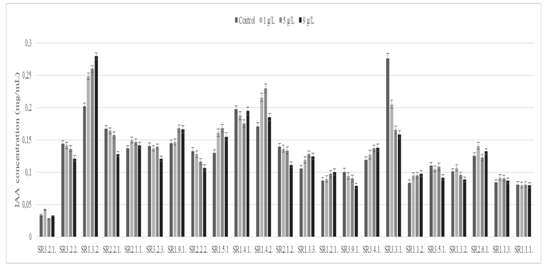


Fig. 1. Indole acetic acid (IAA) production of salt tolerant bacterial strains

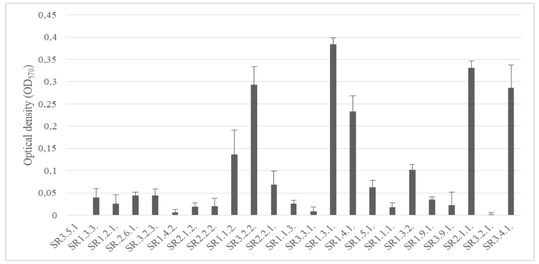


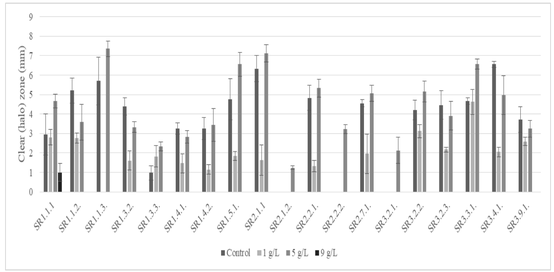
Fig. 2. Exopolysaccharide (EPS) production of salt tolerant bacterial strains

Results and discussions

From 25 (92%) bacterial strains in case of 23 isolates indole acetic acid and exopolysaccharide production was observed.

The inorganic phosphorus solubilisation capacity was observed for 19 (76%) bacterial strains.

From 25 isolated salt tolerant bacterial strains 3 (12%) isolates were able to produce siderophore.



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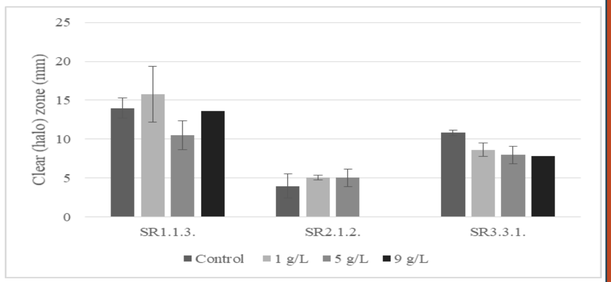

Fig. 4. Siderophore production of salt tolerant bacterial strains

Conclusions

- Based on our results most of the bacterial strains maintained at least one plant growth promoting property even in the presence of high salt concentration
- From the 25 tested bacterial strains, we obtained positive results for all plant growth promoting properties in case of 3 bacterial strains: SR1.1.3, SR2.1.2 and SR3.3.1. The above listed bacterial strains with high PGP potential were selected for plant experiments.

Acknowledgment

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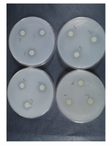

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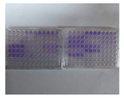

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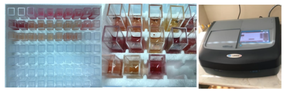

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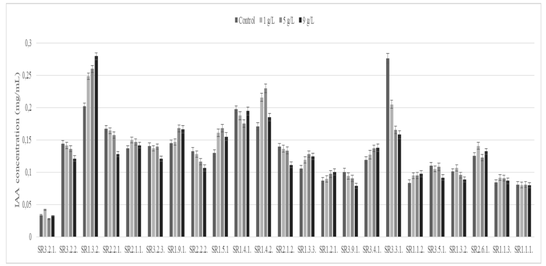

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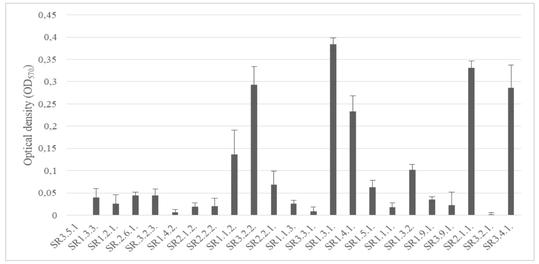

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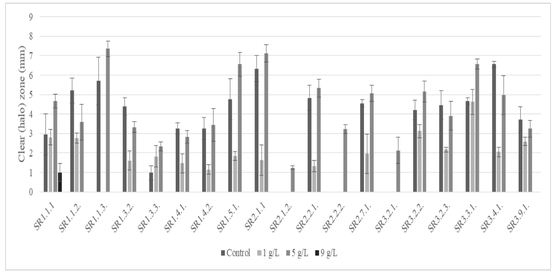

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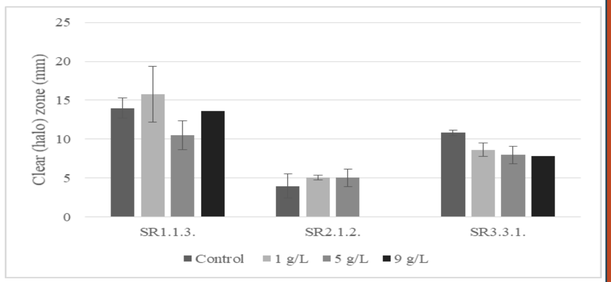

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Conclusions

- Based on our results most of the bacterial strains maintained at least one plant growth promoting property even in the presence of high salt concentration
- From the 25 tested bacterial strains, we obtained positive results for all plant growth promoting properties in case of 3 bacterial strains: SR1.1.3, SR2.1.2 and SR3.3.1. The above listed bacterial strains with high PGP potential were selected for plant experiments.

Acknowledgment

The authors are grateful to Sapientia University and Corax Bioneer CEU SA for making available the lab equipment, and to University of Pécs, Faculty of Sciences, Institute of Chemistry, Chemical Doctoral School for financial support.

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Valorization of food industry byproducts towards polyhydroxyalkanoates production by mixed microbial cultures

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Introduction

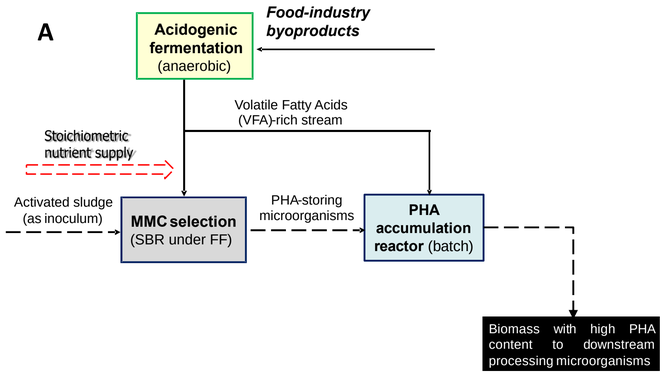
The valorization of byproducts deriving from food industry towards the production of value-added compounds perfectly fits with the idea of circular economy. As an example, byproducts from wine, cereals and vegetables industries, can be transformed into short and medium chain volatile fatty acids (VFA) through, for instance, an acidogenic fermentation process (*Lee et al., 2014*). These acids can be in turn exploited for various applications, including the production of biopolymers such as polyhydroxyalkanoates (PHA) via Mixed Microbial Culture (MMC). PHA are polyesters defined three times *bio* since, besides being biologically produced, they are completely biodegradable in the environment and can also be bio-based (*Valentino et al., 2017*)

Materials and method

The MMC-based PHA production process involves several stages (**Figure 1A**). Here, the acidogenic fermentation step has been performed through batch experiments by using 9 types of food industry byproducts, at room temperature and acidic pH (i.e. 5.5), in order to inhibit the methanogenic activity. As for the MMC selection towards PHA-storing microorganisms, a lab-scale SBR (1L working volume) was inoculated with an activated sludge and operated with a cycle length of 12 hours with an uncoupled feeding of the carbon and nitrogen source in order to trigger the establishment of the feast and famine conditions (**Figure 1B**), essential to guarantee a good selection (*Reis et al., 2011*). The SBR was operated in fully aerobic conditions (ADF) or alternating aerobic and anoxic conditions (AE/AN). In both cases an organic load rate of 2.12 gCOD/L d, consisting of a synthetic mixture of VFA, was applied. Both acids and PHA were measured by means of gas-chromatography analysis.

Results and discussions

Batch tests on the acidogenic fermentation of food industry byproducts, in particular cereals, revealed a high yield of conversion (over 70% in terms of Chemical Oxygen Demand, COD) into fermentation products. These mainly consisted of VFA, especially acetic and propionic acids. Based on these results, a mixture of these two acids was used to feed the sequencing batch reactor (SBR) operated under both the ADF and AE/AN conditions. In all cases a microbial culture able to store the poly(hydroxybutyrate/hydoxyvalerate) (PHBV) copolymer was selected. This is particularly interesting, since the PHBV has properties similar to polypropylene.



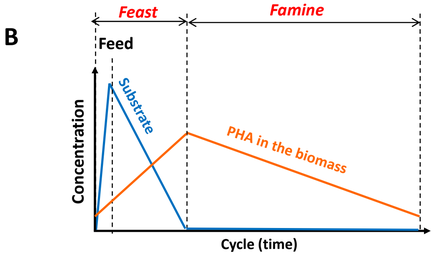
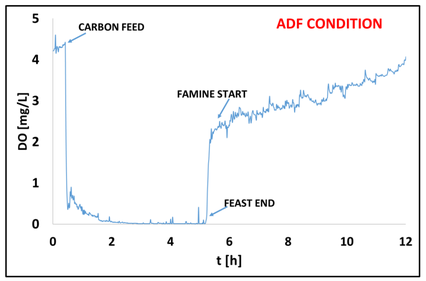


Figure 1. Scheme of the multi-stage process for MMC-PHA production (**A**). Establishment of feast and famine conditions for the selection of PHA-storing microorganisms (**B**).

The profile of the Dissolved Oxygen (DO) concentration in the SBR operated with ADF or AE/AN conditions is reported in **Figure 2**. In the ADF approach, the DO concentration rapidly decreased in correspondence to the feed of acids, due to the increase of the microbial activity, and it suddenly increased once all acids were depleted (end of the feast phase). On the contrary, when the AE/AN condition was applied, oxygen was supplied only during the feast phase since nitrite was used as electron acceptor for PHA consumption during the famine phase, characterized by the absence of an external carbon source. As a main result, in both cases it was possible to establish the required feast and famine conditions.



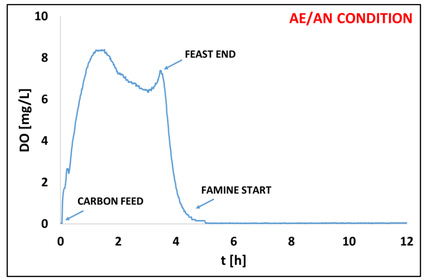


Figure 2. Profile of the Dissolved Oxygen (DO) concentration in the SBR operated in fully aerobic (ADF) or aerobic/anoxic (AE/AN) conditions

Conclusions

The results of this research clearly indicate the possibility to easily ferment food industry byproducts into VFA, that are direct substrate for PHA production with MMC.

In terms of microbial selection towards PHA-storing microorganisms, it was found that both ADF and AE/AN conditions can be used to establish the required feast and famine conditions. However, microorganisms selected in the ADF-SBR showed a higher storage ability.

Importantly, in all conditions the PHBV copolymer was produced.

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Autoinduction fermentation: a new viable pathway for biosynthesis

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Introduction

The biosynthesis of recombinant proteins with non-native structure and expressed in a non-soluble state is a major challenge. More than 30% of the recombinant protein produced by the most used prokaryotic expression system (*Escherichia coli*) is produced in insoluble form, further steps are required to solubilize it.

In our experiments, we compared the inducer effect, the monitored growth parameters of the conventional broth (LB, 2YT) and the auto-induction broth.

Materials and method

Recombinant plasmid and bacterial strains

E. coli strain BL21 STAR (DE3) (F-ompT hsdSB (rB-, mB-) galdcmrne131) was used as the host for protein expression. Plasmid pGEX-4T1 – which carries the tac promoter, an GST tag coding sequence and ampicillin resistance gene – was used as expression vector for GST protein.

Bacterial growth curve analysis

Fresh cells of each strain were resuspended in the required medium to the initial A595 of 0.1. Wells in the microplate were filled with this suspension (200 µL in each well). The absorbance in each well was measured at 595 nm at 20 min with intensive shaking of the microplate. Data are shown either as the average of 4–8 parallel growth curves.

Calculations and graphs were performed with Microsoft Excel 2013 and MARS Data Analysis Software v.1.10.

Results and discussions

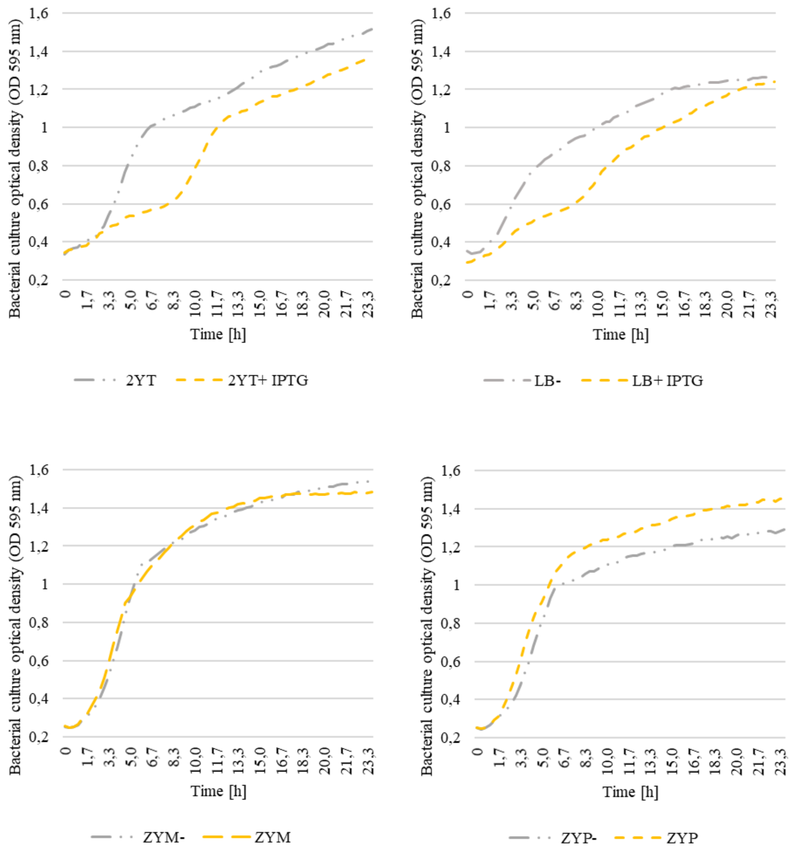

Fig. 1. Bacterial culture growth curves under different conditions: conventional LB and 2YT, IPTG induction broths, and ZYM and ZYP autoinduction broths. The notation "-" in the caption indicates the absence of an inducing agent (IPTG, lactose)

Figure 1 shows the growth curve of the bacterial culture under different conditions: conventional LB and 2YT, IPTG induction broths, and ZYM and ZYP autoinduction broths. The notation "-" in the caption indicates the absence of an inducing agent (IPTG, lactose).

The effect of the added inducing agent is examined in Figure 2. In the conventional IPTG induction method, the inducer has a negative effect on the growth curve of the bacterial culture. The addition of the inducer delays the onset of the exponential phase, while also negatively affecting the maximum growth rate.

The presence of the inducer shows a similar trend for LB and 2YT medium.

Unlike media using the traditional IPTG induction method, the autoinduction method does not show a negative effect of the inducing agent on the growth of the bacterial culture.

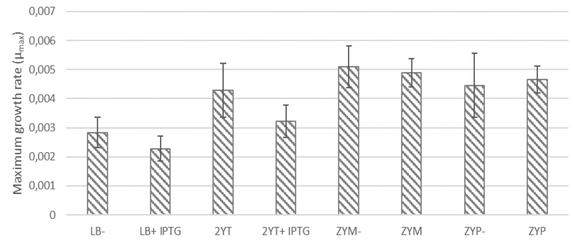

Fig. 2. Maximum growth rate of bacterial cultures

Conclusions

Figure 2 shows the maximum growth rate of bacterial cultures tested under different conditions. As shown in Figure 2, a higher maximum growth rate can be achieved by using autoinduction broth.

No negative effect of the inducer on either the bacterial growth curve or maximal growth was detected with ZYM and ZYP autoinduction broths.

Acknowledgment or Contact

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GENETIC MARKERS ASSOCIATIONS IN ENDANGERED CATTLE BREEDS TO RESISTANCE ON ENVIRONMENTAL CONDITIONS

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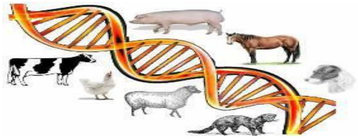
INTRODUCTION

Many local cattle breeds have become endangered due to their substitution by high-yielding breeds. Populations of many local breeds have dangerously decreased and some of them are even threatened by extinction. By now two local cattle breeds from Romania are listed as endangered livestock breeds, respectively Grey Steppe cattle breed and Pinzgau cattle breed.

The systemic usage of molecular markers will promote the thorough management of vulnerable species which should be paired with breeding schemes to enhance the economic characteristics, preventing the loss of breeds. In the present review, we have described vulnerable cattle breeds based on the analyzes nuclear microsatellites, chromosome markers and mtDNA sequences.

MATERIALS AND METHOD

The purpose of this study is to investigate the genetic status of threatened with extinction cattle breeds in Romania through molecular data analysis, the desire to protect their genetic purity being generally recognized for environmental, economic and cultural purposes.



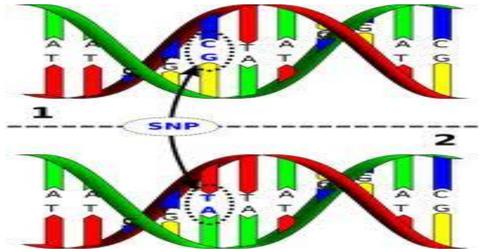
RESULTS AND DISCUSSIONS

According to the Food and Agriculture Organization of the United Nations (FAO), about one third of the recorded livestock species are listed as having a significant chance of extinction and about 1,000 have vanished in the last 100 years. All over the world, many local cattle breeds became endangered due to their substitution by high- yielding breeds. In Romania, for example, two local cattle breeds, Grey Steppe and Pinzgauer, are listed as endangered (Scherf, 2000).

Although, they have low milk and meat yields, they are very resistant to diseases and temperature changes. Reducing the size of their populations and their genetic purity, many desirable allelic complexes for environment resistance will be lost (Demir and Balcioglu, 2019).

Thus, there is an urgent concern about the survival of their „wild" genes pool, the depletion in individuals' number contributing to a decline in the biological diversity of animal genetic capital and even to the reduction of the national cultural heritage.

In this respect, in developed countries, native breeds were included in various genetic preservation programmes, assessing their genetic structure using molecular markers in order to facilitate the creation of strategies for their management and protection (Davidescu et al., 2019; Grădinaru et al., 2018).



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CONCLUSIONS

The genetic analysis is the first step in these programmes, molecular methods being used to investigate the individual DNA. Now, a broad variety of genetic markers are used, and microsatellites are especially sensitive for the assessment of genetic diversity and, in addition, to approximate phylogenetic connections between various breeds.

Furthermore, whatever their type, genetic markers may be used to reduce the risk of inbreeding and to maintain genetic variability in these kind of populations.

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INFLUENCE OF EXTRACTION CONDITIONS ON PROPERTIES OF *SALVIA OFFICINALIS L.* POLYPHENOLIC EXTRACTS, FREE AND ENCAPSULATED INTO MESOPOROUS TITANIA MATRICES

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Introduction

Extracts obtained from various medicinal herbs present antioxidant, antimicrobial, anti-inflammatory or even antitumoral properties and their benefits for human health are well known [1,2].

Salvia officinalis L. (common sage) that belongs to the *Lamiaceae* family is a valuable source of phytochemicals with radical scavenger properties, which are promising ingredients for cosmetics, nutraceuticals, supplements, or food industry [3,4].

Also, the common sage polyphenolic extracts exhibited a good antimicrobial activity on various strains and fungi. This can be associated with the presence of carnosol or carnosic acid in the extract [4].

It was reported that rosmarinic acid is stable in ethanol or ethanol-water mixture, irrespective the temperature of extract preparation, while carnosol and carnosic acid were degraded at temperature higher than 50 ºC [5].

Purpose of the work

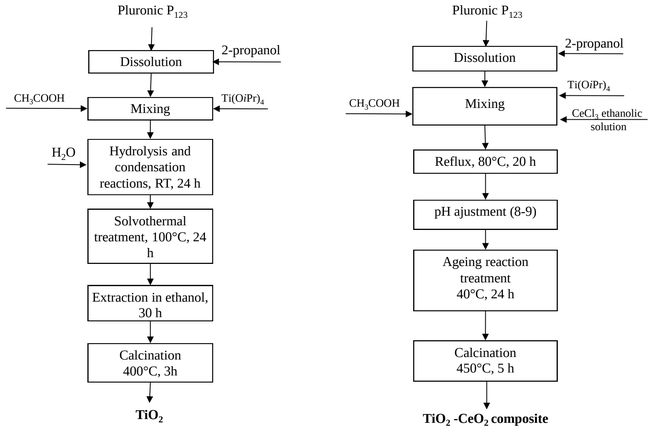
The aim of this research was to assess the influence of the extraction conditions on chemical profile, radical scavenger capacity and antimicrobial potential of common sage polyphenolic extracts. To improve the phytochemical stability, selected polyphenolic extracts were embedded into mesopores of titania-based nanomaterials

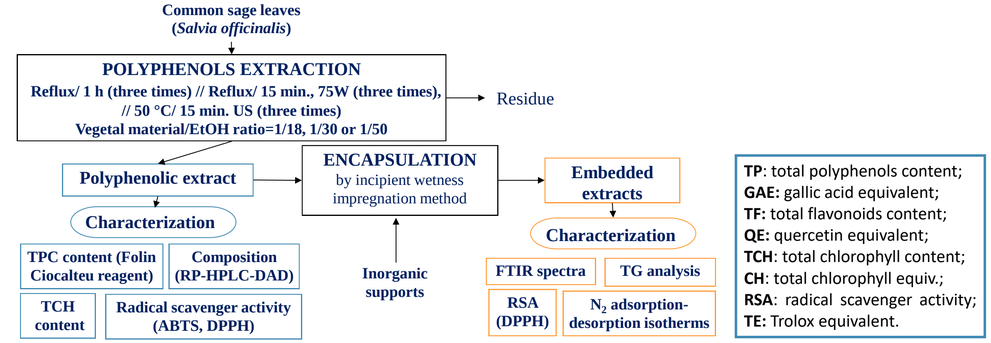
Materials and method

The ethanolic and hydroalcoholic (ethanol-water 1/1 v/v) polyphenolic extracts from *Salvia officinalis* were prepared at different plant/solvent weight ratios, at reflux or 50 ºC, either by conventional method, microwaves- or ultrasound-assisted extraction.

Mesoporous titania with anatase structure or titania-ceria composite powders were loaded with selected common sage extract through incipient wetness impregnation method, followed by solvent evaporation in vacuum.

Mesoporous titania or composite nanoparticles were prepared by sol-gel method in the presence of triblock copolymer Pluronic P123 according to the following schemes:



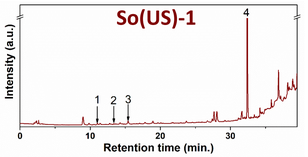


TP: total polyphenols content;
GAE: gallic acid equivalent;
TF: total flavonoids content;
QE: quercetin equivalent;
TCH: total chlorophyll content;
CH: total chlorophyll equiv.;
RSA: radical scavenger activity;
TE: Trolox equivalent.

Results and discussion

EXTRACTS CHEMICAL PROFILE DETERMINATION

Extract	Solvent	T (°C)	plant/ solvent (g/v)	Extract (%wt)	TP (mg GAE/ $g_{extract}$)	TCH (mg CH / $g_{extract}$)	TF (mg QE/ $g_{extract}$)	RSA_{ABTS} (mg TE/ $g_{extract}$)	RSA_{DPPH} (mg TE/ $g_{extract}$)
So(US)-1	EtOH	50	1/30	13.00	192.81±5.43	12.86±0.61	24.35±0.20	245.68±6.28	201.29±16.36
So(MW)-2	EtOH 50%	reflux	1/50	19.92	168.97±1.57	0.53±0.01	26.52±0.20	232.32±0.73	211.86±4.45
So(Conv)-3	EtOH 50%	50	1/30	31.71	145.40±2.31	0.57±0.08	25.11±0.49	215.20±4.22	298.34±10.42
So(Conv)-4	EtOH	reflux	1/18	13.95	129.20±5.59	4.4±0.40	36.98±1.22	128.89±4.80	249.44±11.55
So(Conv)-5	EtOH 50%	reflux	1/18	24.46	165.52±2.99	4.19±0.15	23.62±0.06	249.07±6.93	268.11±11.22
So(Conv)-6	EtOH	50	1/30	8.18	138.11±2.45	3.56±0.12	15.42±0.11	113.36±2.40	98.22±8.72

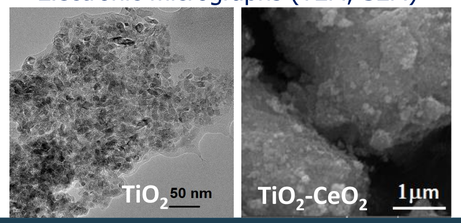


Compound	Concentration in extract (mg/g extract)					
	So(US)-1	So(MW)-2	So(Conv)-3	So(Conv)-4	So(Conv)-5	So(Conv)-6
protocatechuic acid	n.d.	0.571±0.003	0.235±0.007	n.d.	0.569±0.014	n.d.
caftaric acid (1)	0.760±0.000	0.587±0.001	n.d	n.d.	0.746±0.003	n.d.
chlorogenic acid (2)	0.330±0.000	0.675±0.00	0.828±0.004	1.194±0.008	0.753±0.005	0.094±0.000
caffeic acid (3)	0.552±0.000	2.494±0.019	2.175±0.000	0.984±0.000	2.632±0.000	0.174±0.001
p-coumaric acid	n.d.	n.d	n.d	0.108±0.012	n.d	n.d
rosmarinic acid (4)	35.335±0.000	14.861±0.008	22.877±0.004	33.094±0.024	20.542±0.009	5.673±0.025

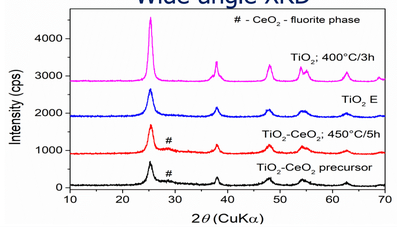
n.d – not detected;

SUPPORTS CHARACTERIZATION

Electronic micrographs (TEM, SEM)



Wide-angle XRD



CHARACTERIZATION OF ENCAPSULATED EXTRACTS

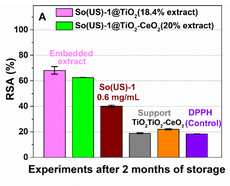
Textural parameters

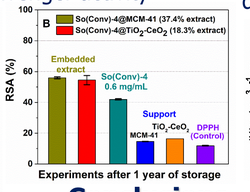
Sample	%extract	S_{BET} (m^2/g)	V_p (cm^3/g)	$d_{BJH\ des}$ (nm)
TiO_2	-	124	0.26	7.43
So(US)-1@TiO_2	18.4	48	0.16	7.40
TiO_2 –CeO_2	-	150	0.54	13.18
So(US)-1@TiO_2-CeO_2	20	78	-	-
So(Conv)-4@TiO_2-CeO_2	18.3	78	0.35	11.4
MCM-41	-	976	0.88	2.67
So(Conv)-4@MCM-41	37.4	-	-	-

Antimicrobial activity

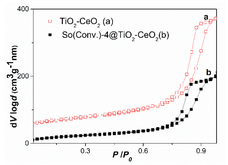
The inhibition zone diameters of sage polyphenolic extracts (20 mg/mL) against *P. Aeruginosa* and *S. Aureus* were in the range of 15-17 mm and 7-12 mm, respectively, compared to 23.5 mm and 21 mm for gentamicin and doxycycline, respectively.

In vitro radical scavenger activity





N_2 adsorption/ desorption isotherms



Conclusions

✓A lower temperature, 50ºC, and water-ethanol mixture as solvent favored the extraction of phenolic compounds. The MW-assisted extraction led to an enhanced antioxidant activity of sage extract.

✓ Concerning the radical scavenger properties, the hydroalcoholic extracts exhibited better antioxidant capacity.

✓ In all samples, rosmarinic acid was the most abundant substance, besides protocatechuic, chlorogenic, *p*-coumaric, and caffeic acids that were found in lower amounts.

✓The bactericidal activity of common sage polyphenolic extract was tested against reference bacteria, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, all prepared extracts being active against both tested bacterial strains, the highest values of inhibition zone diameter being observed for the polyphenolic ethanolic extract.

✓ The extract-loaded materials exhibited an enhanced radical scavenger activity than the free extract assessed by DPPH assay after 2-12 months storage at 4 ºC, which means a better stability of phytochemicals when were embedded into a mesoporous matrix.

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High antioxidant capacity of cannabidiol oil extracted from hemp

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Introduction

The antioxidant capacity of vegetal bioactive compounds is related to anticancer activity and can be quantified by the compounds' capacity to neutralize reactive species (RS), including reactive oxygen species (ROS) and reactive nitrogen species (RNS). Often, in tumors and cancer cells the concentration of RNS is higher than in normal tissues, acting as oxidative molecules to influence cancer progression [1]. The aims of this study were: **(a)** to test *in vitro* antioxidant capacity, by different methods, of cannabidiol (CBD) which is found in extracted oil from industrial hemp [2] and **(b)** to increase the CBD concentration of extracted oil by decarboxylation reaction of cannabidiolic acid (CBDA), an inactive form for antioxidant activity [3].

Materials and methods

The extracted hemp oil was decarboxylated at 90ºC in order to obtain oil with maximum active CBD yield.

The antioxidant activity of CBD oil was measured *in vitro* using various antioxidant assays, including

- ferrous ions (Fe^{2+}) chelating activity,
- lipid peroxidation inhibitory assay,
- ferric ions (Fe^{3+}) reducing power,
- superoxide anion radicals ($\bullet O^{2-}$),
- hydroxyl radical (•OH) scavenging activity.

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Acknowledgements

This work was funded by PCCDI – UEFISCDI project no. PN-III-P1-1.2-PCCDI-2017-0697/13PCCDI/2018 and InoMatPol, ID P_36_570, Contract no. 142/10.10.2016).

Results and discussions

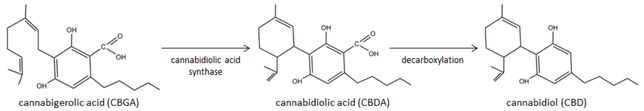

Figure 1. CBD biosynthesis scheme.

Naturally, *Cannabis sativa* L. plant synthesizes CBD as shown in Figure 1. A valuable product has the CBD concentration higher that CBDA concentration. The proper decarboxylation conditions are registered at 90ºC and 1 hour reaction time when 17.2% biologically active CBD and 4.5% crude CBD was obtained, with 97.58% transformation yield (Figure 2).

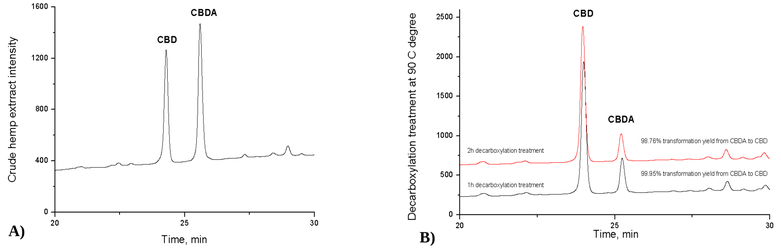

Figure 2. Representative HPLC chromatograms: **A)** Crude hemp extract; **B)** Hemp oil decarboxylated in soft conditions.

The two phenolic groups of CBD have sufficient antioxidant activity to terminate free radical chain reactions by capturing free radicals. *In vivo* studies reveals that this compound decreases oxidative stress induced by oxygen or nitrogen free radical species, thus having chemo-preventive effect [4].

Ferrous ions (Fe^{2+}) chelating activity

The ability of substances to chelate iron represents a valuable antioxidant capability by retarding metal-catalyzed oxidation. The high reactivity of ferrous ions intervenes in lipid oxidation processes by participating in Fenton type reactions and Fe^{2+} chelates are blocking these systems. Fe^{2+} chelating activity was determined using a method published by Gulcin I. et al. [5], with some modifications. The results show that a concentration of 15 µg/mL CBD oil exhibits 27.26 ± 0.2 % chelating activity, a value comparable with other published data [5]. A high chelating activity means a high capacity to bind Fe^{2+} from ferrozine complexes by competition. In the presence of CBD oil, the complex is disrupted resulting in a decrease in color intensity compared with blank sample. The conclusion is that CBD oil can be a protector in peroxidation reactions.

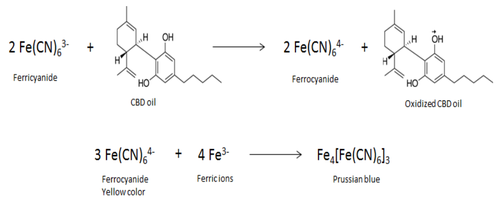

Figure 3. The reducing capability of CBD oil extract

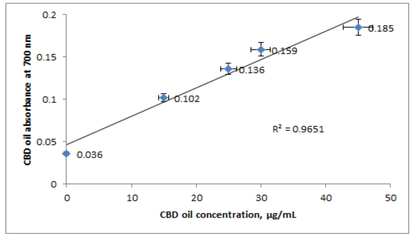

Figure 4. Ferric ions (Fe^{3+}) reducing antioxidant power assay (FRAP) of the CBD oil

Ferric ions (Fe^{3+}) reducing antioxidant power assay (FRAP)

FRAP can be measured by direct reduction of ferricyanide to ferrocyanide with the formation of intense Perl's Prussian blue complex in the presence of ferric ions (Figure 3). An increase in absorbance read at 700nm of the reaction mixture indicates an increase in the reducing capacity due to an increase in the formation of the complex. Figure 4 shows that CBD oil has very effective reducing power, 55.0% calculated from the ratio between tested higher and lower absorbances. By analyzing different concentrations (15-45 µg / mL), CBD oil reveals powerful reducing ability which increases with concentration. The electron donor properties of CBD oil are demonstrated by the reducing capability results, thus neutralizing free radicals by forming stable products [5].

Superoxide anion ($\bullet O_2^-$) and hydroxyl (•OH) radicals scavenging activity

ROS are various forms of activated oxygen including superoxide anion ($\bullet O_2^-$) and hydroxyl radical (•OH). Superoxide anions, formed in different metabolic processes, are toxic and are the precursors of active free radicals, such as hydroxyl (•OH) radicals, that have potential to react with biological macromolecules inducing tissue damage and initiating lipid peroxidation [6].

CBD oil is well known to have anti-inflammatory and antioxidant properties, as well as scavenging activity of superoxide anion ($\bullet O_2^-$) and hydroxyl (•OH) radicals. The high values (69.1 % related to superoxide anion radicals and 221.5% to hydroxyl radicals) demonstrate a very high capability of CBD oil to inactivate super reactive radicals that can extremely damage all living systems. So, the first defense against oxidative stress in living cells as superoxide anions radicals scavenging capacity will be very well accomplished by a low CBD oil concentration.

Lipid Peroxidation Inhibitory Assay

Lipid peroxidation is initiated by oxygen and hydroxyl radicals generated by auto-oxidation reactions of different enzyme systems [5]. The amount of peroxide produced during the initial stages of oxidation is measured by ferric thiocyanate method and the antioxidant capacity of CBD oil was determined. The high index obtained in four reaction days (59.77 % ± 2% for 15 µg/mL CBD oil) suggests a high capacity of CBD oil compared with previously published data [7], to neutralizing peroxide radicals which is the first stage in treatments of diverse human pathologies such as cancer, atherosclerosis, heart disease or aging [6].

Table 1. Antioxidant activity of the CBD oil

	Ferrous ions (Fe^{2+}) chelating activity, (%)	Ferric ions (Fe^{3+}) reducing antioxidant power assay (FRAP), (%)	Superoxide anion ($\bullet O^{2-}$) scavenging activity, (%)	Hydroxyl radical (•OH) scavenging ability, (%)	Lipid Peroxidation Inhibitory Assay, (%)
CBD oil extract	27.26	55.0	69.1	221.5	59.77

Conclusions

Hemp oil with 15 µg/mL CBD showed a high capacity to inactivate both super reactive radicals which are usually produced in cells of patients diagnosed with cancer and therefore could be used as complementary anti-cancer treatment, as well as in other pathologies.

Obtaining and characterization of natural anthocyanin extracts with fluorescent properties

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Introduction

Selected extracts with amplified fluorescent properties were obtained from different parts of various plants. UV-VIS spectrophotometric methods were used for characterization in order to quantify the total anthocyanin content and to determine the total polyphenol content. Fluorescence measurements were performed to highlight the special properties of purified and concentrated selective extracts.

Materials and method

Materials:

-petals of Petunia Night Sky, Pelargonium zonale red, Delphinium grandiflorum,

-fruit cuticles of Parthenocissus tricuspidata and Vittis vinifera.

Method:

-Methods use are: solid-liquid extraction, purification by C18 column liquid chromatography , redissolution in compatible solvents, advanced purifications by HPLC chromatography.

-UV-VIS spectrophotometric methods were used for characterization in order to quantify the total anthocyanin content and to determine the total polyphenol content.

-To highlight the fluorescence of the extracts, qualitative (laser excitation at different wavelengths: 450 nm, 550 nm) and quantitative (Horiba Duetta fluorescence spectrophotometer) investigations were performed.

Results and discussions

The results obtained revealed that from Parthenocissus tricuspidata was obtained the most fluorescent extract.

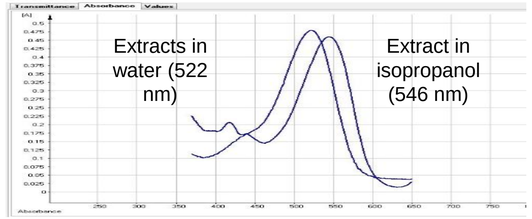

Fig. 1

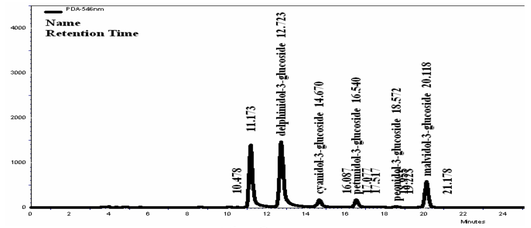

Fig.2.

Tabel 1. Characterization of extracts

Nr.crt.	Extracts	Total polyphenol content (ppm))	Total flavonoid content (mg/L quecertin)
1.	*Parthenocissus tricuspidata* în izopropanol	**2.15**	**0,74**
2.	*Parthenocissus tricuspidata* în etanol	**2.23**	**0,33**

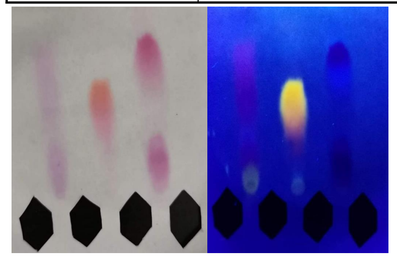

Fig.3a

Fig.3b

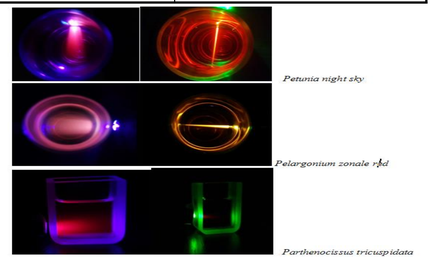

Fig. 4

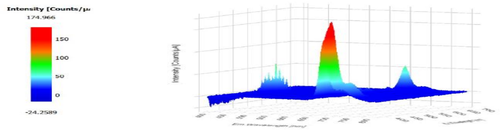

Fig. 5.

Conclusions

The extracts were separated and purified by C18 stationary phase column chromatography and spectrally characterized. It is observed that as the polarity of the extraction solvent increases, the effect is hypsochrome and hypochrome.

The extracts were characterized by determining the total polyphenol content (ppm) using the Folin Ciocâlteu method and total flavonoids (mg / L quecertin),

The fluorescence of the extracts was highlighted - by separation on chromatographic paper and their introduction in an environment with UV light, but also by excitation with green (550 nm) and blue (480nm) lasers.

Fluorescence spectra were made by emission from 300,350 to 800 nm and excitation from 250,300 to 700 nm.

Acknowledgment or Contact

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Improved *E. coli* biomass and enzyme production using oxygen vectors

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Introduction

β-galactosidase, EC 3.2.1.23, is an important enzyme in dairy and medical industry. It is used for obtaining dairy products with very low lactose content used for condensed milk or ice-cream, for speeding the coagulation time for cheese and yoghurt and also for valorization of whey.

E. coli is used for the biosynthesis of several metabolic products at industrial level, as it is easy to cultivate, it requires an inexpensive medium and high product titer can be achieved, but in order to increase the productivity of an aerobic microbial process it is extremely important to optimize the oxygen transfer.

In this communication we are reporting the investigations of n-dodecane as oxygen-vector for the enhancement of β-galactosidase activity and *E. coli* cell mass.

Materials and method

Strain
E. coli ATCC 15224

Media
glucose 3 g, peptone 10 g, meat extract 5 g, sodium chloride 5 g.

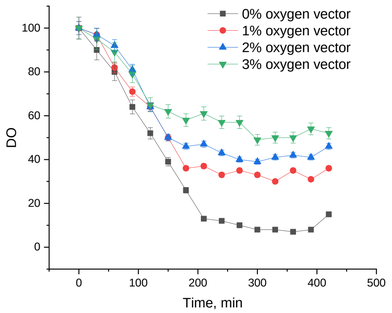
Culture conditions
37 °C, 150 rpm, 2 l/min air, pH of 6.5 (corrected with NH_4OH), harvested by centrifugation after 7 cultivation hours.

Batch Fermentation
2 l autoclavable laboratory stirred bioreactor (Fermac, Electrolab)

Measurement
cell density, OD, glucose analysis, k_La, protein concentration, enzyme activity

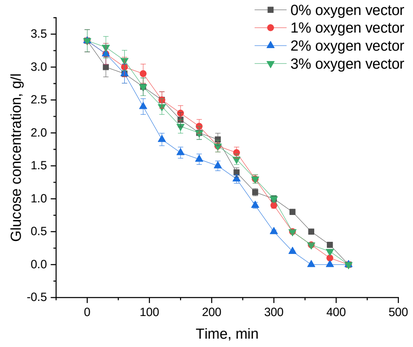
Results and discussions

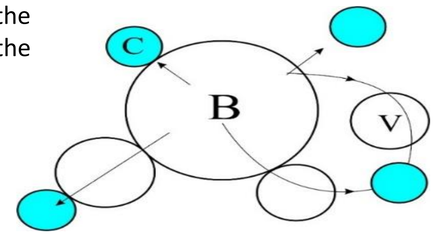
The experimental results for E. coli broths indicated a significant increase of DO, by adding n-dodecane in a proportion between 1 and 3%, without intensification of mixing or aeration compared with the control.



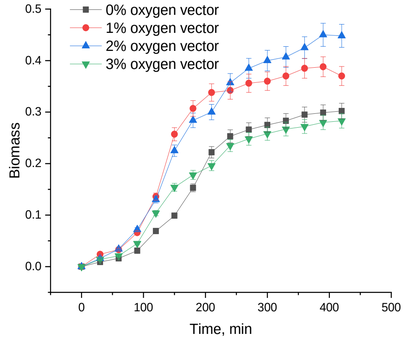
The highest cell density was attained for 2% n-dodecane in the broth, for which rapid growth of bacteria with a shorted lag phase allowed it to enter the logarithmic phase earlier leading to an increased productivity.

The n-dodecane addition leads to a superior rate of glucose consumption for 2% oxygen vector, due to an increased rate of bacterial growth under higher dissolved oxygen concentration, while for 1 and 3%, the final glucose consumption was above the value obtained in the absence of oxygen vector, proving the positive effect of its addition in the fermentation broth.

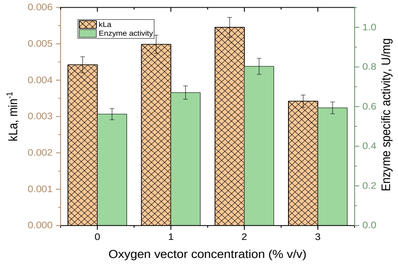




The values of dissolved oxygen concentration are significantly higher in the presence of hydrocarbon, due to its positive effect on oxygen transfer from air bubble to liquid phase, effect that is maintained for all the duration of the fermentation.



The maximum for enzyme activity and also kLa (the volumetric mass transfer coefficient) was obtained for the addition of 2% v/v n-dodecane in the bioreactor.



Conclusions

- ✓ The effect of n-dodecane as oxygen vector on the *E. coli* fermentation revealed that the relatively high DO levels induced during fermentation lead to an increase in biomass productivity and faster glucose consumption.
- ✓ Results on β-galactosidase biosynthesis suggest that n-dodecane addition markedly promotes biomass and enzyme biosynthesis compared with the control.
- ✓ Dodecane offers several advantages compared to other oxygen vectors: is nontoxic for the biomass, chemically inert and stable (can be easily recovered), inexpensive (requiring low initial investment costs).
- ✓ Under relatively high DO conditions, *E. coli* may modulate the metabolic flux in favor of biomass growth.
- ✓ Addition of n-dodecane in *E. coli* medium created a high dissolved oxygen fermentation environment which elevated intracellular oxidative metabolism that will promote a biomass accumulation and a more accurate protein folding of β-galactosidase that would increase its activity.

References

C.P. Ciobanu, A.C. Blaga, R. Froidevaux, F. Krier, A-I. Galaction, D. Cașcaval, Enhanced growth and β-galactosidase production on Escherichia coli using oxygen vectors, 3 Biotech 2020, (10), 298.

Synthesis and Characterization of Novel Cryogels Based on Dextran and Polyphenolic Extract from Spruce BarK

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Introduction

Spruce bark (*Picea Abies*) represents a waste in the wood forestry industry, whereas the complex chemical composition recommends this feedstock as a valuable source of polyphenols. The polyphenolic extract (PF) includes compounds with well-known biological properties [1] that can be embedded into natural polymers (such as polysaccharides) for designing new materials and new applications with high-added value. Among polysaccharides, dextran (Dx) is an important water-soluble natural polymer composed of linear α-l,6-linked D-glucopyranose residues that has been used widely in various applications starting from medicine to wastewater treatment [2]. In this context, the objective of this study was to prepare novel cryogel films based on Dx embedding PF from spruce bark.

Materials and method

Dx , PF/water, NaOH 5M, EGDGE 50%

- Temperature 22°C
- Continuous magnetic stirring

Precooling in Liquid Nitrogen, cryogenation at -18°C

- Freeze / thaw technique

Purification

- 20% NaCl - samples loaded with polyphenols
- Ultrapure water - control evidence

Liofilization

Stored in a desiccator with phosphorus pentoxide

Caracterization

FTIR, SEM, GFY% , P%, SR

Gel Fraction Yield (GFY, %) was calculated: $GFY(\%) = \frac{W_d}{W_m} \times 100$

W_d - the weight of dried sample;
W_m - the total weight of compounds used in synthesis.

The porosity (P,%) was calculated: $P\,\% = \frac{V1-V3}{V2-V3} x\ 100$

V1 - the volume of isopropanol; V2 - the total volume of isopropanol;
V3- the volume of isopropanol measured after removal of the cryo-beads.

Results and discussions

Table 1. Influence of the volume ratio PF :EG (v/v) on the gel fraction yield (GFY%) and porosity (P%) of cryogels.

Dx conc. wt.%	Sample	PF:EG (v/v)	GFY %	P%
20%	PF1EG0.375	1: 0.375	62.19	96.36
	PF0EG0.375	-	94.23	58.74
	PF1EG0.25	1: 0.25	57.34	57.23
	PF0EG0.25	-	87.78	48.36
	PF1EG0.5	1: 0.5	69.62	94.24
	PF0EG0.5	-	91.1	54.27

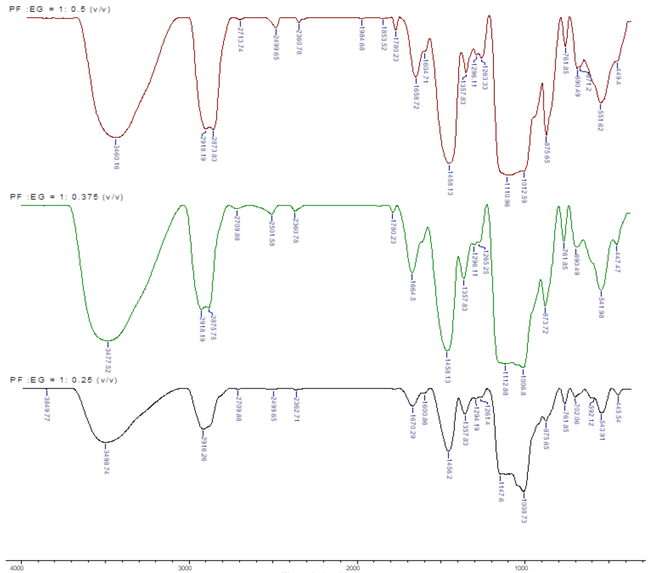

Figure 1. FTIR spectrum of PF1EG2.5, PF1EG0.375 and PF1EG0.5 cryogel films.

Characteristic peaks of Dx (1261cm^{-1} resulted from the primary O-H in plane bending and the bands at 1004 and 1145 cm^{-1} assigned to stretching vibration of C-O-C bridge in anhydroglucose units; 3462 cm^{-1} broad band due to the hydroxyl stretching vibration of the polysaccharide) and PF (new peaks at 761 cm^{-1} and 873 cm^{-1} (stretching vibration of aromatic rings) were identified in the FTIR spectra of all cryogels (Figure 1) and indicated the successful entrapment of PF within cross-linked Dx-based cryogel networks.

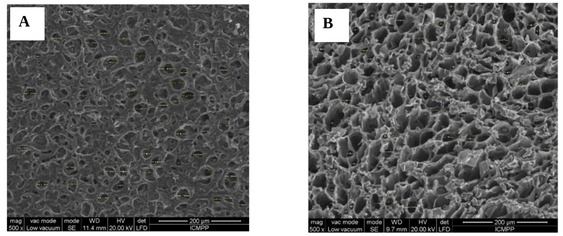

Figure 2. SEM images of cryogels PF1EG0.25 (A) and control sample without PF (B).

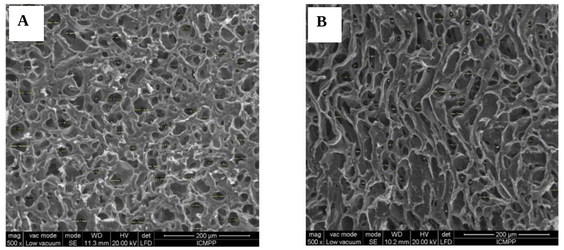

Figure 3. SEM images of cryogels PF1EG 0.375 (A) and control sample without PF (B).

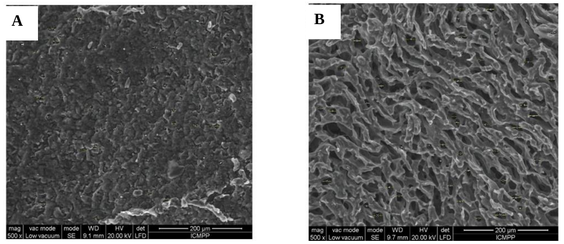

Figure 4. SEM images of cryogels PF1EG0.5 (A) and control sample without PF (B).

The SEM micrographs reveal a heterogeneous morphology consisting of polyhedral pores with an average size of 40 μm to 100 μm depending on the cryogel film composition. A less compact morphology with larger interconnected pores was observed for the Dx-based cryogels entrapping PF.

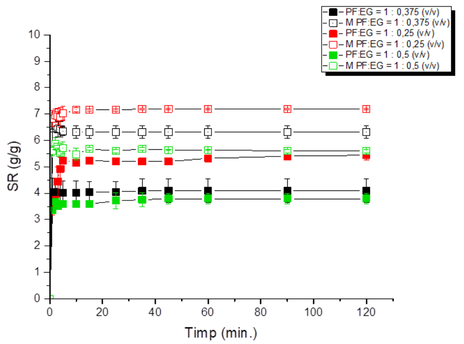

Figure 5. Degree of swelling for polyphenol-loaded gels as a function of cross-linker amount.

- The Dx-based cryogel films exhibited a swelling behavior characteristic for macroporous morphologies with interconnected pores. The swelling equilibrium was attained in about 10 min irrespective of cryogel film compositions.
- The values of swelling ratio (SR) were influenced by the cross-linking degree, the increase of the cross-linker ratio decreasing the SR values. A higher cross-linking hinders mobility and relaxation of the polymer chains, which in turn impedes the mobility of water, hence lowering the SR and equilibrium water content.

Conclusions

- Porous cryogel films based on Dx and PF with superfast swelling properties were successfully prepared using the freeze/thawing technique.
- Their formation was proved by evaluation of GFY, porosity measurements, SEM analysis, FTIR spectroscopy and swelling degree.
- One future application envisage for these biomaterials includes food packaging because the incorporation of antioxidant agents in the package could be a way of improving the stability of oxidation-sensitive food products.

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VEGETAL EXTRACT FROM SPONTANEOUS ROMANIAN FLORA WITH BIOINSECTICIDAL EFFECT USED IN THE PESTS CONTROL DURING THE SEEDS STORAGE

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Introduction

Intensive use of pesticides (persistent and non-biodegradable), often irrational, has led to increased productivity in the agricultural sector, but at the same time has led to increased deep soil degradation, groundwater pollution, surface and air pollution, and thus deterioration of flora and fauna. The contamination of crops grown on these soils has also been identified, which negatively influences food quality. Recent studies have shown that an increasing number of plant extracts have been tested on a wide range of pests, demonstrating a high efficacy, multiple mechanism of action and low toxicity to vertebrates.

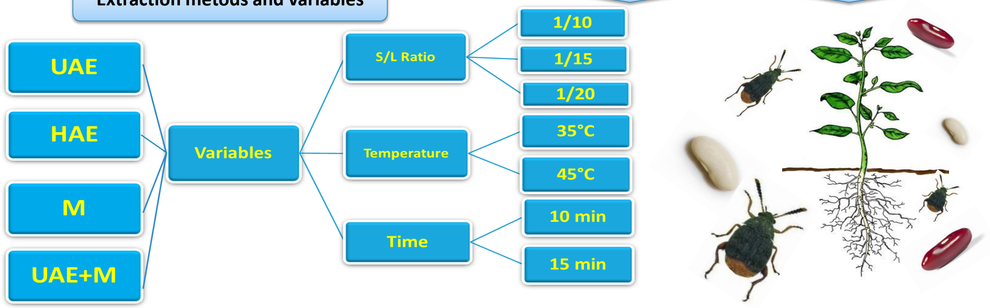
Aim

The aim of our paper is to present the results of experimental research on investigating the bioinsecticidal efficiency of plant extracts.
In the experiments were used plant extracts from the spontaneous flora of Moldova and Bukovina (Romania) (wormwood - *Artemisia absinthium*; common marjoram - *Origanum vulgare*) to control pests during seed storage (bean insect - *Acanthoscelides obsoletus*).

Artemisia absinthium

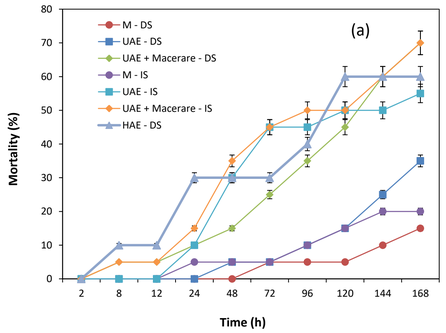
Origanum vulgare

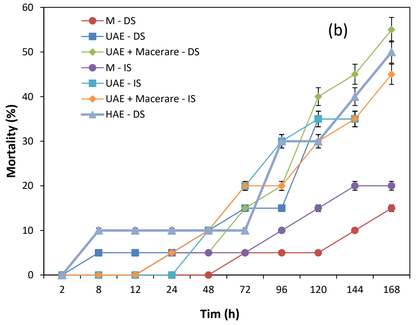
Extraction metods and variables



Results and discussion

• Obtaining of the plant extracts was achieved by three extractive techniques, the efficiency of the processes (expressed by extraction yield) being studied according to a series of physical parameters, such as solid/liquid ratio, extraction time, temperature.
• The experimental results showed that the most efficient method of obtaining plant extracts with a high content of bioactive substances is the combined method ultrasound assisted extraction (UAE) + Maceration (M), followed by heat reflux extraction (HAE), ultrasound assisted extraction, and the maceration method.

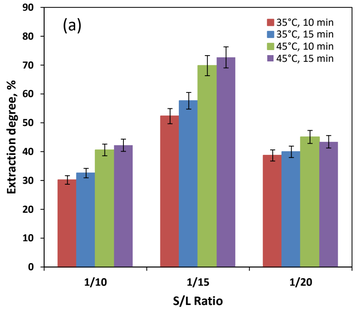


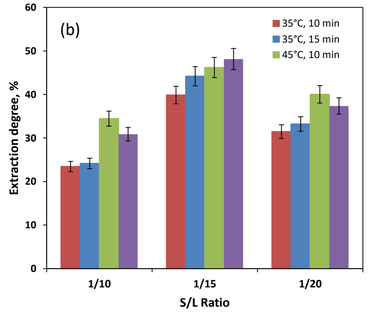


Legend

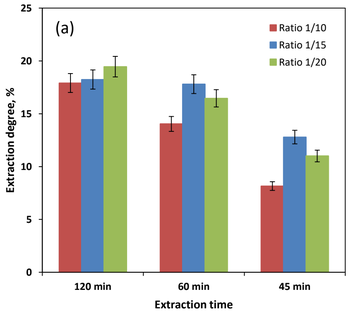
- Control sample – DS = Control sample – Direct Spraying;
- HAE – DS = HAE - Direct Spraying;
- UAE – DS = UAE - Direct Spraying;
- UAE + Maceration – DS = UAE + Maceration - Direct Spraying;
- Control sample – IS = Control sample – Indirect Spraying;
- UAE – IS=UAE - Indirect Spraying;
- UAE + Maceration – IS = UAE + Maceration - Indirect Spraying

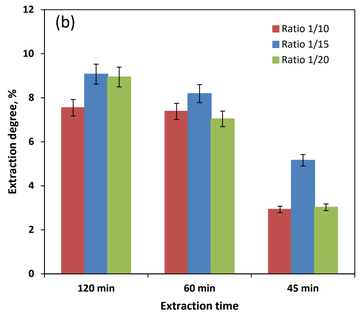
The bioinsecticidal action of *Origanum vulgare (*a) and *Artemisia absinthium* (b) extracts on *Acanthoscelides obsoletus* (using the direct or indirect treatment technique and different extraction variants)





Extraction degree (%) using the **UAE+M** method for obtaining the *Artemisia absinthium* (a) and *Origanum vulgare* (b) extracts





Extraction degree (%) using the **HAE** method for obtaining the *Artemisia absinthium* (a) and *Origanum vulgare* (b) extracts

CONCLUSIONS

The factors that were taken into account in the study of extractive techniques had a significant and different influence on the extracts composition and on its effectiveness in relation with the bioinsecticidal effect on studied deposit pests.

We can rank the magnitude of the influence of these parameters, presenting a descending order: temperature, S / L ratio (1/10, 1/15 and 1/20), and time, which played a less significant role (15 and 10 min).

The most effective plant for controlling pests of the species *Acanthoscelides obsoletus* was *Origanum vulgare* followed by *Artemisia absinthium* judging on the mortality rates.

The most effective method of treatment administration was spraying on an adsorbent surface (indirect spraying), compared with direct spraying on seeds (beans).

Synergic reactive extraction of fumaric acid obtained by fermentation with *R. oryzae*

R.A. Ilica[1], A.I. Galaction[2], D. Cascaval[1], L. Kloetzer[1]

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Introduction

Fumaric acid is a naturally occurring organic acid, an intermediate in the citric acid cycle and it has many potential industrial applications, from the manufacture of chemical products (synthetic resins, biodegradable polymers) to food and pharmaceutical products (additive, therapeutic drugs). It is produced on a large scale by the petrochemical route but the current tendency is towards implementing environmental friendly technologies like biotechnological production of fumaric acid using low-cost raw materials.

Current research trends focus on improving the fermentation process and also, on developing and applying different downstream techniques for easy recovery of fumaric acid from the fermentation broth.

Materials and method

The experiments have been carried out using an extraction column with vibratory mixing, which offers high interfacial area and the possibility to reach rapidly the equilibrium state.

Aqueous phase:

- solution of fumaric acid 5 g/l
- pH of aqueous phase = 2 – 8

Organic phase:

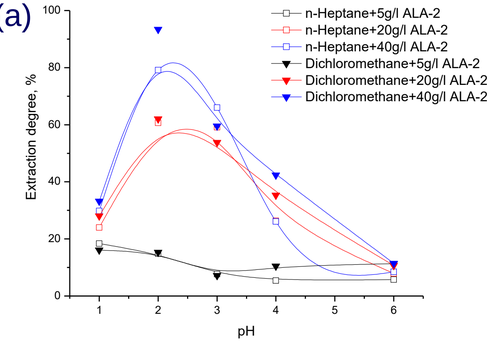
- solvent: n-heptane, dichloromethane (DCM)
- extractant: Amberlite LA-2: 0 – 80 g/l
- phase modifier: 1-octanol

Results and discussions

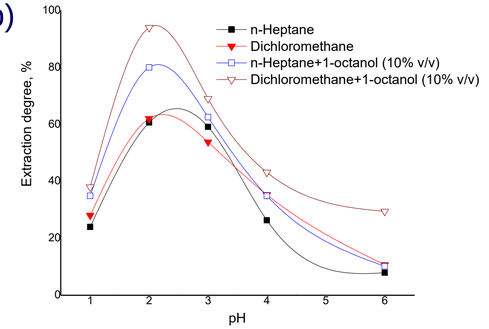
This work investigates the possibility of separation of fumaric acid from biosynthetic broth obtained by fermentation with *Rhizopus oryzae* using reactive extraction with Amberlite LA-2 (ALA-2 dissolved in different solvents (dichloromethane and n-heptane) in absence and presence of 1-octanol as phase modifier.

❖ Influence of pH-value of aqueous phase on the efficiency of fumaric acid reactive extraction with Amberlite LA-2 (a) without 1-octanol and (b) with 1-octanol

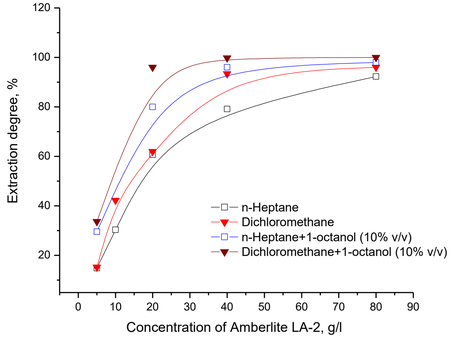
(a)



(b)



❖ Influence of extractant concentration on efficiency of fumaric acid synergic reactive extraction



❖ Number of Amberlite LA-2 molecules included in the interfacial compound structure, n, for analyzed extraction systems

Solvent	n
n-Heptane	2.12
Dichloromethane	1.36
n-Heptane + 1-octanol 10 % vol.	1.32
Dichloromethane + 1-octanol 10 % vol.	1.30

❖ Expressions and values of extraction constants for the studied extraction systems

Extractant	Solvent	Extraction constant	Value
Amberlite LA-2	n-heptane	$K_E = \frac{[FA(COOH)_2 \cdot Q_{2(o)}]}{[FA(COOH)_{2(aq)}] \cdot [Q_{(o)}]^2}$	$1.31 \cdot 10^3$ ($L^2\ mol^{-2}$)
	DCM	$K_E = \frac{[FA(COOH)_2 \cdot Q_{(o)}]}{[FA(COOH)_{2(aq)}] \cdot [Q_{(o)}]}$	$1.89 \cdot 10^2$ ($L\ mol^{-1}$)
	n-heptane + 10 vol. % 1-octanol	$K_E = \frac{[FA(COOH)_2 \cdot Q_{(o)}]}{[FA(COOH)_{2(aq)}] \cdot [Q_{(o)}]}$	$1.85 \cdot 10^2$ ($L\ mol^{-1}$)
	DCM + 10 vol. % 1-octanol	$K_E = \frac{[FA(COOH)_2 \cdot Q_{(o)}]}{[FA(COOH)_{2(aq)}] \cdot [Q_{(o)}]}$	$2.55 \cdot 10^2$ ($L\ mol^{-1}$)

Conclusions

- For synergic reactive extraction of fumaric acid the highest extraction efficiency (93 %) was obtained for the extraction system with dichloromethane and 60 g/l extractant concentration.
- The addition of 1-octanol into the solvent phase led to the improvement of extraction efficiency.

ANALYSIS OF THE POLYPHENOLS IN ALCOHOLIC BASIL EXTRACTS

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Introduction

Usually, polyphenols are found in plants as mixtures of compounds with very different chemical structure. They are responsible, not only for the color and aroma of the plants, but they also exhibit antioxidant properties, which have been previously studied [1]. Consumption of phytotherapeutic products, especially those rich in polyphenolic compounds, is correlated with a number of positive health effects, such as reducing the risk of diabetes, obesity, coronary heart disease, cancer and gastrointestinal dysfunction [2 - 5].

Basil (*Ocimumbasilicum*) is one of the spices rich in polyphenolic compounds and recognized for its antibacterial, antioxidant and antiseptic properties. Recently, essential oil and basil extracts have been the subject of several studies due to their potential to be sources of biologically active compounds and antioxidants [6,7].

In this study three concentrated alcoholic extracts of basil, obtained by three different methods (maceration, Soxhlet and ultrasound) and concentrated using a rotary evaporator were analyzed.Measurements were compared with the calibration curve of Gallic acid (25, 50, 100, 250, 500 ppm) and the results were expressed as Gallic acid equivalents.

Materials and method

For the study dried and chopped basil was used, without other additives, commercially purchased.

Ethyl alcohol of agricultural origin, 96°, Prodvinalco, also commercially purchased, was used as solvent for both the maceration and the Soxhlet and ultrasound extractions.

For the maceration of basil in 50°, 60° and 70° alcoholic solutions, the concentrated alcohol of 96° was diluted according to the specialized literature [8].

The Soxhlet extraction was performed in a laboratory installation, and the ultrasonic extraction was performed at a Digital Ultrasonic Cleaner with 50W power and 42000 Hz frequency. The concentration of basil extracts and the recovery of the solvent were performed on a rotary evaporator RV 10.IK.

The analysis of polyphenols in basil extracts was performed using the Folin-Ciocalteu method [9] which is based on the fact that polyphenols from the plant extracts react with specific redox reagents (Folin-Ciocalteu reagent) and form a blue complex, that can be quantified by visible light spectrophotometry. Thus, 0.5 ml of the sample was measured and put into a 10 ml volumetric flask with 0.5 ml of Folin-Ciocalteu solution, 5 ml of ultra-pure water and 1.5 ml of 20% sodium carbonate solution.

The flask was filled to the mark with ultrapure water. The volumetric vial samples were allowed to time for 90 minutes and then the absorbance was measured, at 765 nm wavelength, using a UV / VIS spectrophotometer. The measurements were compared with a gallic acid calibration curve (25, 50, 100, 250, 500 ppm), and the results were expressed in mg of gallic acid (GAE) / ml.
The extraction yield was calculated using a formula from literature [10]:

$$\text{Yield } (\%) = (W_1 * 100)/W_2$$

where,
W_1= weight of the extract residue obtained after solvent removal,
W_2= weight of the plant powder.

Results and discussions

1. Obtaining the basil extracts by maceration in ethanol.

In order to obtain the basil alcohol extracts, in three 500 ml bottles, the basil, previously grounded in a mortar with a pestle, in order to release volatile oils, then of 50°, 60° and 70° ethyl alcohol was added to a mass ratio, plant : solvent = 1:10. The three vials were left to soak for 4 weeks, at room temperature, each being stirred periodically. After this period, the mixture in each vial was filtered to finally obtain three alcoholic extracts of 50, 60 and 70° (photo 1). The extraction yield for each extract was as follows:

- Macerated alcoholic extract of basil 50°, η=42,91%,
- Macerated alcoholic extract of basil 60°, η=50,03%,
- Macerated alcoholic extract of basil 70°, η=51,49%.

Photo 1.

2. Obtaining the basil extract using the Soxhlet apparatus

In this case, the ratio of plant : solvent was 1:20. The extraction was performed for 5 hours, with 2 refluxing cycles / hour and an extraction efficiency of63,5%.

3. Obtaining the basil extract using ultrasounds.

Plant: solvent ratio of 1 : 10 was used. The extraction was performed for 30 minutes in a yield of 75%.

4. Concentration of obtained alcoholic extracts.

The previously obtained basil extracts were subjected to concentration by using a rotary evaporator, and the following yields were obtained:

- Macerated alcoholic extract of basil 50°, η=48,82%,
- Macerated alcoholic extract of basil 60°, η=36,96%,
- Macerated alcoholic extract of basil 70°, η=26,89%,
- Alcoholic basil extract from Soxhlet, η=6,77%,
- Alcoholic basil extract from ultrasound, η=2,66%.

2. Analysis of polyphenols in the alcoholic basil extracts

Table 1 shows the amount of polyphenols present in the studied extracts, according to the analysis.

Table 1. Polyphenol content of the analyzed extracts.

Nr. Crt.	Extract	Polyphenols, mg/L
1.	Macerated alcoholic extract of basil 50°	2160
2.	Alcoholic basil extract from Soxhlet	480
3.	Alcoholic basil extract from ultrasound	630

According to the data from Table 1, the macerated alcoholic extract of basil has a much higher polyphenol content than other extracts. This can be attributed to the extraction process, which takes place at room temperature, for four weeks. Even if both extractions were performed at high temperatures, still the alcoholic extract of basil obtained by ultrasound has a higher content than that obtained on the Soxhlet apparatus. This may be possible due to the short extraction time (30 minutes).

Conclusions

In this study, three basil alcoholic extracts were analyzed using Folin-Ciocalteu method, in order to determine the total polyphenols content. Measurements were compared with the calibration curve of Gallic acid (25, 50, 100, 250, 500 ppm) and the results were expressed as Gallic acid equivalents. Values obtained ranged within very large limits of 630-2160 mg/L GAE. The lowest concentration of polyphenols was found in basil alcoholic extract obtained with the ultrasonic method and the highest concentration in the basil alcoholic extract obtained through the maceration method.

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Separation of itaconic acid by reactive extraction

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Introduction

Itaconic acid is a versatile organic compound with many interesting industrial applications (monomer for plastics, resins and synthetic fibres, paints, films, detergents, cleaners, thickeners or bioactive components with anti-inflammatory and analgesic properties).

It can be obtained by fermentation processes which provide a green, renewable and environmentally favourable route to this industrially important metabolite. In the recent years there has been an increasing interest in process optimization by integration of fermentation and separation (downstream) units, known as *in-situ* product recovery processes. Therefore, the most important challenge is applying efficient methods for separation and recovery of itaconic acid from the fermentation broth. The conventional method of precipitation followed by acidification for recovery of acids has many disadvantages regarding the environmental protection. An efficient alternative is represented by reactive extraction separation method for itaconic acid.

The present study focuses on the development of an efficient reactive extraction system for the separation and recovery of itaconic acid from the fermentation broth. Reactive extraction was performed by using tri-n-octylamine as extractant dissolved in organic solvents.

Materials and method

The fermentation processes were carried out in 2 l laboratory stirred bioreactor (Fermac, Electrolab), provided with computer-controlled and recorded parameters. The experiments for reactive extraction processes have been carried out using an extraction column with vibratory mixing, which offers high interfacial area and the possibility to reach rapidly the equilibrium state.

Fermentation processes

Microorganism: Aspergillus terreus ATCC 32588

Cultivation parameters: 30 °C, air volumetric flow rate 1 L/min, impeller rotation speed 400 rpm, pH value 3.4, inoculum volume 10 %, fermentation time 190 hours.

Extraction processes

Aqueous phase:
itaconic acid 50 g/L, pH values 2 - 3.4

Organic phase:
Extractant: tri-n-octilamine (TOA)
Organic solvents: dichloromethane (DCM), n-heptane
Phase modifier: 1-octanol

Results and discussions

Fig.1 indicates the significant decrease of glucose concentration during 160 hrs, the substrate being consumed very slowly till the end of fermentation. The maximum itaconic acid concentration was reached after 140 hours of fermentation.

The increase of extractant concentration in the solvent phase exhibits a favorable effect on the acid extraction, due to the increase of the interfacial amount of one of the reactants. For the solvent with the highest polarity, namely dichloromethane, Fig. 2 indicates that the extraction degree continuously increases with the increase of tri-n-octylamine concentration only for extractant concentration below 50 g/L.

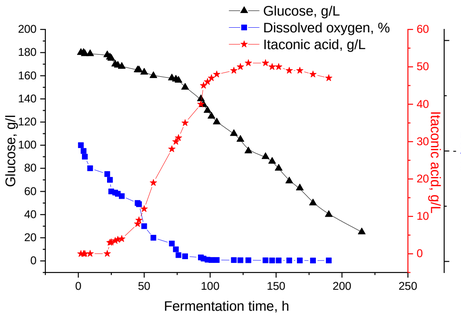
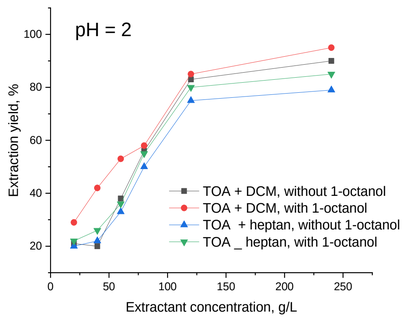


Fig.1. Variation of parameters during batch fermentation processes



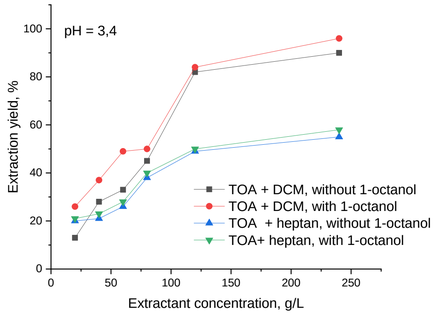


Fig.2. The influence of extractant concentration on extraction yield

Conclusions

- For reactive extraction of itaconic acid the highest extraction efficiency (93 %) was obtained for the extraction system with dichloromethane and 240 g/l extractant concentration.
- The addition of 1-octanol generates the reduction of the number of tri-n-octylamine molecules included in the interfacial compound structure, leading to the increase of interfacial reaction rate.
- The efficiency of the reactive extraction system is influenced by solute acidity, extractant concentration, and solvent polarity.

Growth optimization of algae for biodiesel production

A.C. Blaga[1], A. Tucaliuc[1], A.I. Galaction[2], D. Cascaval[1]

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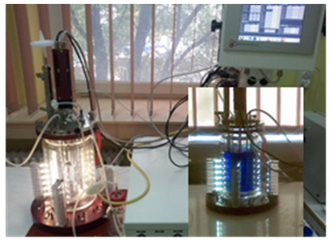
Introduction

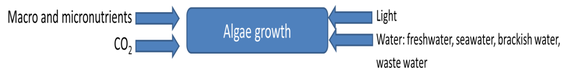
Sustainable sources of energy developed through economically viable processes are nowadays considered an alternative to fossil-based fuels, due to environmental effects of greenhouse gas emissions and the high cost of diesel price. Biodiesel represents in EU 82% of biofuels production with a total cost that implies 60–75% the price of the feedstocks.

Microalgae offers great potential as a sustainable feedstock for biodiesel production, due to its many advantages: **high lipids content (15 and 75% d.w.)**, **fast growth rate**, a lower land area requirement compared to the use of crops, the production process can use existing technologies and the available distribution system can be maintained.

Materials and method

The studies objectives include the optimization of algae growth conditions for biodiesel production using two types of photobioreactos: stirred tank and flat-plate, in order to obtain high biomass productivities.





Results and discussions

Microalgae are capable to grow rapidly due to their simple structure in very different conditions, but in order to obtain high productivity and high bioactive compounds concentration, it is important to have a very good process control. The controllable environment in which the algae can be cultivated, allows the regulation of the supply of light, nutrients, carbon dioxide, air, pH and temperature.

Microalgae strain	Proteins	Carbohydrates	Lipids
Schizochytrium sp.	13.2	19.4	**50-77**
Nannocholoropsis sp.	18-46	3	**31-68**
Botrycoccus braunii	5-45	15-20	**35-75**

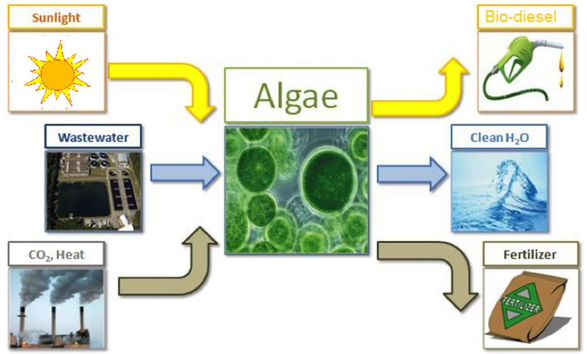
Microalgae cultivation is extremely important for the production of lipids (used for biodiesel), due to an extremely important effect on productivity of the environmental conditions.

The lipids production and the different fatty acids concentrations in microalgae are strongly influenced by the composition of culture media. Nutrient limitation (nitrogen or phosphorous) can be used to increase the accumulation of bioactive compounds, but with a negative effect on cell growth. In order to maximize the lipid content, different conditions of the culture medium such as: carbon dioxide supply, pH and temperature can be used.

Microalgae	Environmental changes	Lipids modifications
Schizochytrium sp.	Salinity at 9–36 g/L at temperature range of 16–30 °C	Saturated FA C15:0 and C17:0 was greatly increased
Nannochloropsis sp.	Increase from 20 °C to 25 °C Nitrogen limitation	Lipid production increased by 14.92% Total lipid increased by 15.31%
Botryococcus braunii	Increase in temperature	Saturated FAs increased

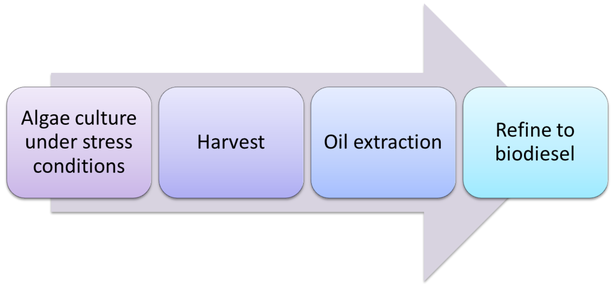
The investigated PBR offer advantages :

- high productivity,
- low contamination,
- continuous operation and
- controlled growth conditions,
- but with some limitations regarding:
- low light penetration



Conclusions

Microalgae appear to be a considerable source of biodiesel with potential to make it competitive with petrodiesel, but the cultivation systems still require attention in order to obtain productivities that could make microalgal biodiesel economically competitive.



Acknowledgment

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Separation of 7-aminocephalosporanic acid by reactive extraction

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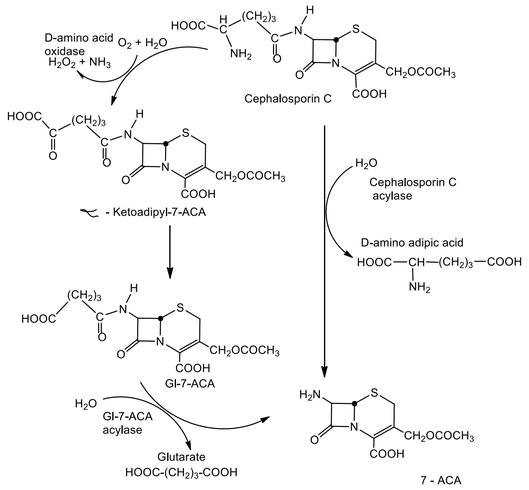
Introduction

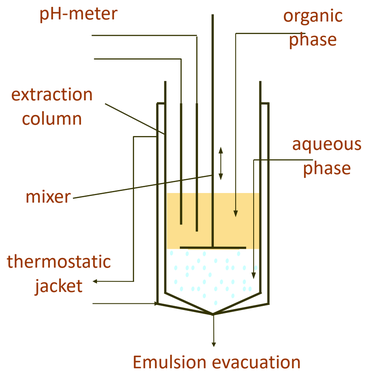
7-Aminocephalosporanic acid is the core chemical structure for the synthesis of cephalosporin antibiotics and intermediates.

Chemical deacylation of cephalosporin C, a fermentation product, is the primary method used to produce 7-ACA industrially. In the past decade, enzymatic methods for deacylation have attracted more attention in the manufacturing of cephalosporin antibiotics and several enzyme-based methods have been developed. Following the enzymatic conversion process of cephalosporin C to 7-aminocephalosporanic acid, the product must be isolated from the reaction mixture.



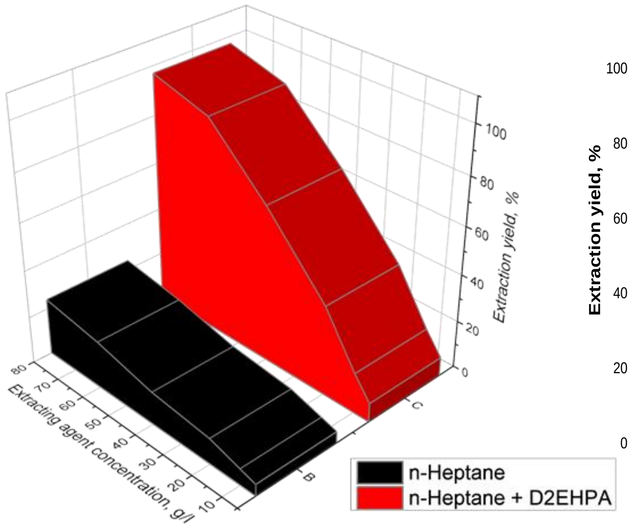
The physical solvent extraction raises a number of difficulties, because of the amphoteric character of the molecule. 7-ACA could be found in different charges of ionic forms depending on the pH of the media. Therefore, a viable method for the separation of 7-ACA is extraction accompanied by chemical reaction with an extractant, namely reactive extraction.

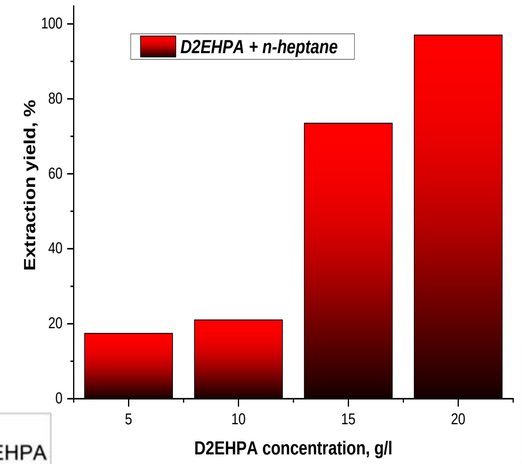
Materials and method



- Extraction column with vibratory mixing
- Aqueous phase: 7-aminocephalosporanic acid
- 7-ACA pH 2 - 11
- Organic phase: n-heptane
- Extractant: di-(2-ethylhexyl) phosphoric acid (D2EHPA) 5 – 20 g/l
- HPLC concentration determination

Results and discussions





Conclusions

The physical extraction of 7-ACA with n-heptane is achieved by diffusion and solubilization processes, with low separation rates.

Adding D2EHPA as extractant leads to the formation of a miscible compound only with the solvent, thereby increasing its ability to retain the solute and increasing the selectivity of the separation, therefore the extraction yield.

97%
Extraction yield

Brilliant Red HE-3B dye biosorption by immobilized *Bacillus* sp. residual biomass: Fixed–Bed-Column Studies

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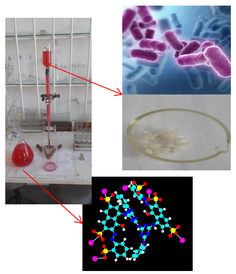
Introduction

The biosorption potential of *Bacillus* sp. residual biomass for reactive Brilliant Red HE-3B textile dye removal from aqueous media was studied. The waste biomass, resulting from a process of removing fatty acids from wastewater, was immobilized in sodium alginate and used for biosorption of the dye from aqueous solution in a fixed-bed column.

The effects of various experimental operating parameters, such as bed depth, flow rate were investigated.

The obtained results reconfirm that the studied residual biomass can be considered as a good biosorbent but only in a static operating system, and this can be used in the treatment of wastewater containing small quantities of organic dyes.

Materials and method

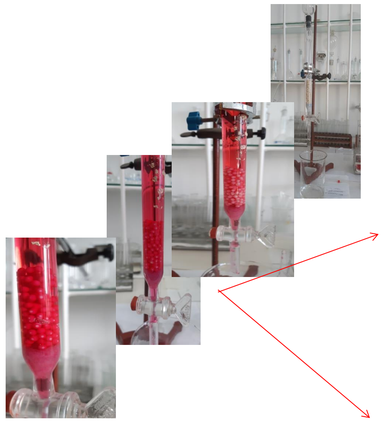


Dye: The Brilliant Red HE-3B (Procion Red HE-3B, C.I. Reactive Red 120) reactive dye (MW = 1463, λ_{max} = 530 nm).

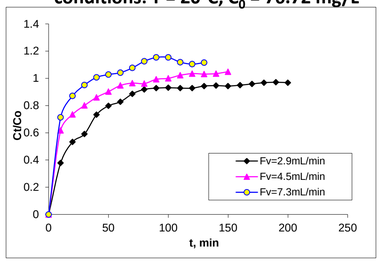
Biosorbent: a mixture comprises in equal ratios the following strains: *Bacillus subtilis, Bacillus megaterium, Bacillus licheniformis* and *Bacillus ortoliquefaciens* was used for the removal of Fats, Oils, and Grease (solid waste) **FOGS** from wastewater, at 35 °C and 150 rpm, in an aerobic system for 96 hours. The biomass was separated by centrifugation (8000 rpm), dried at 80 ^{0}C and immobilized by cell inclusion into sodium alginate

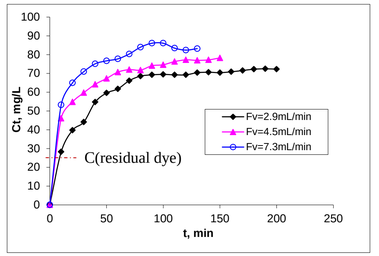
Results and discussions

1. Breakthrough curves



The breakthrough curves for the biosorption system: BRed dye – *Bacillus sp*. residual biomass immobilized in alginate. Operating conditions: T = 20^{0}C, C_0 = 76.72 mg/L





Biosorption dynamic procedure

- The column was packed with known varying amounts of residual biomass immobilized in alginate in the form of granules with a diameter of 0.5 mm (3.8 – 6.77 g) providing a packed bed height of biosorbent between 3.8 - 7 cm.
- A dye solution of known concentration (usually 76.72 mg/L) was introduced on the top of the column by means of a feeding funnel to ensure uniform continuous flow.
- The passing of the dye solution through the column was done freely, gravitationally, and the effluent was collected from the bottom for further analyzing and control.
- At 10 min time intervals , samples of 5 mL-effluent were taken from column outlet (the bottom of the column at different time intervals) and analyzed with the UV-VIS Digital Spectrophotometer, model S 104D/ WPA, especially for the residual dye concentration in the treated effluent.

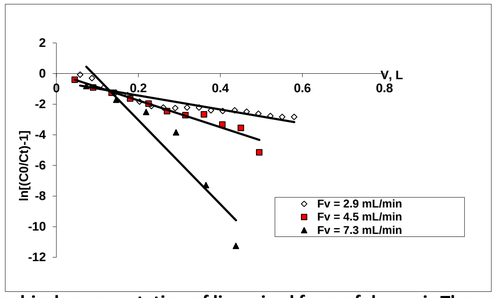
This work was supported by a Grant of The Romanian Ministry of Research and Innovation, CCDI-UEFISCDI, project number PN-III-P2-2.1-PED-2019-1063, within PNCDI III.

Characteristic operating parameters of the breakthrough curves

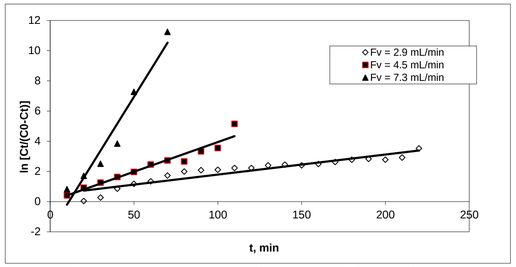
Parameter	Significance and characteristics	Experimental values for each studied flowrate (F_v), [mL/min]		
		2.9	4.5	7.3
Biosorbent bed height – h (cm)	Height in column of each added adsorbent amount	4.0	7.0	3.8
Breakthrough time - t_b (min)	Time required for attaining the breakthrough point, when the dye concentration has the value of 0.1C_0 (C_b)	7.0	4.0	1.5
Saturation time - t_s (min)	Time required for attaining the saturation point, where dye concentration has a value of 0.9C_0 (C_s)	55.0	32.5	16.5
The length of mass transfer zone – L (MTZ) (cm)	$L(MTZ) = h \cdot \left(1 - \frac{t_b}{t_s}\right)$ where, h - the height of adsorbent bed	3.49	6.138	3.45
Breakthrough volume – V_b (mL)	Volume of working solution at breakthrough point, calculated as $V_b = F_v \cdot t_b$, where F_v is the volumetric flow rate (mL/min).	20.3	18.0	10.95
Saturation volume – V_s (mL)	Volume of working solution at saturation point, calculated as $V_S = F_v \cdot t_s$, where F_v is the flow rate (mL/min)	159.5	146.25	120.45
Breakthrough capacity - q_b (mg/g)	Amount of BRed dye retained per biosorbent mass at breakthrough point. $q_b = \frac{(C_0 - C_b)\cdot V_b}{m}$ where, m - adsorbent mass, g.	7.293.10^{-3}	3.89.10^{-3}	4.2.10^{-3}
Saturation capacity - q_S (mg/g)	Amount of MB dye retained per adsorbent mass at saturation point. $q_S = \frac{(C_0 - C_S)\cdot V_S}{m}$ where, m - adsorbent mass, g.	57.307	34.81	46.17
Rate of exhaustion - R_{AE} (g/L)	Amount of exhausted adsorbent (g) per volume of working solution at the breakthrough point. $R_{AE}(g/L) = \frac{\text{mass of exhaused adsorbent}}{\text{volume of working solution}}$	0.2114 4.482	0.556 11.783	0.208 4.409
bi (mL/g.min)	Mean flow rate per adsorbent mass $b_i = \frac{V_n + n_i \cdot v}{m \cdot t_{ni}}$ Where, V_n is the dye solution volume passing through the fixed adsorbent bed (mL); n_i - the number of samples ; v - the sample volume (v=5 mL); m – the adsorbent mass (g) and t_{ni} is the total adsorption time(min).	1.8757	0.6396	1.1248
Vi (mL/g)*	Mean volume of dye solution passing through the fixed adsorbent bed (mL) per adsorbent mass (g) The adsorption time till maximum residual concentration attaining (tri, (min)) was varied as 10.00, 7.86 and 5.652 min, respectively.	10.149	5.027	11.248

* The optimal wastewater volume passing through the adsorbent mass (**Vopt**) was found to be **5.0273 mL/g**,or 5.0273x10^3 mc/kg .

- **Bulk density** of residual biomass was found to be **576.65 kg/mc**.
- For a common industrial effluent flowrate (textile industry) **of 50 mc/day (**or 2.083 mc/h), the **design data** of a **dynamic adsorption column reactor/tank** and a **static adsorption basin/tank**, considering a minimum of **one day** of adsorption, were calculated, and correspond to the values presented as



Graphical representation of linearized form of dynamic Thomas biosorption model



Graphical representation of linearized form of dynamic Yoon-Nelson biosorption model

Conclusions

- The obtained results reconfirmed that the studied residual biomass immobilized in alginate can be considered as an efficient biosorbent in **static regime** but not for the **dynamic operating systems**, especially when is intended to be used for the treatment of effluents containing organic dyes.
- The **adsorption time** till adsorbent regeneration step in dynamic operating regime (column reactor) is **very low** (10-15 min), thus better results are obtained in static regime as previous data shown.

Adsorption column reactor/tank (dynamic regime) (Vads =17.251 mc; Mads =9945.7 kg)	Dr = 1.671 m	H (adsorb. bed) = 2.507 m Hr = 3.76 m Efficiency : low
Radial adsorption basin/tank (static regime)	D tank = 4.10 m	H tank = 4 m (agitation by air) H useful = 3. 6 m

Investigation of the potential use of Ratanhia CO_2 extract-based herbal oral products in treatment of oral mucositis

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Introduction

Oral mucositis appears as erythematous and ulcerative lesions of the oral mucosa, that can be observed at patients with cancer who are being treated with chemotherapy and / or radiation therapy in regions involving the oral cavity. Oral mucositis lesions are often painful and affect oral nutrition and hygiene, and increase the risk of local and systemic infections. Recently, various natural agents in plants have been noticed in mucositis, which may improve the symptoms through different interventions. The purpose of this investigation is to focus on the potential use of Ratanhia CO_2 extract-based herbal oral products to prevent, alleviate and treat the oral mucositis.

Materials and method

Materials: ***Ratanhia CO_2 extract*** have a biological action due to its high content of tannins such as rhataniatannic acid, which are responsible for the astringent, hemostatic, disinfectant, tonic and firming effects on the mucous membranes of the mouth. ***Aloe barbadensis*** contains polysaccharides, anthraquinone, lectin, superoxide dismutase (an antioxidant enzyme), glycoprotein, amino acids, vitamins C and E and minerals. Due to the fact that *Aloe bardadensis* gel contains multiple pharmacologically active compounds have healing proprieties as anti-inflammatory, analgesic, anticarcinogenic effects. ***Matricaria recutita flower essential oil*** is used in traditional medicine for its antioxidant, antimicrobial, and anti-inflammatory action. ***Citrus bergamia***, freshens up breath and stops tartar formation. ***Pelargonium graveolens*** essential oils has antibacterial, antioxidant and antifungal effects.

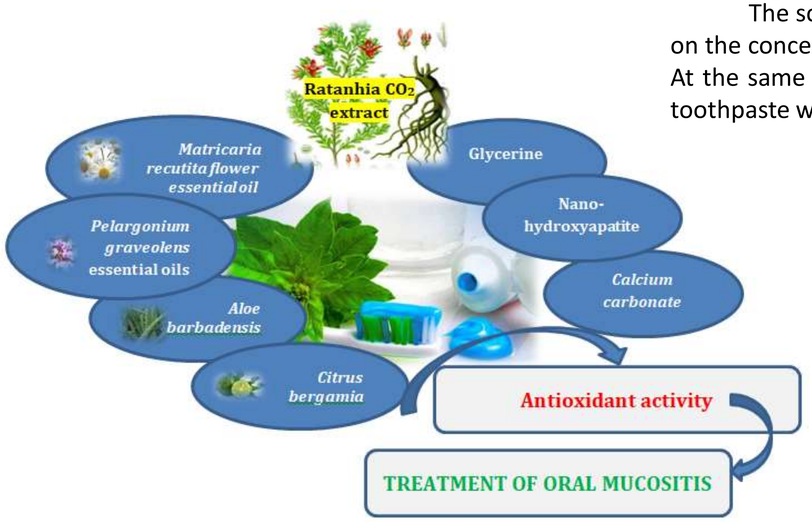
Nano-hydroxyapatite was prepared by sol-gel technique and added to the toothpaste with 20% concentration. The purpose of using nano-hydroxyapatite in our product is for the remineralization effect and strengthens tooth enamel. Other inorganic material content in herbal oral product is ***natural calcium carbonate*** used in smaller quantities for adjust texture and viscosity. ***Glycerine*** is used as humectant and also inhibit bacterial growth and provide flowability to the dentifrice.

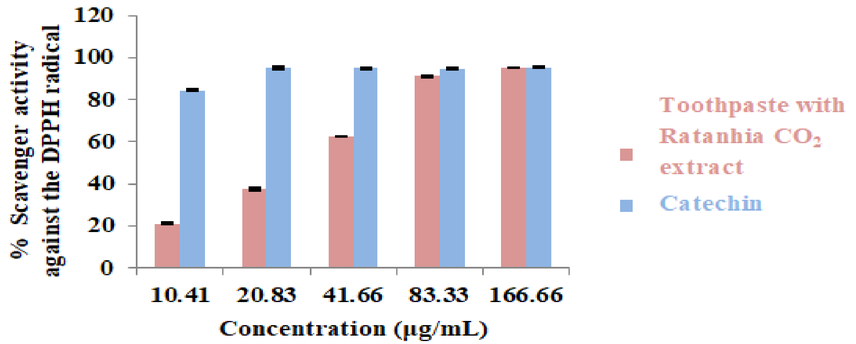
No sodium lauryl sulfate, fluoride, sugar, no artificial flavors, colors or preservatives.

Method: The percentage of antioxidant activity (AA%) of toothpaste with ***Rathania CO_2*** extract and catechin was assessed by DPPH free radical assay. The measurement of the DPPH radical scavenging activity was performed according to methodology described by Brand-Williams et al.. The samples were reacted with the stable DPPH radical in an ethanol solution. The changes in color (from deep violet to light yellow) were read at 517 nm using a UV-VIS spectrophotometer .

Results and discussions

The scavenger activity against the DPPH radical of the toothpaste with ratanhia CO2 extract, as well as the positive control, catechin, varied depending on the concentration. At a concentration of 166.66 µg / mL, the toothpaste with ratanhia CO2 extract inactivated the radical in a proportion of 94.85 ± 0.16%. At the same concentration (166.66 µg / mL), the catechin activity was 95.48 ± 0.51%. At 10.41 µg / mL, the scavenger activity of the DPPH radical of the toothpaste with ratanhia CO2 extract was 22.12 ± 0.34%, respectively, while the catechin inactivated the DPPH radical in the proportion of 85.54 ± 0.45%





Conclusions

This study suggests that the use of Ratanhia CO_2 extract-based herbal oral products such as toothpaste may have a positive influence on the oral side effects of cancer chemotherapy due to its demonstrated antioxidant effect, and that further investigations might be desirable.

Contact

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References: Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. Lebenson Wiss Technol 1995;28:25-30

Spectroscopic investigation and chemical fingerprint of *Datura ionnoxia* dry biomass

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Introduction

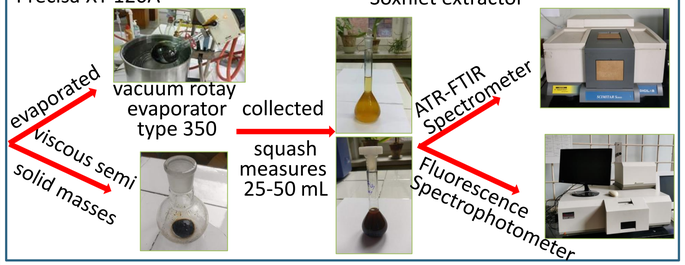
Plant species represent a **rich source of bioactive compounds**.

Nowadays, **scientists** from various fields **are interested** for **advanced vibrational spectroscopic methods** in **fingerprinting secondary metabolites from plants** which can either positively or negatively affect human health.

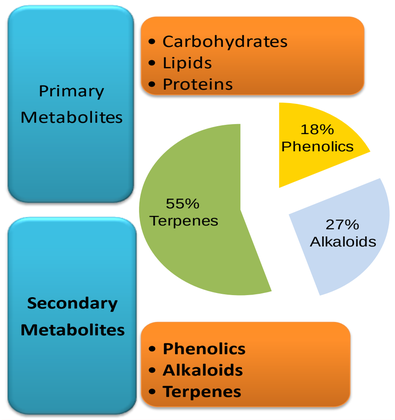
Non-chromatographic techniques are **extremely valuable** for **identification, isolation and characterization** of **bioactive compounds** from **vegetal biomass extracts**.

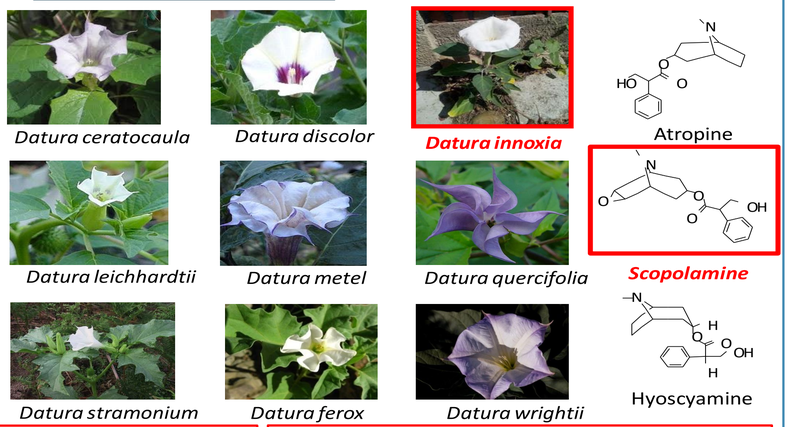
Datura innoxia belong to the **Solanaceae** family. Nowadays, is becoming **important for medicine for its rich tropane alkaloids content, especially hyoscine (scopolamine).**

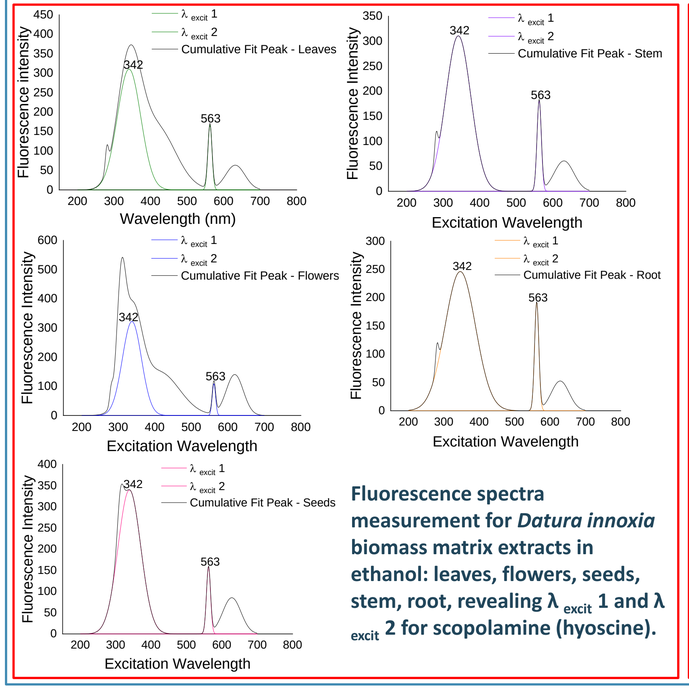
Materials and method



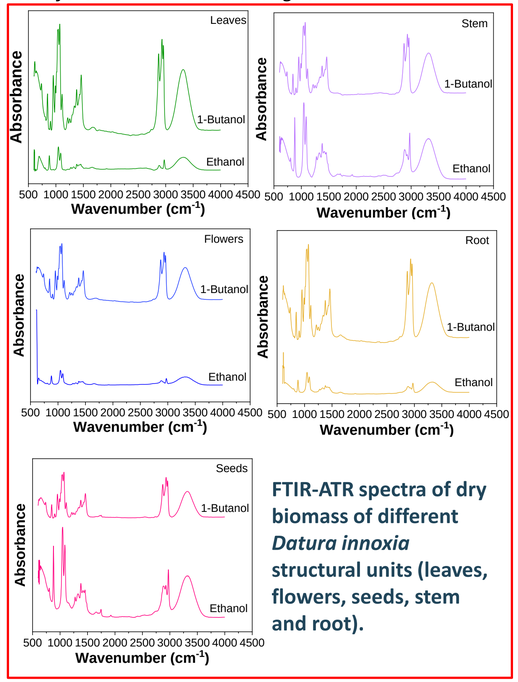
Results and discussions







Fluorescence spectra measurement for *Datura innoxia* biomass matrix extracts in ethanol: leaves, flowers, seeds, stem, root, revealing λ excit 1 and λ excit 2 for scopolamine (hyoscine).



FTIR-ATR spectra of dry biomass of different *Datura innoxia* structural units (leaves, flowers, seeds, stem and root).

Conclusions

- The present **results** revealed that **scopolamine** has been identified from **ethanolic** and **1-butanolic** extracts in **dry biomass** of different ***Datura innoxia*** structural units using **Soxhlet extraction** method fallowed by **fluorescence** and **ATR-FTIR** analysis.
- **ATR-FTIR analysis results** confirmed the presence of secondary metabolites, mainly alkaloids e.g. **scopolamine or hyoscine**, from *Datura innoxia* dry biomass extracts.
- Results showed the presence of hyoscine **in all vegetative organs (leaves, flowers, stem, root).**
- Regarding **fluorescence study**, λ excit 1 and λ excit 2 for hyoscine were found to be 342 and respectively 563 nm.
- **Experimental** results confirm the fact that this **plant posse's important classes of secondary metabolites** useful in medicine domain.
- **Further** scientific **investigation** for **intensification** of **extraction** by **nonconventional methods** is needed.

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