CORPUS PUBLISHERS

Current Trends in Engineering Science (CTES)

ISSN: 2833-356X

Volume 3 Issue 6, 2023

Article Information

Received date : September 15, 2023

Published date: October 05, 2023

*Corresponding author

Vasile Staicu, Hofigal Export-Import SA, Research Development Patent Department, Romania

DOI: 10.54026/CTES/1046

Keywords

Adsorption chromatography; Mineral adsorbents; Plant extracts; Adsorption columns; Purification of extracts

Research article

Separation and Purification of Plant Extracts by Removal of Chlorophyll, Xanthophylls and Pigments using Mineral Adsorbents in Liquid Chromatography (CLS)

Vasile Staicu[1,2*], Ciprian Gabriel Chisega Negrilă[2], Justinian Andrei Tomescu[1,2], Mihaela Neagu[1] and Ioan Călinescu[2]

[1]*Hofigal Export-Import SA, Research Development Patent Department, Romania*

[2]*Faculty of Chemical Engineering and Biotechnologies, Romania*

Abstract

This study focused on the purification of plant extracts, with the help of adsorption chromatography (liquid chromatography with a solid stationary phase - CLS) so that the purified plant extracts lead to obtaining bioactive compounds in a pure state or with advanced purity. The studied extracts can be divide into categories as follows:

a) The purification of extracts from perilla frutescens
b) The purification of extracts from rosmarinus officinalis
c) The purification of extracts from artemisia annua (pelinița)

For the perilla frutescens extract, dried leaves, the purification aimed at obtaining pure crystals of ursolic acid and the physico-chemical analysis of the plant extracts before and after extraction, to determine the loss of the important compounds of the plant, due to their retention in the adsorbent layer. For the rosemary and wormwood (artemisia annua) extracts, dry leaves, the purification aimed at obtaining pure crystals of bioactive compounds (ursolic acid, artemisinin) and their gravimetric dosing to determine the bioactive compound content of the original plant. Mineral adsorbents and combinations of mineral adsorbents in the form of adsorbent mixtures have been used. Glass liquid columns of various sizes were used. For each experiment, the working times (saturation-activation time, elution and column washing time) were noted. All experiments were performed with the adsorption chromatography procedure, with gravity flow column (in adsorption chromatography with gravity flow column), without using Sephadex LH-20 macroporous resins as a stationary phase.

Introduction

The first research on liquid chromatography is attributed to Tswett, 1906 [1], a talented Russian botanist who made it possible, for the first time, to separate natural compounds on a column filled with calcium carbonate using a mobile phase made up of organic solvents [1]. This simple technique, called at the beginning chromatographic procedure, or in short Chromatography it was made known thanks to numerous studies and inventions carried out by Tswett [2]. Starting from his first experiments, Winterstein and Stein [3,4] extracted from green plant leaves the two components of chlorophyll, chlorophyll A and B, violaxanthin, xanthophylls and carotenes, using a glass column (10x1 cm) with a stationary phase consisting of sucrose, calcium carbonate and alumina (Al_2O_3) and as solvents a mixture of petroleum and benzene (Figure 1). After removing the stationary phase from the glass tube, the column was cut and the coloured portions were eluted separately with a mixture of light petroleum and methanol to isolate and dose compounds from each coloured area [3,4].

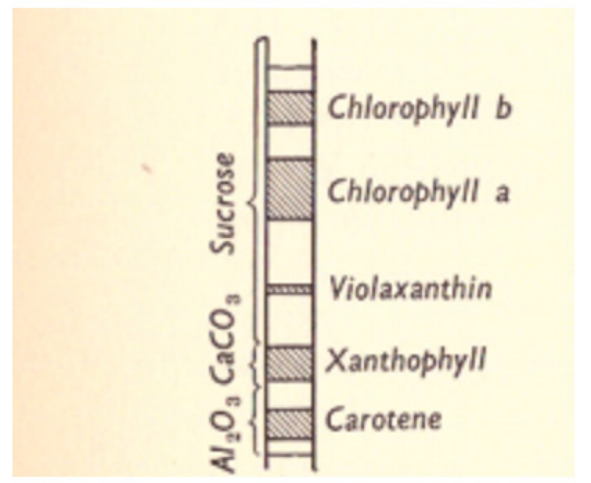


Figure 1: Chromatogram of green leaf extract [3,4].

Mohler & Hämmerle [5,6] used liquid chromatography to examine foodstuffs. Using a glass column adapted to a trunk vessel and as an alumina adsorbent, Al_2O_3, they used a Bordeaux red wine, passing it through the column and obtaining a chromatogram with three zones: bright red, slightly darker red and blue greenish fringe (Figure 2). The filtrate had a very faint bluish tint.

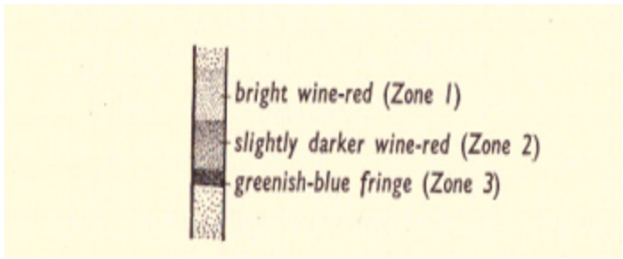


Figure 2: Red wine chromatogram [5,6].

At the 214th communications meeting of the British Society of Biochemistry in June 1941, Martin and Synge, two young chemists, presented a paper on the separation and determination of N-acylated amino acids in wool samples by a new analytical method [7]. They separated acetyl-proline from acetyl-leucine, using a column filled with silica gel impregnated with water (a highly polar material) and chloroform as the mobile phase. Thus the chromatography of partition [7] was born, later recognized as chromatographic separation from liquids in normal phase. Martin and Synge's key contributions to the development of chromatography were awarded the Nobel Prize in Chemistry in 1952.

In chapter Adsorption Chromatography [8], the diagram of the adsorption chromatography is presented schematically, (Figure 3), and in chapter Adsorption Chromatography [9] we have the representation of the preparatory chromatography diagram (Figure 4).

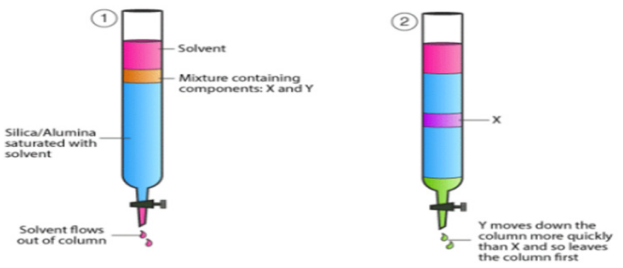


Figure 3: Diagram of adsorption chromatography [8].

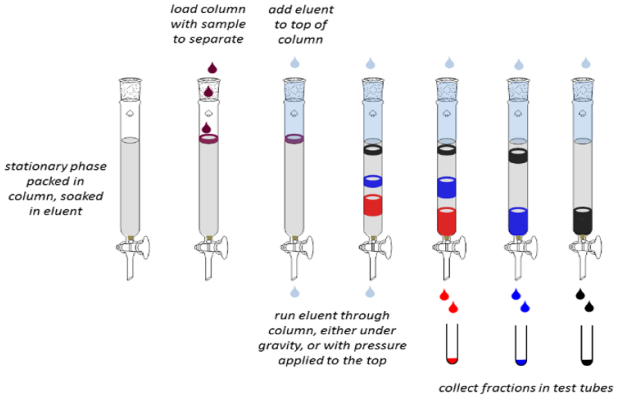


Figure 4: Diagram of preparative chromatography [9].

It is observed that the stationary phase formed by silica gel or alumina, Al_2O_3, is saturated in the solvent, and the mixture of components is above the stationary phase. The solvent elutes the mixture (mixture of components), resulting in their separation, depending on the affinity of the component to the stationary phase or solvent. This affinity makes it possible for the components to be separated and collected separately in preparative chromatography.

Chavan & Kengar [10] makes a classification of the most used mineral adsorbents, encountered in adsorption chromatography, establishing the following ranking

a) Silica gel
b) Activated alumina balls
c) Activated carbon
d) Cation exchange column
e) Aluminum oxide, etc.

Amuda & Ibrahim [11] provide a description of an activated carbon adsorbent obtained from coconut shells for the treatment of industrial waste water, which can retain organic materials and thereby help purify such waste water.

In another work [12] an adsorbent composed of sea buckthorn meal waste (from the food supplement industry) which retains heavy metals from aqueous solutions is presented.

In chapter Separation of Materials by Column Chromatography [13] a mixture of estriol, estradiol, estrone is presented, which are separated, in a silica gel column, due to their different polarity: estriol>estradiol>estrone, using as solvent a mixture of ethyl acetate and hexane (Figure 5).

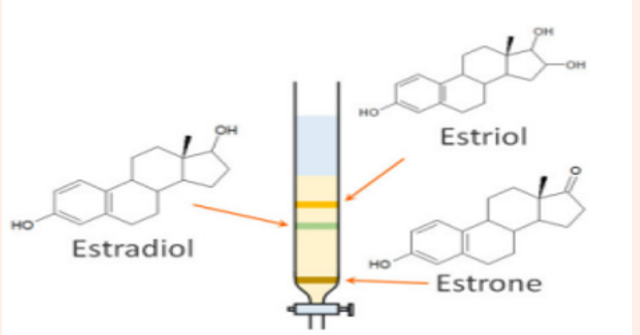


Figure 5: Separation of the mixture of estriol, estradiol, estrone [13].

In our study we used the principles of adsorption chromatography to separate and purify plant extracts by removing chlorophyll, xanthophylls or pigments, using mineral adsorbents or mixtures of mineral substances, providing a simple method of purifying raw materials (plant extracts) for industrial research and development. We have proposed, through analyses, to verify that the processes of separation and purification of extracts taken into work, eliminate or not eliminate the main compounds of plants, seeking to obtain purified plant extracts. The studied extracts can be divided into categories as follows:

a) The purification of extracts from perilla *frutescens*
b) The purification of extracts from *rosmarinus* officinalis
c) The purification of extracts from artemisia *annua* (peliniță)

For the perilla *frutescens* extract, dried leaves, the purification aimed at obtaining pure crystals of ursolic acid and the physico-chemical analysis of the plant extracts before and after extraction, to determine the loss of the important compounds of the plant, due to their retention in the adsorbent layer.

For the rosemary and wormwood (artemisia annua) extracts, dry leaves, the purification aimed at obtaining pure crystals of bioactive compounds (ursolic acid, artemisinin) and their gravimetric dosing to determine the bioactive compound content of the original plant.

Mineral adsorbents and combinations of mineral adsorbents in the form of adsorbent mixtures have been used. Glass liquid columns of various sizes were used. For each experiment, the working times (saturation-activation time, elution and column washing time) were noted. All experiments were performed with the adsorption chromatography procedure, with gravity flow column (in adsorption chromatography with gravity flow column), without using Sephadex LH-20 macroporous resins as a stationary phase.

To prepare the column, a small cotton wool plug is put at the lower end of the column [14] then a small gauze stopper and segregation material (2-7mm pebbles or glass spheres) to ease the gravitational flow. The adsorbent or adsorbent mixture is poured dryly into the hollow tube. The amount of adsorbent is calculated at around 20-50 g per gram of active substance to be separated [14]. The ratio of the diameter of the column to its length should be from 1:5 to 1:20. The column is activated/saturated with a volume of solvent, usually the solvent used for plant extraction. If the adsorbent occupies 2/3 of the column length, the solvent used for activation will fill the column. After draining the solvent, at the top of the column introduce the sample in the form of a concentrated solution, diluted in the activation solvent in a ratio of 1:1. Allow to distribute evenly over the surface of the column and time the separation time. If necessary, further add the sample solution. When the working sample has finished draining, add fresh solvent (eluent). The stage corresponds development, when the components of the sample to be analysed are distributed over the column in relation to their affinity to the stationary phase. After elution with solvent, the compounds in the mixture can be separated as coloured strips [14] along the column, each coloured strip can be further sectioned and analysed, the method being applicable to the mixture of pigments of different colours (e.g. at the separation of chlorophylls and accompanying pigments, carotenes and xanthophylls). Another method involves elution with solvents until the eluent brings to the end of the column various fractions that are collected separately and analysed in terms of composition [14]. The second variant applies to the extraction of b-carotene from plants, flavones from plant materials, HCH isomers, phytosterols, peptides etc. The adsorbent in the column can be regenerated and reused. To increase the elution rate at the height of the column, it can be attached to a trunk vessel connected to vacuum or work under compressed air (flash chromatography) [14].

Methods and Materials

The following 4 types of columns were used, with dimensions in cm (Figure 6):

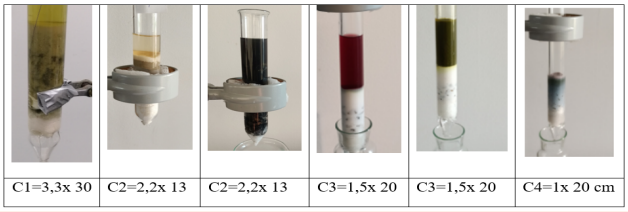


Figure 6: Adsorption columns.

To separate plant pigments and chlorophylls from plant extracts, adsorbing mixtures of mineral compounds chosen after numerous trials were used so as to ensure the removal of plant pigments, chlorophylls and fat/volatile substances and to result in purified extracts, which, after evaporation of the solvent, lead to dry residues or crystals of advanced purity. Depending on the affinity of the bioactive compound to the stationary phase, these dry residues or resulting purified crystals represent a higher or lower percentage of the bioactive compound content of the plant under construction. If the percentage is very small, the stationary phase must be removed from the column and eluted with a specific solvent to recover the bioactive compound from the adsorbing mixture, followed by further purification of the liquid obtained after elution of the column.

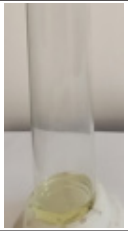
The following four types of adsorbing mixtures were used:

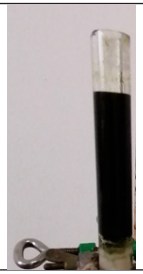
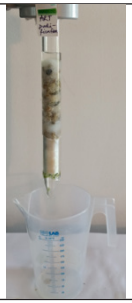
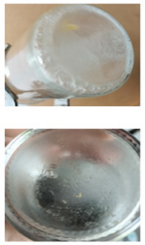
A1=Sucrose+$CaCO_3$+Al_2O_3(alumina)-all in powder form layered.
A2=Sucrose+silica gel (60-200 mesh) +activated carbon 1-4 mm (import), in layers.
A3=mixture 50 cm3 of: 25p construction stone 3-8 mm (segregating agent) +6p natural bentonite 1-5 mm, Rm.Vâlcea area+2p dolomite ($CaCO_3$*$MgCO_3$) powder, Filipesti-Bacău area+4p activated carbon 1-4 mm, import+3p construction sand (segregating agent)+2p natural clay, powder, Calarasi area+2p red clay, powder, Oradea area+2p blue clay, powder, Ricin-Mures area + 2p green clay, import Sicily + 2p Kisselgur, import Hungary, company Biorganic (p = 1 cm^3).
A4 = mixture 35 cm3 of: 20p construction stone 3-8 mm (segregating agent) + 2p dolomite ($CaCO_3$*$MgCO_3$) powder + 2p Kisselgur, imported from Hungary, Biorganic company + 4p sucrose + 1p blue clay, powder, Ricin-Mures area + 2p activated carbon 1-4 mm, import + 2p Al2O3 (alumina) powder + 2p silica gel, 60-200 mesh (p = 1 cm^3).

A1 is formed by introducing into the column alumina having direct contact with the cotton wool, from the bottom end of the column, followed by the introduction of calcium carbonate, and sucrose is introduced on top. The result is a column with 3 distinct layers of adsorbents. A2 is formed in the same way as A1, with activated carbon in contact with cotton wool. A3 and A4 are mixtures of mineral adsorcants, which are introduced into the column in direct contact with cotton wool from the bottom end of the column and can occupy 1/4, 1/3, 1/2 or 2/3 of the height volume of the column. The free volume is for the solvent or extract used. After wetting the column (activation of the column) the collected fraction is composed of the liquid collected after passing through the column of the plant extract, as well as of the fraction resulting from the column washing operation. Sucrose usually retains chlorophylls and violoxanthin from extracts, calcium carbonate retains xanthophylls, and alumina retains carotenoids, forming an adsorption-separation chromatogram for the plant extract. Bentonite and dolomite retain vegetable pigments and include traces of water that damage separation, clays, regardless of colour, retain fatty substances and volatile oils from extracts, and kieselgur, silica gel and activated charcoal retain plant pigments and yellow-xanthophyll pigments. The segregating agent-building stone, 3-8 mm, has the role of loosening the stationary phase formed to shorten the leakage time through the column and prevent the formation of plugs by clogging the stationary phase.

Table 1 below shows the purified plant extracts together with the recorded working parameters.

Table 1: Purified plant extracts by removing chlorophylls and plant pigments with mineral adsorbents.

Extract name	Filter layer	Column size cm	Wetting solvent/ quantity	Wetting time, hours	Purified extract volume/time, hours	Washing solvent/ quantity	Column washing time, hours	Amount of puri of purified extract, hours	Color crystals after evap. solv	Analysis type	Photo column	Photo extract/ crystals	Photo extract/ crystals
Perilla extract with alcohol 960, dark green	A3	C2 2,2x13 1/3 solv	alc.96% 15 mL	6 hours	17mL/15 hours	alc.96% 17 mL	6 hours	26 mL very light green	-	HPLC for raw material, primary extract and very light green and physicochem analysis			-
The very light green perilla extract above	A2	C2 2.2x13 1/5 solv	alc.96%	0,25	25 mL/	alc.96%	0,5hour	40 mL/	White	HPLC for the colorless extract and physicochem analysis	-		
Rosemary extract with alcohol 960, dark green brown	A3	C3 1,5x20 1/3 solv	alc.96% 15 mL	0,35 hours	30 mL 5 hours	alc.96% 30 mL	10 hours	55 mL Green brown (does not purify)	-	-		-	-
Green-brown rosemary extract, which has not been purified	A1	C3 1,5x20 2/3 solv	alc.96% 10 mL	0,75 hours	20 mL 1,5 hours	alc.96% 10 mL	0,75 hours	20 mL yellow	The yellow solution obtained was evaporated, the residue was redissolved in alc.96% resulting in white crystals of ursolic acid	HPLC for the raw material and gravimetric for the white crystals of ursolic acid obtained			

Wormwood extract (artemisia annua) with alcohol of 960, dark green	A4	C1 3,3x30 1/3 solv	alc.96% 50 mL	2,0 hours	60 mL 12 hours	alc.96% 35 mL	9,0 hours	100 mL yellow solution	-	-			-
Yellow worm-wood extract purified above	A3	C4 1x 20 1/3 solv	alc.96% 10 mL	1/2 hours	25 mL 3,5 hours	alc.96% 15 mL	0,75 hours	40 mL colorless	White crystals of arte-misinin	Gravimetric for the white crystals of artemisinin obtained.			

Physico-Chemical Analyses for Perilla and Perilla Extracts

For perilla frutescens and perilla extracts obtained with 96% alcohol, physicochemical analyses were performed to determine the content of polyphenols, flavones, hydroxycinnamic derivatives [15] and antioxidant capacity of primary extract and purified perilla extract as well as determination of the content of these bioactive compounds in the working raw material, dried perilla leaves. These analyses are necessary to see the degree of purification of the perilla extract, the amount of active compound remaining in the stationary phase, and the separation yield of pure crystals from the original extract, all compared to the initial content of these compounds in the original plant.

Total polyphenol content (PCT), expressed as gallic acid equivalent

Total content of polyphenols (TPC) was evaluated by the Folin–Ciocâlteu method [16]. The absorbance was measured at 765 nm against ultrapure water with a Jasco Model V-530 Vis UV Spectrophotometer and the total polyphenol content was calculated using the standard gallic acid calibration curve with concentrations between 5 and 100 μg/100 mL and expressed as gallic acid equivalent (mg/g).

Total flavone content (TFC), was carried out by the method described by Marinova et al. [17] i.e. the method spectrophotometric in the presence of aluminium chloride, using as analytical standard Quercetin (λ= 510 nm).

Total antioxidant capacity (Ac AO) by CUPRAC method (CAT – CUPRAC – Reducing Antioxidant Power)

The Cuprac method described and modified by Apak R. et al. [18], is based on total antioxidant capacity (CAT–CUPRAC – Reducing Antioxidant Power) by reducing cupric ion Cu2+ to cupros ion Cu+. It is achieved by measuring the antioxidant activity determined by means of a Jasco Model V-530 UV-VIS spectrophotometer, based on a calibration curve using Trolox (antioxidant substance) of known concentrations as standard, at wavelength λ= 450 nm [19].

Determination of antioxidant capacity FRAP II λ=593nm (by reducing Fe3+ to Fe2+) (Ferric Reducing Antioxidant Power)

This Frap II method is based on the reducing power of biologically active compounds associated with their electron donation capacity, thereby reducing ferric ions to ferrous ions, which form at acidic pH a complex coloured blue-intense purple [20,21].

Determination of the content of total hydroxycinnamic derivatives expressed as rosmarinic acid

The principle of the method. The total amount of phenolic acids was determined following the method of European Pharmacopoeia 7.0, Rosmarini folium monograph, page 1227 [15] at 505 nm using a JASCO 530 UV-Vis spectrophotometer The content of hydroxycinnamic derivatives was expressed as rosmarinic acid equivalent (RAE) in milligrams per gram of dry extract.

HPLC analyses for the determination of ursolic and oleanolic acid from perilla and vegetable extracts obtained from perilla

For the determination of ursolic/oleanolic acid in perilla, dried leaves (raw material) and perilla extracts, obtained with 96% alcohol, the modified method adapted from Chen, Xia, 2003 was used [22] HPLC analysis was performed with Thermo Fisher Scientific Vanquish Core HPLC system, equipped with a Vanquish Core Dual Pump C (VC-P32-A-01), Vanquish Split autosampler (VC-A12-A-02), Vanquish column compartment (VC-C10-A-03), and Vanquish™™™™ Diode Array Detector (VC-[19] D11-A-01), the separation column was ZORBAX Eclipse Plus C18 (4.6 x 150 mm, 3.5µm). The detection was reached at a wavelength of 210nm. For perilla the plant was used a plant/solvent ratio = 1g:20, and for extracts a sample meal of approx. 0.8 g. HPLC analysis for the determination of ursolic/oleanolic acid in rosemary, dried leaves, raw material. The method modified and adapted from Chen, Xia, 2003 [22], above, was used. The HPLC chromatograms obtained for the two plants analysed, perilla and rosemary, are given below.

Looking at the data in table 1, we see the following:

a) The total purification time of the presented extracts is between 20-30 hours, a relatively long time. But, taking into account the simplicity of the separation and purification process, as well as the low costs incurred with the acquisition of mineral adsorbents, this process is a solution for obtaining purified extracts. One kg of bentonite or dolomite can cost one euro, while 1 kg of macroporous resin for adsorption can cost 1000 euros.

b) Purification of extracts using mineral adsorbents as above cannot be achieved in a single pass through the column. Only a semi-purified extract is obtained, which is freed from chlorophylls and pigments, is light in colour and is used for the second column, chosen in such a way as to eliminate yellow-xanthophyll pigments, fatty substances and volatile oils and to lead to colourless extracts and/or white crystals of advanced purity, which are weighed and gravimetrically determined the purification yield.

Following physicochemical analyses to determine the content of polyphenols, flavones, hydroxycinnamic derivatives and antioxidant capacity of the primary extract and purified perilla extract as well as the determination of the content of these bioactive compounds in the working raw material, dried leaves of perilla, the results obtained are presented below in table 2.

a) Sample No 1. Primary perilla extract, alcohol 96%, dark green -(40mL)
b) Sample No 2. Purified perilla extract (very light green – 40mL)
c) Sample No 3. Perilla powder, Hofigal greenhouses -2021

Table 2: Results of physicochemical analyses for perilla frutescens.

Item no.	Analysis performed	Sample No. 1	Sample No. 2	Sample No. 3
1	Total polyphenol content (CTP) (expressed as gallic acid), mg/g	1.79	0.11	45.24
2	Total flavone content (CTF) (expressed as quercetin), mg/g	10.73	Below detection limit	126.14
3	Total hydroxycinnamic content (exp in rosmarinic acid) mg/g	1.24	0.042	24.41
4	Antioxidant capacity 'CURAC method' (expressed as Trolox), mg/g	58.96	0.15	119.68
5	Antioxidant capacity 'FRAP 2 method (expressed as Fe2+), mg/g	50.18	0.18	166.09

The graphical representation of the data in table 2 is shown in figure 7 below. Table 2 and the graphic show the following:

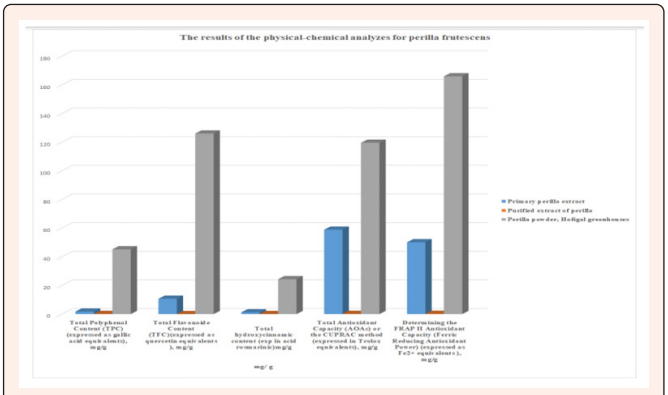


Figure 7: Graphic representation of the results of physio chemical analyzes for perilla frutescens extracts.

a) The values of polyphenols, expressed as gallic acid, mg/g, in the primary extract, dark green colour, represent 3.95% of their values in the raw material, ie 25 times lower.

b) The values of polyphenols, expressed in gallic acid, mg/g, in the purified, very light green extract, represent 0.24% of their values in the raw material, i.e. 400 times lower.

c) The values of flavones, expressed in querticin, mg/g, in the primary extract, represent 8.5% of their values in the raw material, i.e. 12 times lower.

d) Flavone values, expressed in querticin, mg/g, in purified extract, represent 0.0% of their values in the raw material, below the limit of detection.

e) The values of hydroxycinnamic derivatives, expressed as rosmarinic acid, mg/g, in the primary extract, represent 5% of their values in the raw material, i.e. 20 times lower.

f) The values of hydroxycinnamic derivatives, expressed as rosmarinic acid, mg/g, in the purified extract, represent 0.17% of their values in the raw material, i.e. 600 times lower.

g) CUPRAC values, expressed in Trolox, mg/g, in the primary extract, represent 50% of their values in the raw material, i.e. 2 times lower.

h) CUPRAC values, expressed in Trolox, mg/g, in purified extract, represent 0.125% of their values in the raw material, i.e. 800 times lower.

i) FRAP values, expressed in Fe2+, mg/g, in the primary extract, represent 30.2% of their values in the raw material, i.e. 3.3 times lower.

j) FRAP values, expressed in Fe2+, mg/g, in purified extract, represent 0.108% of their values in the raw material, i.e. 925 times lower.

In this case, the physicochemical analyses performed show us that the stationary phase retained most of the bioactive compounds analyzed, these being found in the purified solution in percentages hundreds of times lower than the primary extract and the raw material analyzed, which leads to the conclusion that for these compounds the proposed method of purification does not give results. In addition, for the evaluation of these compounds, additional operations of elution of the stationary phase with specific solvents are required to separate the respective compounds.

Also, physicochemical analyses show that the extraction of bioactive compounds analyzed, with 96% alcohol, from perilla leaves, led to extraction yields between 4-8.5% for polyphenols, flavones and hydroxycinnamic derivatives and between 30-50% for CUPRAC and FRAP. Therefore, 96% alcohol extraction of perilla leaves yields poor results for polyphenols, flavones and hydroxycinnamic compounds and satisfactory yields for antioxidant activities, CUPRAC and FRAP. Following HPLC analyses to determine the ursolic/oleanolic acid content of the primary extract, purified perilla extracts and working raw material, dried perilla leaves, the results obtained, after interpretation of the HPLC chromatograms obtained (shown below), are presented in the following table (Table 3).

a) Sample No. 1. Primary perilla extract, alcohol 96%, dark green
b) Sample No 2. Purified perilla extract, very light green
c) Sample No.3. Purified perilla extract, colourless
d) Sample No 4. Perilla powder, Hofigal greenhouses -2021

Table 3: HPLC test results for perilla frutescens.

Item no.	HPLC analysed compound mg/g	Sample 1	Sample 2	Sample 3	Sample 4
1	oleanolic acid	0.247	0.044	0.041	2.338
2	Ursolic acid	0.692	0.107	0.08	3.591

The graphical representation of the data in table 3 is shown in figure 8 below.

From the analysis of the data from the chromatograms obtained, the data from table 3 and figure 8, the following can be seen:

a) the primary extract, dark green colour, obtained from the dried leaves of perilla with 96% alcohol, has an oleanolic acid content of 10.56% compared to the content existing in perilla leaves, i.e. 9.5 times less than the oleanolic acid content existing in the raw material.
b) the purified extract, very light green in colour, has an oleanolic acid content of 1.88% compared to the content existing in perilla leaves, i.e. 53 times less than the oleanolic acid content existing in the raw material.
c) the purified, colourless extract has an oleanolic acid content of 1.75% compared to the content existing in perilla leaves, i.e. 57 times less than the oleanolic acid content existing in the raw material.
d) the primary, dark green extract, obtained from dried perilla leaves with 96% alcohol, has an ursolic acid content of 19.28% compared to the content existing in perilla leaves, ie 5.18 times less than the ursolic acid content existing in the raw material.
e) the purified extract, very light green in colour, has an ursolic acid content of 2.98% compared to the content existing in perilla leaves, ie 33.5 times less than the ursolic acid content existing in the raw material.
f) the purified, colourless extract has an ursolic acid content of 2.23% compared to the content existing in perilla leaves, ie 49 times less than the content of ursolic acid existing in the raw material.

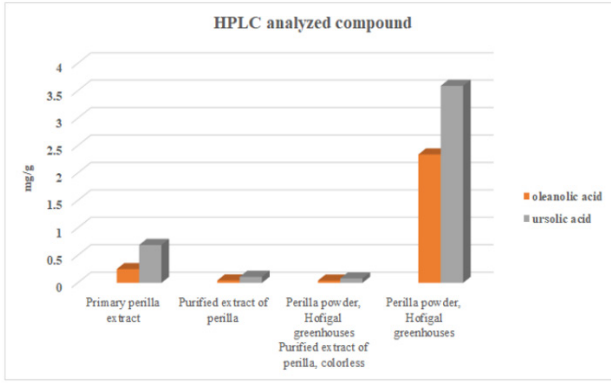
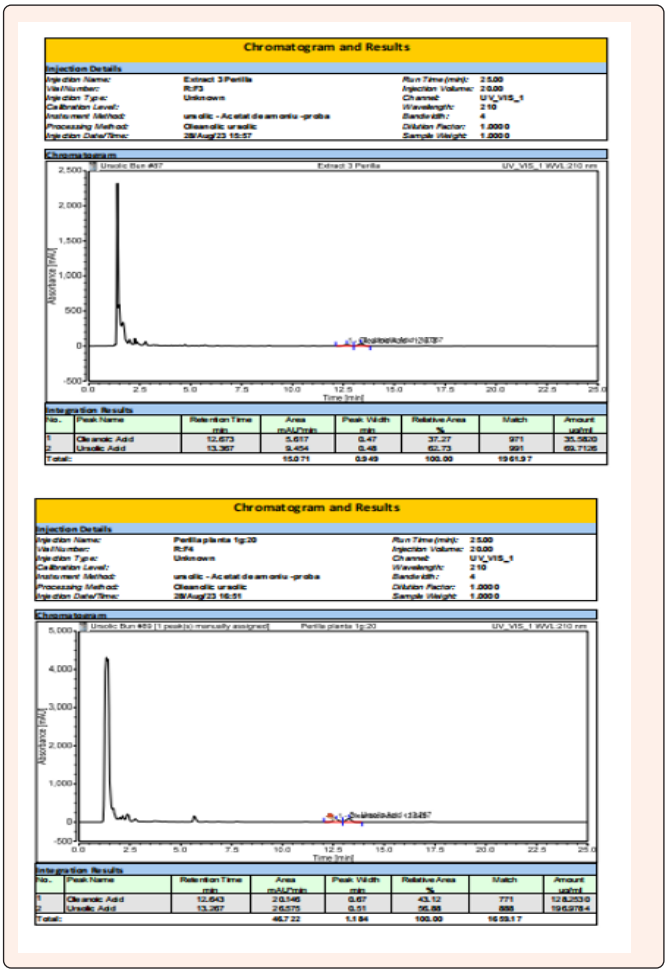


Figure 8: Graphic representation of HPLC analyzes for perilla frutescens.

Following HPLC analyses to determine the ursolic / oleanolic acid content of the primary extract, purified perilla extracts and the working raw material, dried perilla leaves, it is found that the results obtained are superior (20-40 times higher) to those obtained from physicochemical analyses to determine the content of polyphenols, flavones, hydroxycinnamic derivatives and antioxidant capacity of extracts. This shows us that ursolic/oleanolic acid passes easily through the column and this variant can be a method of purifying extracts and obtaining crystals of advanced purity. A percentage of 19% (for ursolic) of extraction from the raw material and a percentage of 2.22% of ursolic in the final purified, colourless extract, are good results for the simplicity of the proposed method. In other words, if we start from a sample mass of 40g perilla leaves and obtain by extraction (maceration), with 96% alcohol, 120 mL primary extract (containing 29mg pure ursolic acid) after purification we obtain approximately 3 mg of ursolic acid crystals of advanced purity, in the form of white crystals (or starting from 100 g plant. We obtain, after purification, 8 mg of ursolic acid crystals of advanced purity, in the form of white crystals). For rosemary, HPLC analysis has been performed for the raw material, dried rosemary leaves and the chromatogram is given below. The content of ursolic acid in the raw material is 16mg/g. And here, if we start from 100 g of the plant and make an extract with 96% alcohol that we undergo purification by the above method, we obtain about 35 mg of ursolic acid crystals of advanced purity, in the form of white crystals.

For wormwood we obtained a quantity of 5 mg artemisinin, crystals of advanced purity, in the form of white crystals, following the purification process, starting from 50 g of the plant macerated in 96% alcohol, which represents an artemisinin content of 0.05% in the plant, at an extraction yield of 20%. This results is comparable to values in the literature [23,24], (Figures 9 & 10).



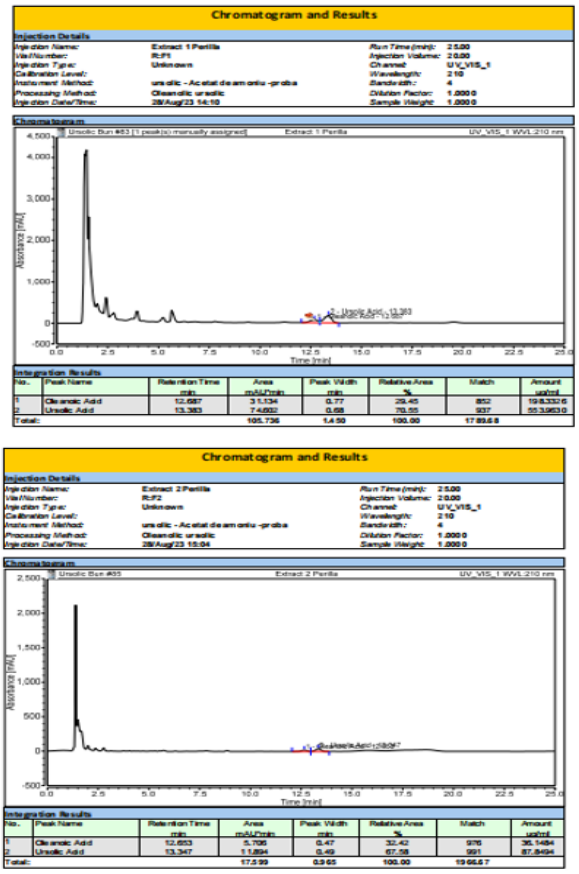


Figure 9: Perilla HPLC chromatograms.

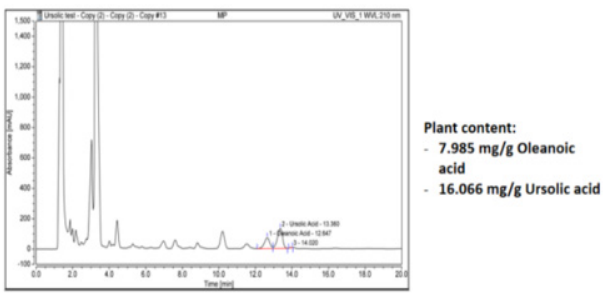


Figure 10: Rosemary plant HPLC chromatograms.

Conclusion

The need to use natural, cheap mineral adsorbents, which do not require their thorough activation and additional processing, has led to this method of purification of plant extracts, simple and without high costs. It is necessary to refine the method as well as to perform a comparative study with the use of macroporous resins as an adsorbent layer or stationary phase. Not all experiments performed in the experimental part yielded the expected (satisfactory) results, especially green leaf extracts could not be purified. The results of this study show that the proposed method is sustainable and economical for purifying plant extracts and obtaining crystals of advanced purity for bioactive compounds in medicinal plants (dry).

Thanks

The authors thank for the financial support received through the Human Capital Operational Program, Priority Axis 6 - Education and Skills, Project: Preparation of PhD students and postdoctoral researchers in order to acquire applicative-SMART research skills, MySMIS Code:153734.

References

1. Zechmeister LLC (1941) Principles and Practice of Chromatography. Made in England ed. John Wiley& Sons Inc. New York.
2. Tswett M (1910) Chromophylls in Plant and Animal World., Warschau. Warshawskago utschebnago Okruga.
3. Stein GAW., 1. Z. physiol. Chem, 1933. 220(247).
4. Stein G, A W, 2 Z physiol Chem (1934) 220(263).
5. Mohler H, W H (1933) 1 Z Unters Lebensm (70(193).
6. Mohler H, W H (1936) 2 Z Unters Lebensm 71(186).
7. Martin AJ, Synge RL (1941) A new form of chromatogram employing two liquid phases: A theory of chromatography. Application to the microdetermination of the higher monoaminoacid in protein. Biochem J 35(12): 1358-1368.
8. https://courseware.cutm.ac.in/wp-content/uploads/2020/06/Adsorbtion-Chromatography-METHODOLOGYpdf, a., Adsorption chromatography.
9. https://chembam.com/definitions/adsorbtion-chromatography/ accesat11.05.2023, Adsorbtion chromatography.
10. Chavan RV, Manohar K, Dhole AR, Sahmkhe VR (2019) A review on Adsorbents in chromatography. International Journal of Scientific Research in Science and Technology 6(3): 36-42.
11. Amuda OS, Ibrahim AO (2006) Industrial Waste Water Treatment Using Natural Material as Adsorbent. African Journal of Biotechnology 5(16): 1483-1487.
12. Kim L, Gina AC, Cernica G, Staicu V, Popescu M, et al. (2021) Removal of Metals from Aqueous Solution Using Sea Buckthorn Waste from Dietary Supplement Technology. Sustainability 13: 1441.
13. https://hatsudy-com/kyuchaku.htmlacces at 22.07.2023, Separation of Materials by Column Chromatography.
14. https://www.qdidactic.com/stiinta-tehnica/tehnica-mecanica/separarea-cromatografica275.phpaccesat2.07.2023, Separarea cromatografica.
15. Europe, C.o., European Pharmacopoeia,7th ed.
16. Kamboj AR, Kaur RD, Jain UK (2015) Application and analysis of the Folin Ciocalteu method for the determination of the total phenolic content from leaves, sterns and seeds of *Cucumis sativus L.* Journal of Pharmacy Research 9(5): 323-329.
17. Marinova D, Ribarova F, Atanassova M (2005) Total phenolics and total flavonoids in Bulgarian fruits and vegetables. Journal of the University of Chemical Technology and Metallurgy 40(3): 255-260.
18. Ozyurek M, Guclu K, Apak R (2011) The main and modified CUPRAC methods of antioxidant measurement. TrAC Trends in Analytical Chemistry 30(4): 652-664.
19. Apak R, *Güçlü* K, Ozy*ü*rek M, Karademir SE (2004) Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. J Agric Food Chem 52(26): 7970-7981.
20. Benzie IF, Strain JJ (1996) The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. Anal Biochem 239(1): 70-76.
21. Benzie IF, Szeto YT (1999) Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay. J. Agri Food Chem 47(2): 633-636.
22. Chen JH, Xia ZH, Tan RX (2003) High-performance liquid chromatographic analysis of bioactive triterpene in Perilla frutescens. Journal of Pharmaceutical and Biomedical Analysis 32(2003): 1175-1179.
23. Nahar L, Guo M, Sarker SD (2020) A review on the latest advances in extraction and analysis of artemisinin. Phytochemical Analysis 31(1): 5-14.
24. Numonov S, Sharopov F, Salimov A, Sukhrobov P, Atolikshoeva S, et al. (2019) Assessment of artemisinin contents in selected Artemisia species from Tajikistan (central Asia). Medicine 6(1): 23.