"BABEȘ-BOLYAI" UNIVERSITY CLUJ-NAPOCA
Faculty of Chemistry and Chemical Engineering
Doctoral School of Chemistry

STUDY OF ISOLATION AND SYNTHESIS OF PHYTOCHEMICAL PRODUCTS OF PHARMACOLOGICAL IMPORTANCE

Summary of Ph.D. thesis

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Key words: betulinic acid, ursolic acid, dipeptides, phytochemical products, biologically active compounds

INTRODUCTION

Motivation of research

There is a particular interest in the use in therapeutics of medicinal plants which contain efficient active principles against cancer and human immunodeficiency virus (HIV). Although in the last years important progress was made in the knowledge and fight against these diseases, there are still enough unelucidated aspects which make the object of specialized research. The high costs and the low availability of current therapies for the majority of population from poor countries determined the carrying out of intense research based on the revaluation of the natural therapeutic potential of medicinal plants and on the use of modern technologies of processing vegetal materials and compounds with biologically active properties.

Across the research conducted at world level in the field of antitumoral and antiviral agents, structural changes were studied or derivatives of compounds from the category of terpenes, to which betulinic and ursolic acids belong, demonstrating that by a change of the "parent" structure we can generate an important number of potential derivatives which significantly improve the profile of selective toxicity. From this point of view, triterpenes represent a promising class of antitumoral and anti-HIV agents, their mode of action being associated to a few steps of the lifecycle of the cell, including merger, reversed transcriptase and their maturation.

Although in last years many synthetic derivatives of the two acids were produced in the attempt to increase their activity, this paper approaches the possibility of obtaining new dipeptide derivatives, capable to protect cellular membrane and inhibit the development and maturation of tumoral formations, blocking the formation of intercellular connections and becoming thus inhibitors of merger.

Purpose and objectives of this work

This work aims to carry out a theoretical and experimental study on the possibility of obtaining semi-synthesis structures, derivatives of natural compounds with bioactive properties and which can be later used as forerunners of preparations with antitumoral and antiviral properties. The predecessors of derivatives are two organic acids, betulinic acid and ursolic acid, both with bioactive properties which are part of the pentacyclic triterpenes class, being related both by their chemical structure and by their therapeutic effects.

The points of reference and the objectives pursued in this study are:

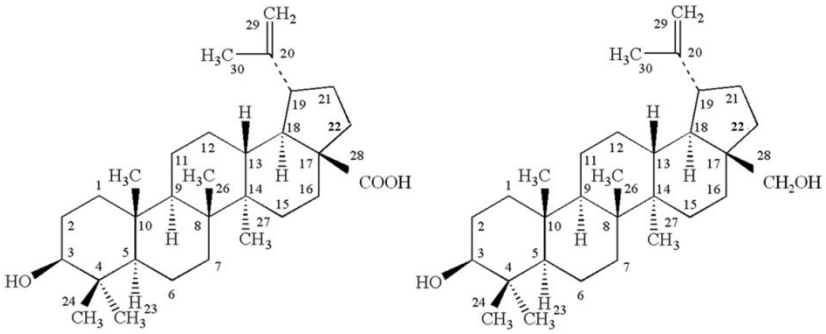
- Extraction of betulinic acid, respectively the ursolic acid from a number of three species of plants present in our spontaneous flora and empirically used: Betula pendula, Roth, (Betulaceae), (White Birch), Calluna vulgaris (L) Hull, (Ericaceae), (Black Grass) and Bruckenthalia Spiculifolia, Salisb., (Ericaceae), (mountain gooseberry bush);
- Use of techniques of natural and artificial drying by convection and in microwave field for fresh vegetal materials and the conducting of a comparative study in view of selecting the optimal drying procedure;
- General presentation of the solid-fluid extraction process and of the main factors which influence this process;
- Phenomenological study of the solid-fluid extraction process used for the recovery of acids from vegetal material;
- Proposal of a model based on dimensional analysis, starting from the phenomenology of the extraction process and its checking by experimental way;
- Derivatization of betulinic and ursolic acids in view of obtaining new biologically active products, by their linking by chemical way to compounds from the dipeptide class: Glycyl-Leucine, alanyl-glycine, respectively leucyl-glycine, structures which are naturally found in the composition of biological fluids, which fulfil a role of vector for active component, towards the areas or tissues infected or affected by some tumoral formations;
- Investigation of the linking procedure and testing of linked structures on several types of carcinogenic/tumoral cells, for the purpose of highlighting the biological activity of synthesized compounds and their therapeutic effects.

DATA FROM LITERATURE

3. VIEWS ON THE NATURAL BIOLOGICALLY ACTIVE COMPOUNDS AND THE VEGETAL SPECIES TARGETED AS RAW MATERIAL FOR THEIR ISOLATION

3.1. Betulinic acid

(3β)-3-hidroxi-20(29)-lupen-28-oic acid or betulinic acid, ($C_{30}H_{48}O_3$), (Fig. 3.1.1.a), is a natural compound derivative of betulin, ($C_{30}H_{50}O_2$), (Fig. 3.1.1.b.), a pentacyclic triterpene which is found abundantly in the peel of birch bark, (Betula pendula, Betula costata, Betula ermanii), [1].



a. **b.**

Fig. 3.1.1. a. Betulinic acid **b.** Betulin

3.1.1. Pharmacodynamic activity of betulinic acid

A wide variety of medicinal properties and biological actions were attributed to betulinic acid, including anticarcinogenic antitumoral properties, [2], anti-inflammatory properties, [3], antimicrobial properties, [4], anti-ischemic properties, [5] antimalarial properties, [6], anti-HIV properties, [7], anti-melanoma properties, [8], cytotoxic properties specific for pulmonary carcinoma, [9], for colon carcinoma, [9] and for neural ectodermic tumours, [10]. Moreover, the compound also develops an antiangiogenic action, [11], antileukaemic and antilymphomatic action, [12], being a good inhibitor of cerebral malignant tumours, [10] by direct activation of apoptosis at mitochondrial level, [13].

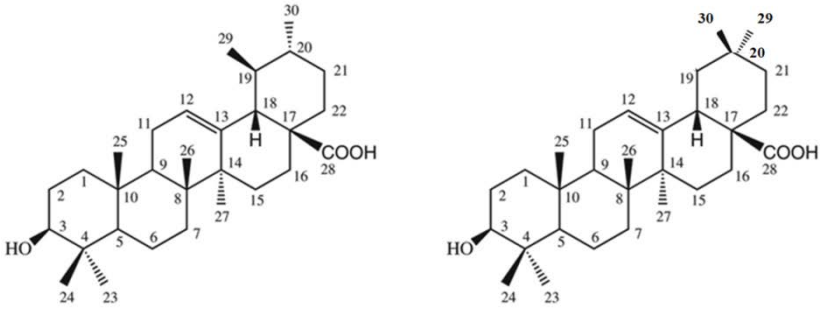
The capacity of betulinic acid to induce apoptosis both in melanoma and its therapeutic index favourable to a low toxicity towards normal cells gives it a high attractiveness, being a promising antitumoral agent.

3.1.2. Synthesis of compounds derivative of betulinic acid

Across the research conducted at world level in the field of antitumoral agents, several structural changes and derivatizations of betulinic acid have been studied, [14], demonstrating that by a simple change of the parent structure we can generate an important number of potential derivatives which significantly improve the profile of selective toxicity, [15] or which can induce a general toxic effect on leukemic cells, [15]. Betulinic acid presents three positions: C3, C20 and C28 where one can make chemical changes in view of obtaining derivatives with important antitumoral activity.

3.2. Ursolic acid

(3β)-hydroxy-urs-12-en-28-oic acid or ursolic acid, ($C_{30}H_{48}O_3$), (Fig. 3.1.1.a), is a hydroxy-pentacyclic triterpene which manifests a good chemoprotective activity for the human body, is widely spread in nature and is isolated from different extracts of plants, alone or conjugated with sugars. Along with its isomer, (3β)-hydroxy-olean-12-en-28-oic acid or oleanolic acid, (Fig. 3.2.1.b.), it is found in numerous plants, including those which have been used for a long time in traditional medicine, [16].



a. **b.**

Fig. 3.2.1. a. Ursolic acid **b.** Oleanolic acid

3.2.1. Pharmacodynamic activity of ursolic acid

Ursolic acid presents remarkable pharmacological properties, the most important ones consisting of antihyperlipidemic effects, [17], anti-inflammatory, [18], hepatoprotective, [19], inhibitor of processes of initiation and proliferation of tumours, [20], analgesic, [21], cardiotonic, [22], sedative and anxiolytic, [23], antiulcer, [24], antimicrobial, [25], hypoglycemiant, [26], antiatherosclerotic, [27], protective against toxicity induced by cyclophosphamides, [28], and anticariogenic, [29], but also tonic and modulating effects of collagen synthesis, [30]. As natural anti-inflammatory and antitumoral agent, the acid acts by the inhibition of tumoral promoters and inflammatory processes due to the presence of tumours.

3.2.2. Synthesis of compounds derivative of ursolic acid

Just like in case of betulinic acid, the ursolic acid presents three positions: C3, C20 and C28 which following chemical changes provided a series of new derivatives with significant biological properties. Following the investigation of a number of over 70 compounds and synthetic derivatives of ursolic acid, we noted the frequency of their biological action of inhibition of proliferation and development of tumoral cells, being considered potential chemo-preventive agents in various types of cancer, [31].

3.3. Vegetal material used as raw material in view of isolation of betulinic acid

In view of extraction of betulinic acid, we used the species Betula pendula Roth, (Betulaceae), a tree which is found under the name of birch or white birch and is specific to hilly areas up to subalpine floor, but it can also grow in plains or grow in the mountain areas up to 1500 m on sunny coasts.

3.4. Vegetal materials used as raw material in view of isolation of ursolic acid

The extraction of ursolic acid was achieved from the species Calluna vulgaris (L) Hull, (Ericaceae), also known as the popular names of *black grass, black currant, and gooseberry*, [32], specific to sub-mountain regions and alpine regions and from the species Bruckenthalia Spiculifolia, Salisb. (Ericaceae), also knows under the popular name of *mountain gooseberry bush*, specific to open or forested areas from the mountain region to the subalpine region.

ORIGINAL CONTRIBUTIONS

6. EXTRACTION OF BETULINIC AND URSOLIC ACIDS FROM VEGETAL MATERIALS AND THEIR CHARACTERIZATION

For the realization of a more efficient separation of biologically active compounds from vegetal matrix, we observed certain technological stages and determined the optimal conditions of work. In this respect, we conceived a scheme of operations in which the work stages are presented in the order of their succession, (Fig. 6.1.1) and applied to the extraction of betulinic and ursolic acids from the vegetal species Betula pendula Roth, (Betulaceae), Bruckenthalia Spiculifolia, Salisb., (Ericaceae) and Calluna vulgaris (L) Hull, (Ericaceae).

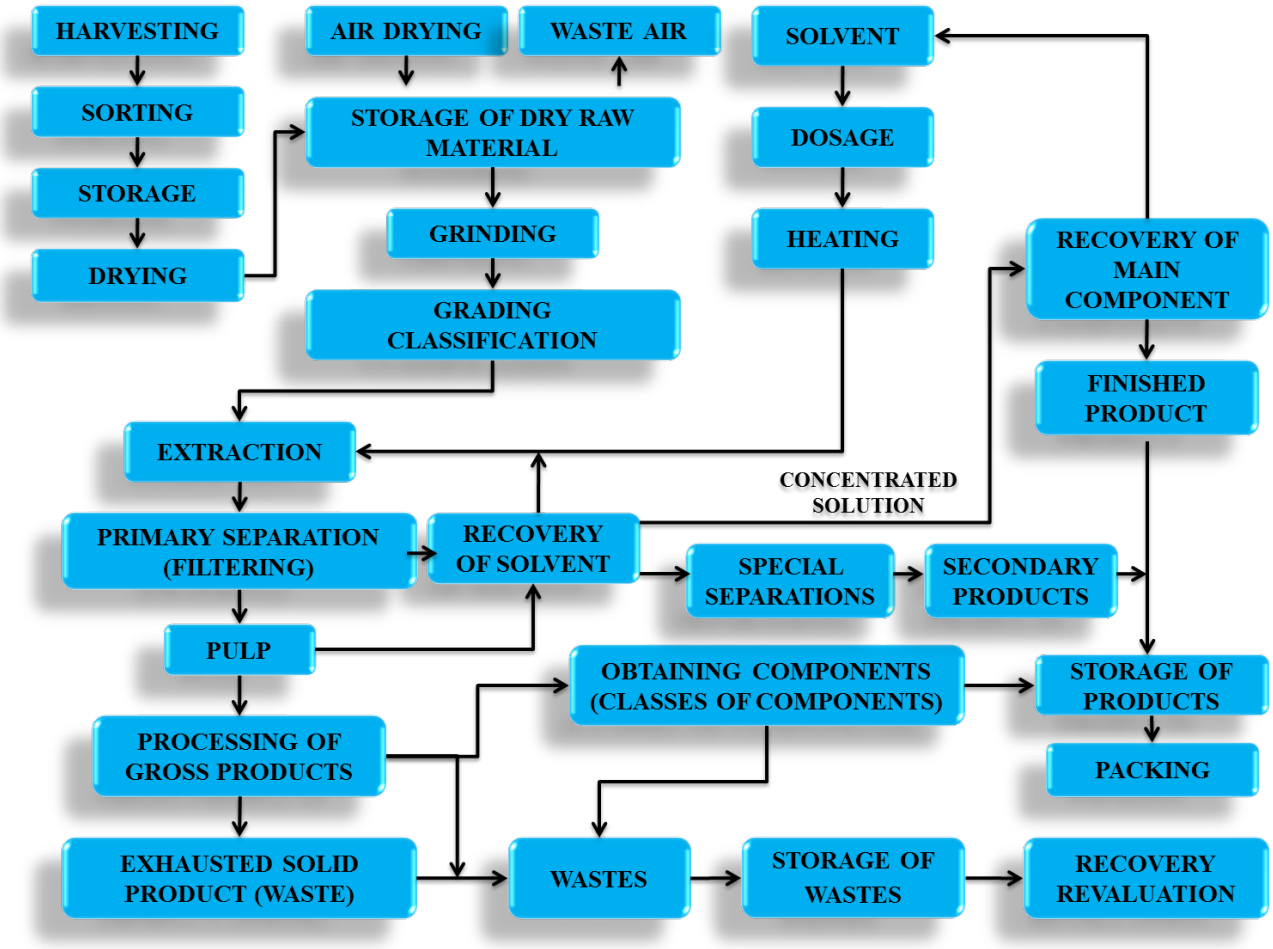


Fig. 6.1.1. General scheme of operations corresponding to the separation process

6.1.3. Drying

The drying of the vegetal material was done immediately after sorting to prevent the triggering of degradation processes, as the process was done by two ways, natural drying and artificial drying.

6.1.3.1. Natural drying

The natural drying was performed immediately after harvesting and sorting, in a well aerated room away from the direct action of sun rays. Throughout the process, the vegetal material was weighed every day, and the room was constantly monitored from the thermal and humidity point of view. We noted after the completion of the process that the drying speed is strongly influenced by the water content from plants, but also by the form, respectively sizes of the vegetal material.

6.1.3.2. Artificial drying

The artificial drying was performed in two ways:

- by convection, in a space provided with a heating and aeration system, using for this purpose a drying closet with shelves;
- in microwave field, in a space provided with a microwave generation system and with ventilation.

In both cases, during the whole period of the process the vegetal material was regularly weighed and constantly monitored from the point of view of morphological aspect.

At drying by convection, after the termination of the drying process and the examination of the morphological aspect, we did not notice any visible degradation of the vegetal product, which did not change colour and smell, but only changed form and sizes.

For the drying in microwave field, we considered as important for the process the following parameters: temperature, humidity of material, power applied to microwaves, the time of exposure to radiative field and morphological evolution of the vegetal material during the process under the action of the microwave energy.

Following the carrying out of the comparative study regarding the drying of vegetal materials by the three methods, *free drying, drying in drying closet and drying in microwave field*, we obtained experimental data which allowed the elevation and interpretation of drying curves for each vegetal species. The variation of the weight of vegetal material after the loss of water by the three drying methods, for each species is represented in the figures below, (Fig. 6.1.3-6.1.5).

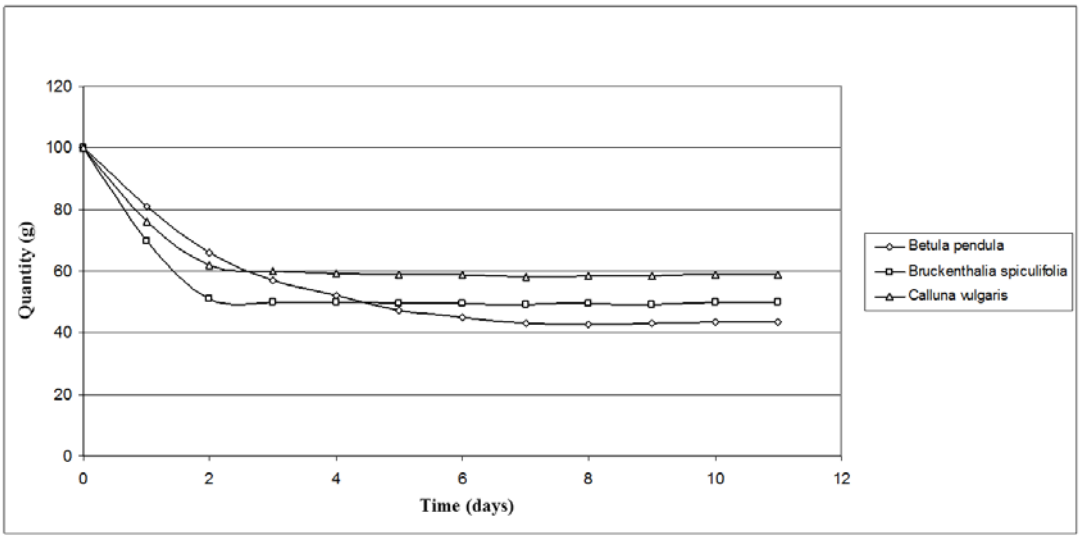


Fig. 6.1.3. Variation of weight of vegetal material following the loss of water by natural drying

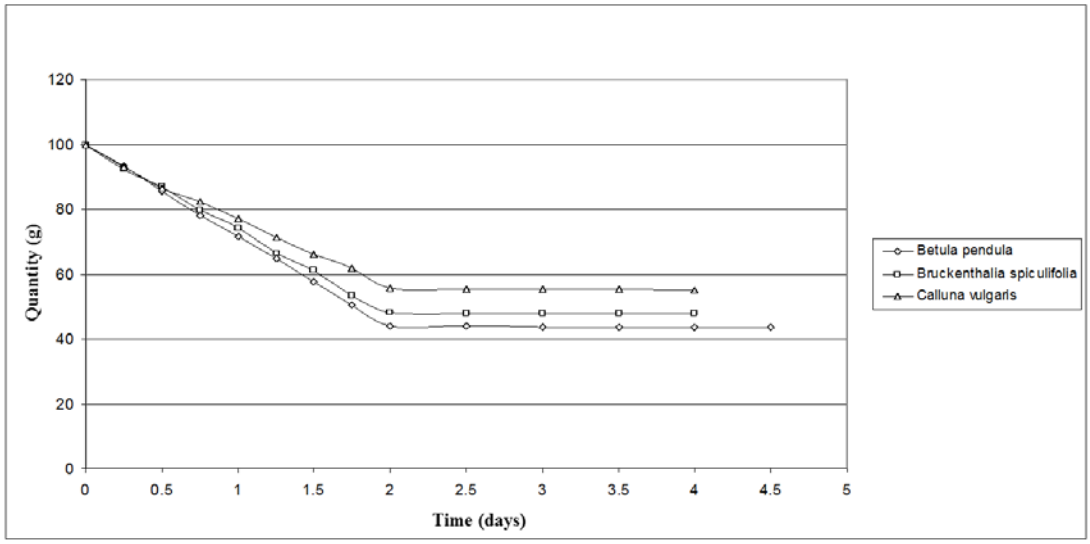


Fig. 6.1.4. Variation of weight of vegetal material following the loss of water by convective drying

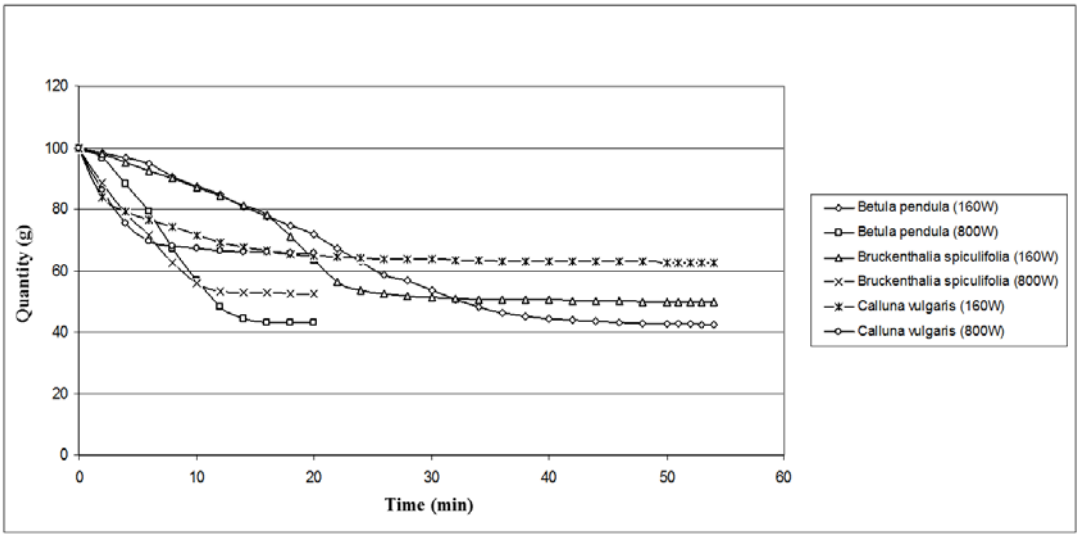


Fig. 6.1.5. Variation of weight of vegetal material in microwave field at 160 W and 800 W

The selection of the optimal drying method for each vegetal species processed was done after the carrying out of the comparative study regarding the drying techniques used and after the interpretation of experimental data, drying diagrams and after considering the advantages and disadvantages involved by each of the drying methods experimentally applied, (Table 6.1.20).

Table 6.1.20. Parameters for the selection of the optimal method of drying vegetal materials

SPECIES	FREE DRYING	CONVECTIVE DRYING	DRYING IN MICROWAVE FIELD
Betula pendula, (Betulaceae)	Duration of process: 8 days Decrease in volume: 43.62% Water loss: 56.38% Yield: 42.31%	Duration of process: 2.5 days Decrease in volume: 43.50% Water loss: 56.50% Yield: 43.50%	Power: 160 W Duration: 50 minutes Decrease in volume: 42.50% Water loss: 57.50% Yield: 42.58%
			Power: 800 W Duration: 16 min Decrease in volume: 43.10% Water loss: 56.90% Yield: 43.12%
Bruckenthalia Spiculifolia, (Ericaceae)	Duration of process: 4 days Decrease in volume: 50.04% Water loss: 49.96% Yield: 49.06%	Duration of process: 2.5 days Decrease in volume: 47.70% Water loss: 52.30% Yield: 47.70%	Power: 160 W Duration: 42 minutes Decrease in volume: 49.70% Water loss: 50.10% Yield: 49.73%
			Power: 800 W Duration: 14 min Decrease in volume: 52.50% Water loss: 47.50% Yield: 52.57%
Calluna vulgaris, (Ericaceae)	Duration of process: 5 days Decrease in volume: 58.74% Water loss: 41.26% Yield: 58.11%	Duration of process: 2.5 days Decrease in volume: 55.11% Water loss: 44.89% Yield: 55.11%	Power: 160 W Duration: 40 minutes Decrease in volume: 62.60% Water loss: 37.40% Yield: 62.68%
			Power: 800 W Duration: 14 min Decrease in volume: 65.50% Water loss: 34.50% Yield: 65.52%

For the characterization of raw material we made a grading study on the dry vegetal material. The grading analysis was performed by sifting with standardized sieves, indicated by Romanian Pharmacopeia and the sifted material was then weighed separately by grading fractions. Based on the results obtained, we drew the histogram of grading distribution in steps, (Fig. 6.1.13).

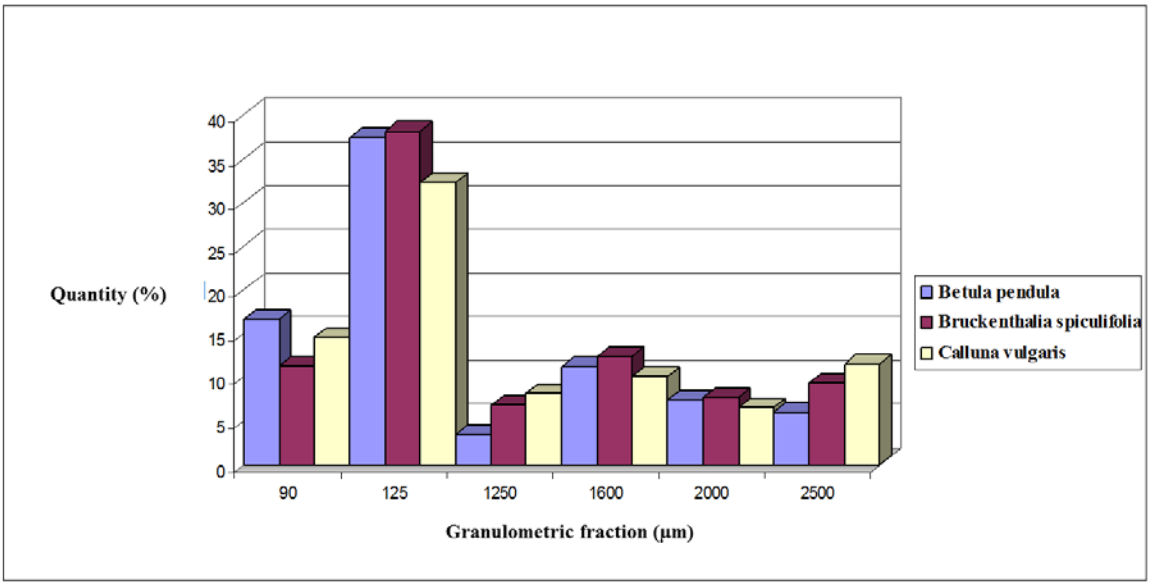


Fig. 6.1.13. Grading distribution after the sifting of vegetal materials

6.2. Extraction of betulinic and ursolic acids

The technologies of obtaining and concentration of bioactive products from the species Betula pendula Roth, (Betulaceae), Bruckenthalia Spiculifolia, Salisb., (Ericaceae) and Calluna vulgaris, (L.) Hull, (Ericaceae) were elaborated based on both classical procedures of extraction and modern methods of separation based on fields of radiative sources in inert natural media, of the type of radiations with microwaves and ultrasonic radiations, (Table 6.2.1.).

Table 6.2.1. Experimental methods used in the isolation of betulinic and ursolic acids

VEGETAL SPECIES	SEPARATION METHOD	SOLVENT	COMPOUND
Betula pendula, (Betulaceae)	Maceration	Ethylic alcohol	Betulinic acid
	Simple extraction with multiple contact in normal conditions		
	Extraction by ultrasounds		
	Extraction in microwave field		
Bruckenthalia Spiculifolia, (Ericaceae)	Maceration	Ethylic alcohol	Ursolic acid
	Extraction by ultrasounds		
	Extraction in microwave field		
Calluna vulgaris, (Ericaceae)	Maceration	Ethylic alcohol	Ursolic acid
	Extraction by discontinuous procedure		
	Extraction by ultrasounds	Petroleum ether, ethylic ether, methylene chloride	Ursolic acid
	Extraction in microwave field		
	Extraction by Soxhlet method		

6.2.1. Extraction of betulinic acid from the species Betula pendula, (Betulaceae)

The extraction procedure aimed at obtaining extracts from the dry birch leaves, in which as few co-extracted compounds as possible are found along with betulinic acid, in the conditions imposed by multiple contact, maceration, the ultrasound field and the microwave field. As solvent we used a watery solution of ethylic alcohol, having different concentrations, of 30, 50, 60, 70, 80 and 95% in volumic percentages.

6.2.1.1. Extraction of betulinic acid by procedure with multiple contact

The extraction was performed at room temperature, and the determinations were done refractometrically up to a constant index which reflects the attainment of balance in working conditions, following which we noted that the most effective solution is the solution with 70% concentration, (Fig. 6.2.1.4).

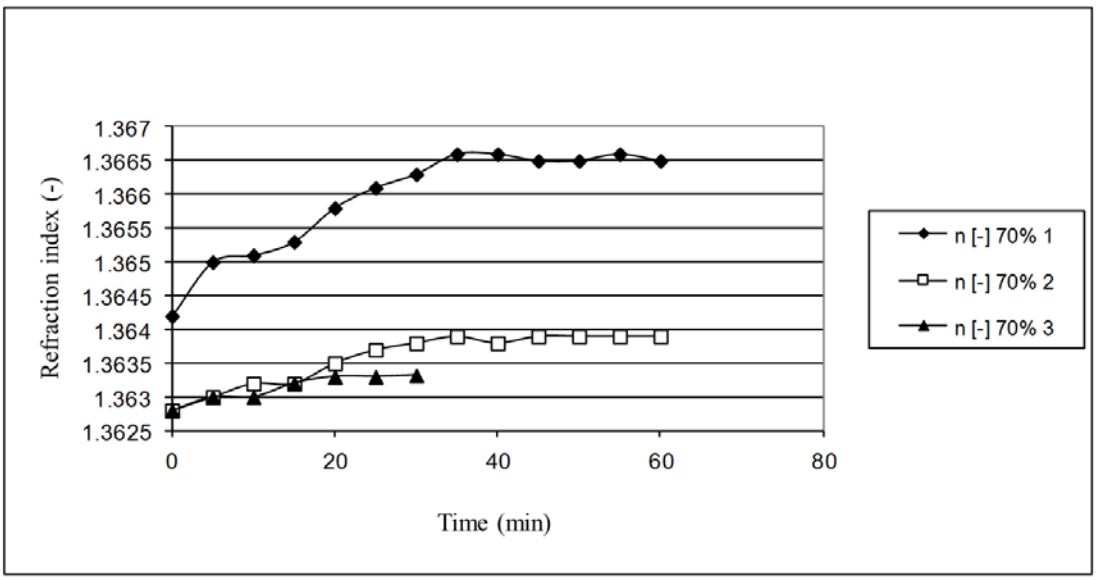


Fig. 6.2.1.4. Variation of refraction index with contact time

6.2.1.2. Extraction of betulinic acid by maceration

The study regarding the extraction of betulinic acid by maceration was performed by watching the influence of several parameters on the process, (Fig. 6.2.1.5-6.2.1.7). The highest efficiency at extraction was represented by the 95% concentration of ethanol-water solvent for a ratio L:S (10:1), [ml:g] and grading fraction – 1250 µm.

6.2.1.3. Extraction of betulinic acid in the presence of ultrasounds

We studied the influence of several parameters on extraction, (Fig. 6.2.1.12 – 6.2.1.16) and the evolution of the process was watched with refractometer up to constant index. We determined the optimal parameters of the process, respectively the fraction + 125 µm, the ratio L:S of 10:1 (ml:g), the solvent concentration of 80%, the temperature of 60ºC and the contact time of 55 minutes.

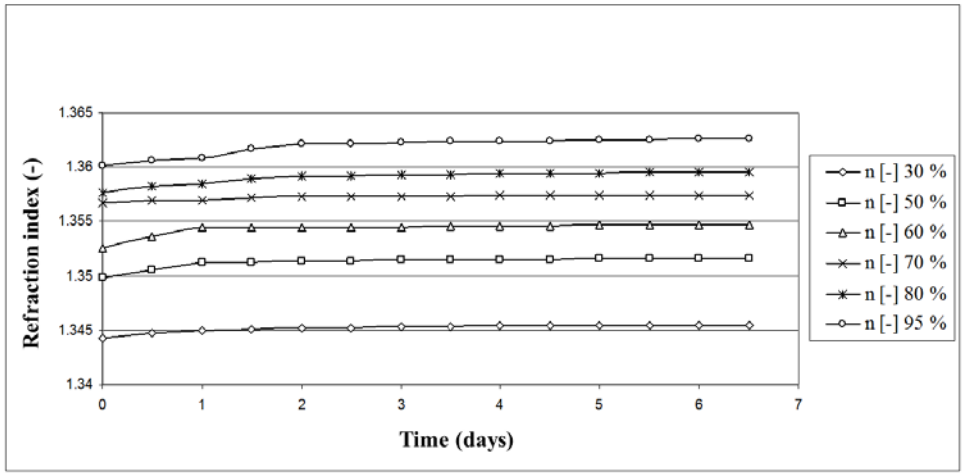


Fig. 6.2.1.5. Influence of solvent concentration on the refraction index

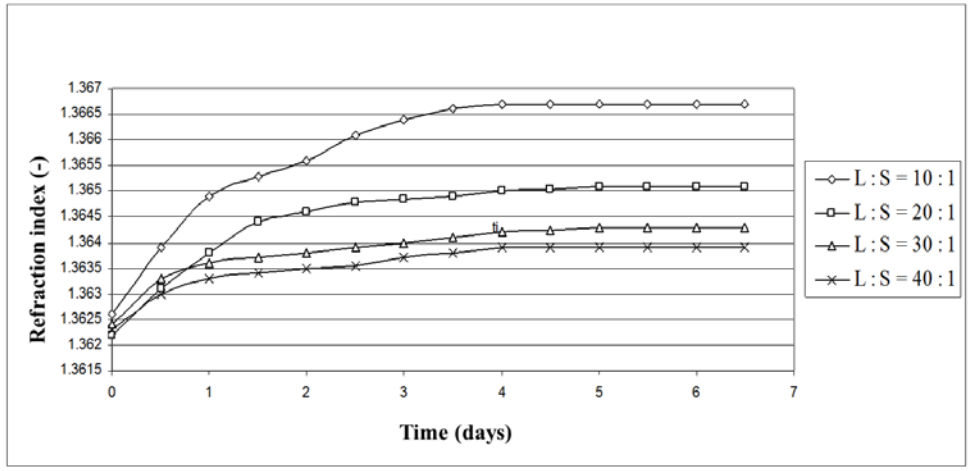


Fig. 6.2.1.6. Influence of fluid-solid ratio on the refraction index

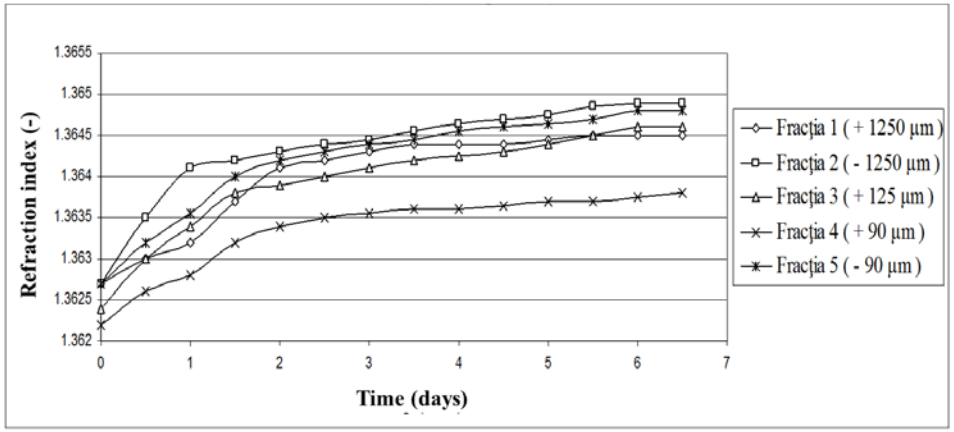


Fig. 6.2.1.7. Influence of the size of grains on the refraction index

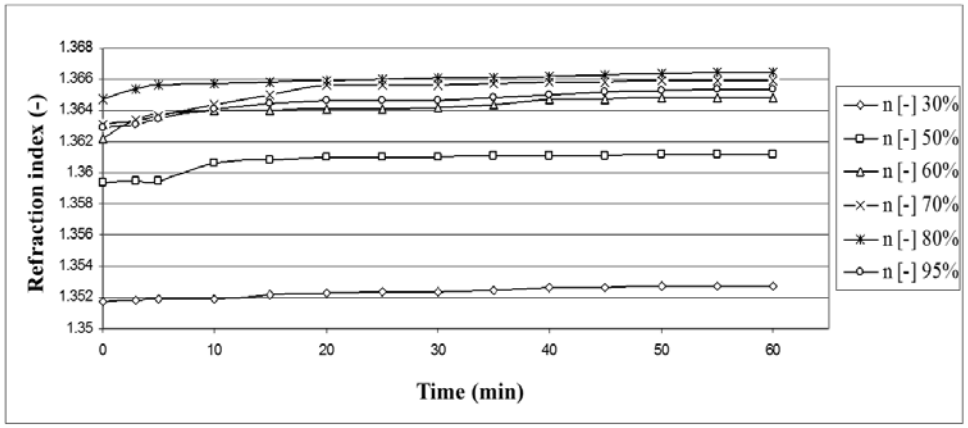


Fig. 6.2.1.12. Influence of solvent concentration on the refraction index

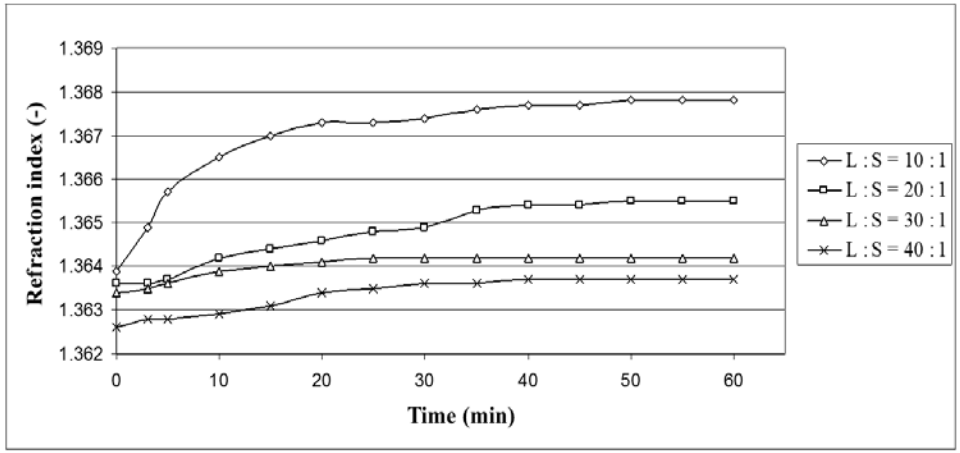


Fig. 6.2.1.13. Influence of fluid-solid ration on the refraction index

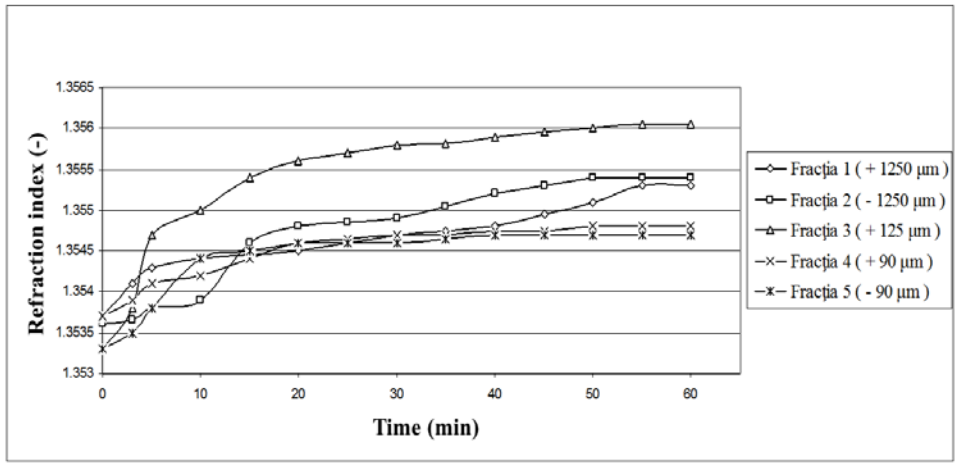


Fig. 6.2.1.14. Influence of the size of grains on the refraction index

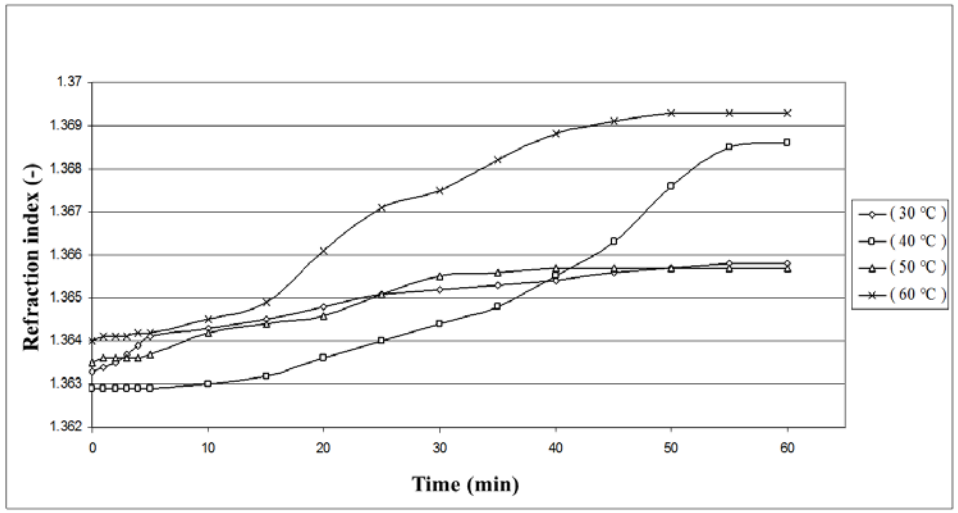


Fig. 6.2.1.15. Influence of temperature on the refraction index

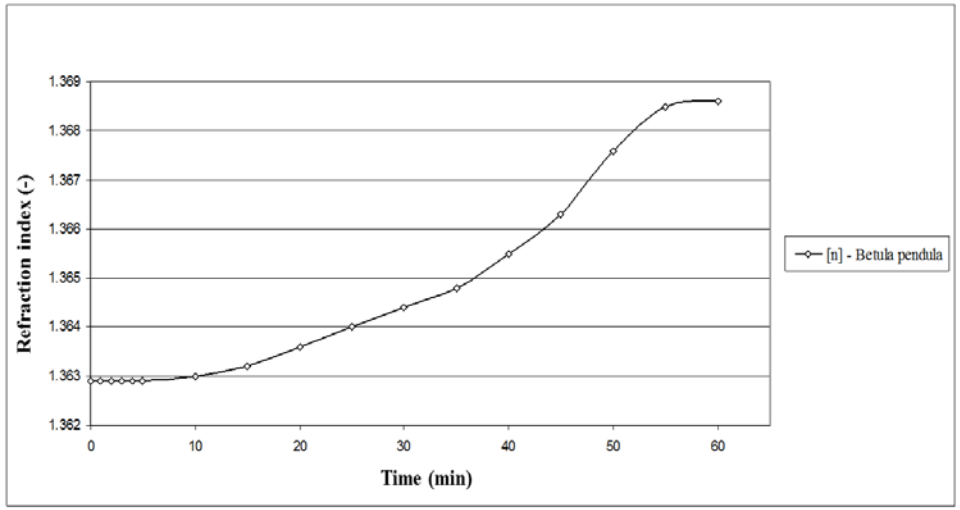


Fig. 6.2.1.16. Influence of contact time on the refraction index

6.2.1.4.Extraction of betulinic acid in microwave field

The study on the extraction of betulinic acid from the birch leaves by the procedure of extraction in microwave field was carried out by watching the influence of several parameters on the process. For carrying out the experiments we resorted to the working variant in compact volume. We determined the optimal parameters for the carrying out of the process, the power of microwaves at Tr H, 800 W, ratio L:S, (10:1), [ml:g], the concentration of ethanol watery solution of 80%, the temperature 60ºC, grading fraction -1250 μm and the duration of the process of 3.5 minutes, (Fig. 6.2.1.22-6.2.1.25).

6.2.2. Extraction of ursolic acid from the species Bruckenthalia Spiculifolia, (Ericaceae)

For the extraction of ursolic acid from the species Bruckenthalia Spiculifolia we used as methods of separation, the extraction by maceration, the extraction with the aid of ultrasonic waves and the extraction in microwave field. The experiments were run in the same conditions as in case of species Betula pendula, (Betulaceae), the difference from the previous case consisted of the form, composition and structure of the solid matrix, of the vegetal material, made up of leaves, flowers and young stems and used in the process under the form of a heterogeneous mixture. We studied the influence of the concentration of ethanol-water solvent, the solid-fluid ratio, the size of the vegetal material, the operation time and the temperature on extraction.

6.2.3. Extraction of ursolic acid from the species Calluna vulgaris (L) Hull, (Ericaceae)

The extraction of ursolic acid from the species Calluna vulgaris, (Ericaceae) was performed by using several techniques of separation, discontinuous extraction, maceration, extraction assisted by ultrasounds and the extraction in microwave field. The experiments were carried out in the same conditions as in the case of species Betula pendula, (Betulaceae) and Bruckenthalia Spiculifolia, (Ericaceae).

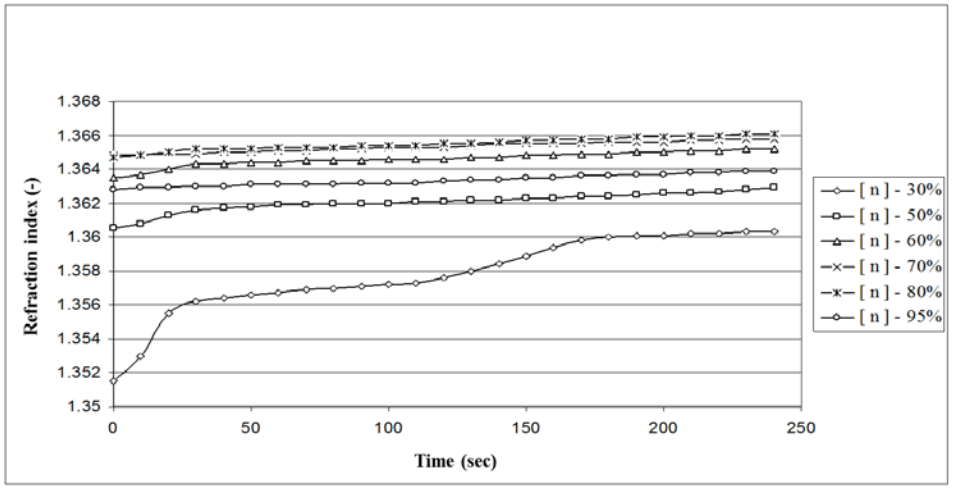


Fig. 6.2.1.22. Influence of solvent concentration on the refraction index

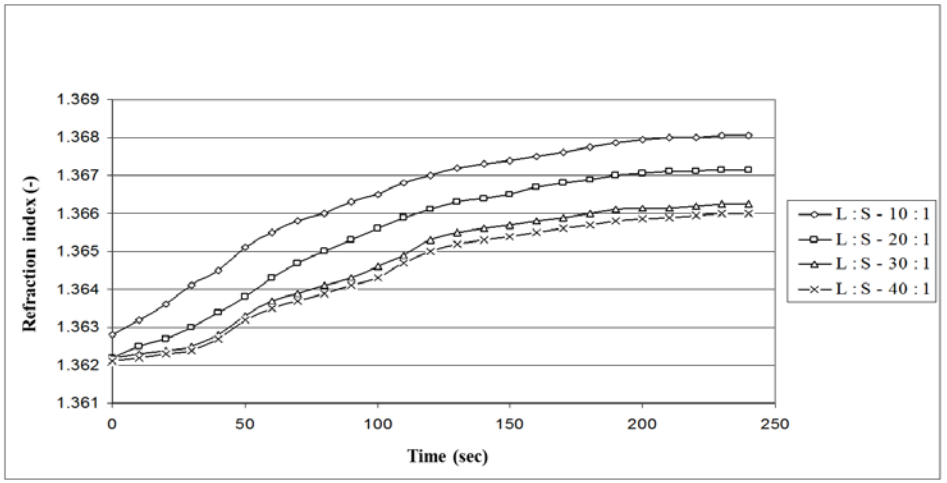


Fig. 6.2.1.23. Influence of L:S ratio on the refraction index

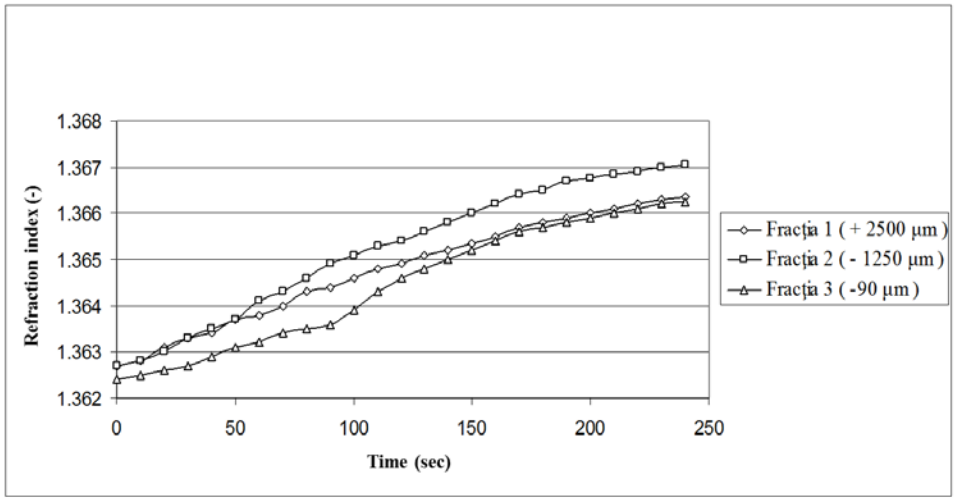


Fig. 6.2.1.24. Influence of the size of grains on the refraction index

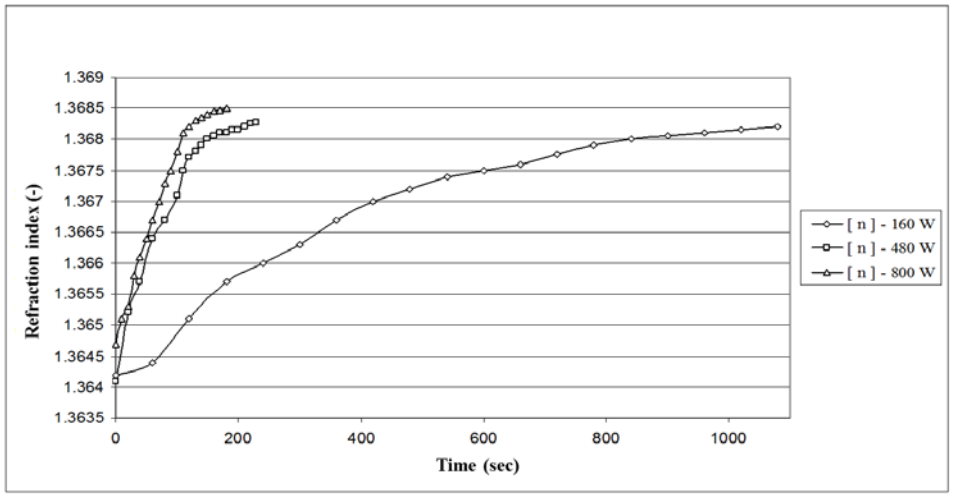


Fig. 6.2.1.25. Influence of radiative power on the refraction index

6.3. Separation, identification and quantification of betulinic and ursolic acids from vegetal extracts

In the experimental study, both qualitative analysis and the quantitative analysis of betulinic and ursolic acids were carried out by the reversed phase high performance liquid chromatography, (RP-HPLC), the method offering a good selectivity and sensitivity to the identification and quantification of the two compounds. In Fig. 6.3.1 and 6.3.2, we illustrated the chromatograms of standard solutions for the compounds of interest and the chromatograms of samples of vegetal extracts, analysed in the same chromatographic conditions.

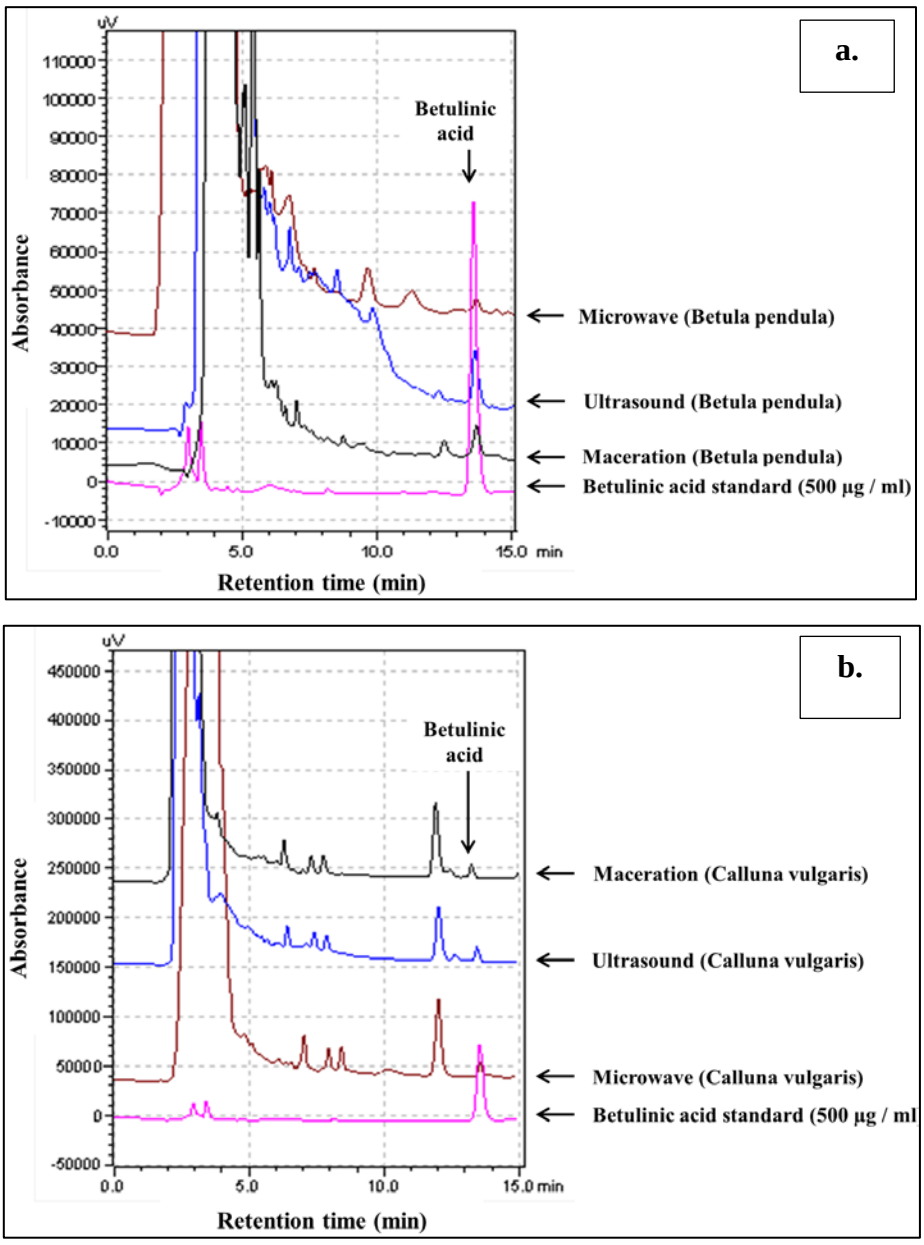


Fig. 6.3.1. Overlapped chromatograms corresponding to the standard solution of betulinic acid and samples of vegetal extract. a. Species Betula pendula, b. Species Calluna vulgaris

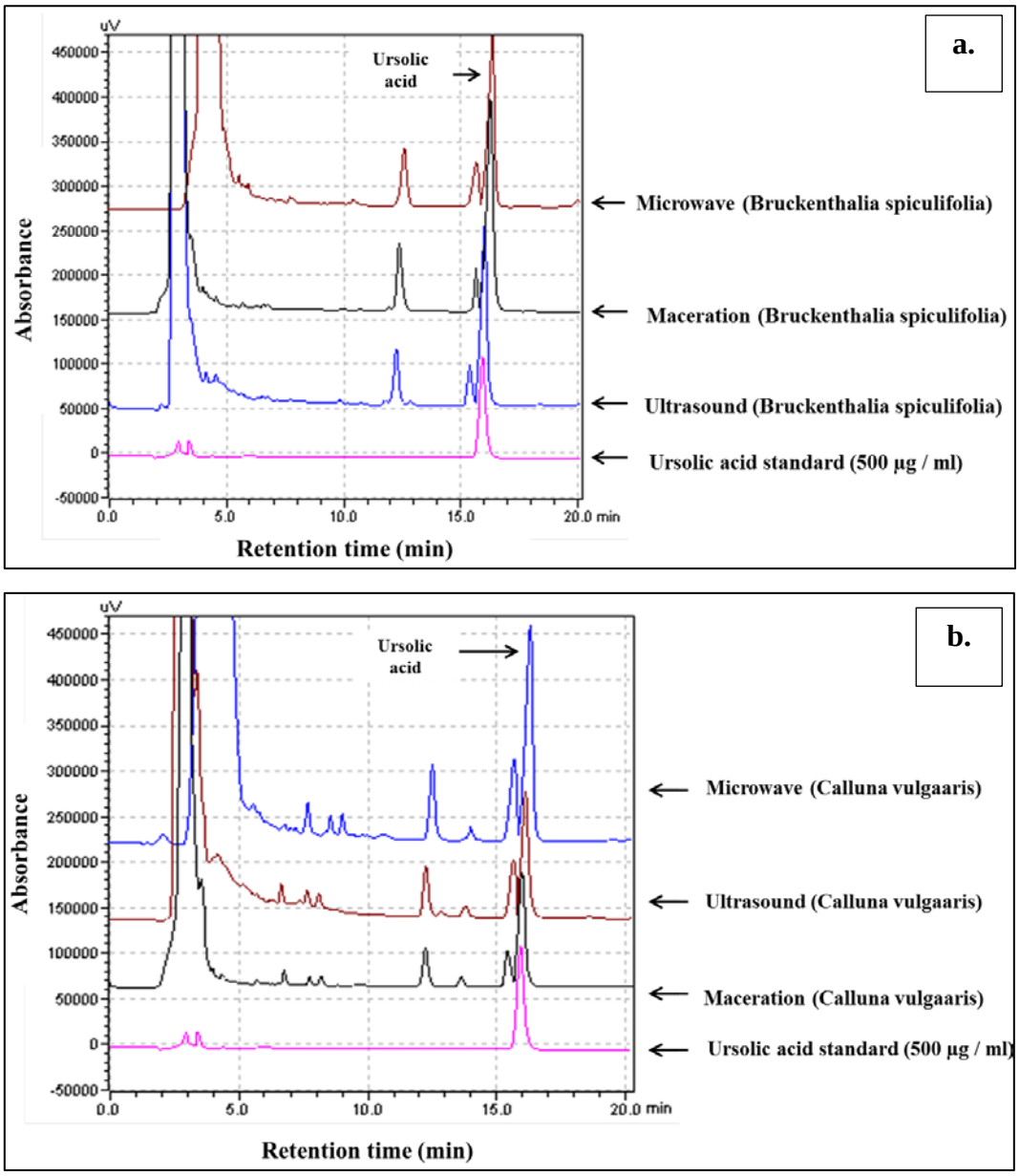


Fig. 6.3.2. Overlapped chromatograms corresponding to the standard solution of ursolic acid and samples of vegetal extract. a. Species Bruckenthalia Spiculifolia b. Species Calluna vulgaris

In order to highlight the optimal conditions of extraction of the studied acids, the results obtained were graphically presented under the form of Boxplot diagrams, (Fig. 6.3.7. - 6.3.8.).

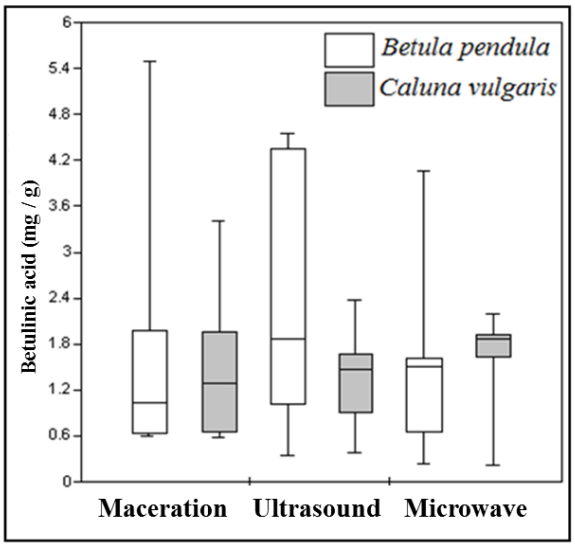


Fig. 6.3.7. Efficiency of extraction of betulinic acid from the species Betula pendula and Calluna vulgaris

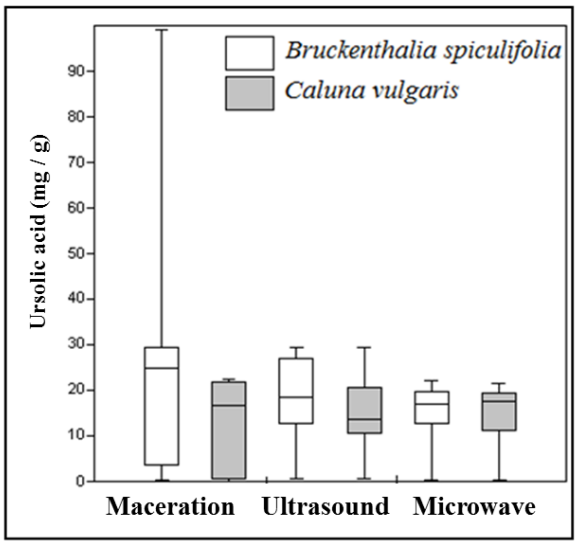


Fig. 6.3.8. Efficiency of extraction of ursolic acid from the species Bruckenthalia Spiculifolia and Calluna vulgaris

The concentration of betulinic acid in the vegetal extract differs considerably depending on the technology applied for its extraction from the vegetal matrix. In case of maceration, except for some isolated situations when high concentrations of betulinic acid were recorded, in general the concentrations varied between 0.64 – 1.97 [mg / g] with an average value of 1.03 [mg / g] for Betula pendula, respectively between 0.66 – 1.96 [mg / g] with an average value of 1.28 [mg / g] for Calluna vulgaris. In case of extraction with ultrasounds, the concentrations increased considerably for Betula pendula, a large part of concentrations ranging between 1.02 – 4.35 [mg / g], with an average value of 1.87 [mg / g]. The extraction in microwave field has improved the extraction yield from Calluna vulgaris, the concentrations of betulinic acid ranging in this case between 1.62 - 1.92 [mg / g] with an average value of 1.87 [mg / g]. In case of ursolic acid, the optimal conditions of extraction turned out to be the extraction by maceration, between 3.71 – 29.41 [mg / g], with an average value of 24.84 [mg / g], in case of extraction from Bruckenthalia Spiculifolia, respectively by microwaves, in case of extraction from Calluna vulgaris, between 11.31 – 19.52 [mg / g], with an average value of 17.55 [mg / g]. The extraction by ultrasounds had a low yield for ursolic acid, obtaining concentrations between 0.58 – 29.54 [mg / g], with an average value of 18.41[mg / g] for Bruckenthalia Spiculifolia, respectively between 0.58 – 29.48 [mg / g], with an average value of 13.63 [mg / g] for Calluna vulgaris.

6.4. Experimental study of solid-fluid extraction by phenomenological analogy

The proposed model can apply to any method of extraction studied above and for vegetal materials with different structures and forms. The hydraulic integrator is the experimental device which can make the analogy between two phenomena and can be successfully used in the simulation or modelling of fast processes or cases in which the studied material has reduced sizes, the case of the leaf. The variation of the solute concentration from inside the leaf during the process can be seen in Fig. 6.4.2.

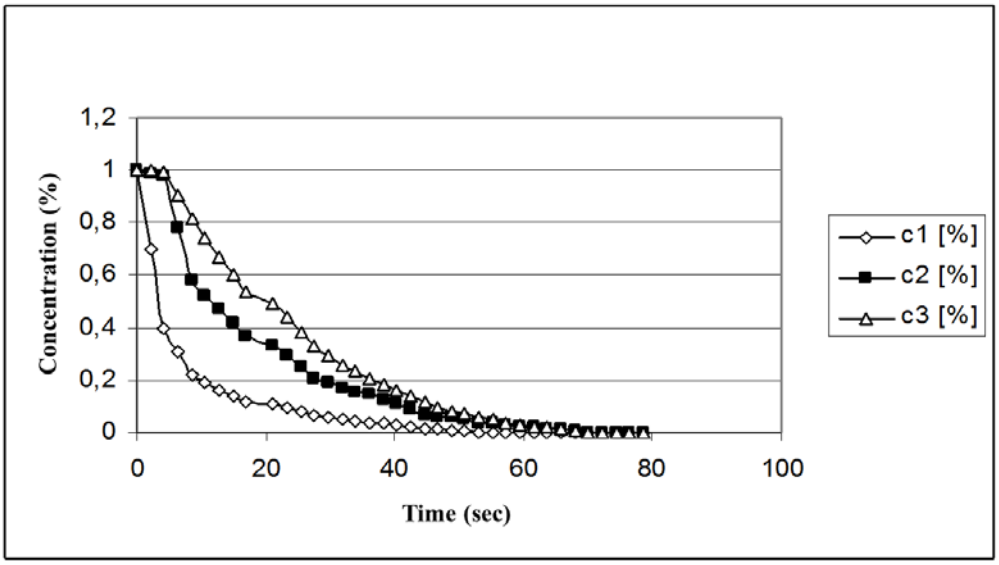


Fig. 6.4.2. Evolution of solute concentration during extraction

Chapter 7. OBTAINING SYNTHESIS DERIVATIVES OF BETULINIC AND URSOLIC ACIDS

7.1. Synthesis of substitute derivatives of betulinic acid

The dipeptide derivatives of betulinic acid were obtained by its condensation with alanyl-glycine, glycyl-leucine and leucyl-glycine by the diagram from Fig. 7.1. The synthesis of betulinic derivatives implied the performance of a series of reactions carried out in four successive stages, among which the first two, respectively the obtaining of intermediary products, (1a) and (2a), are common as method of work with the obtaining of the three betulinic derivatives, (4), (6) and (8).

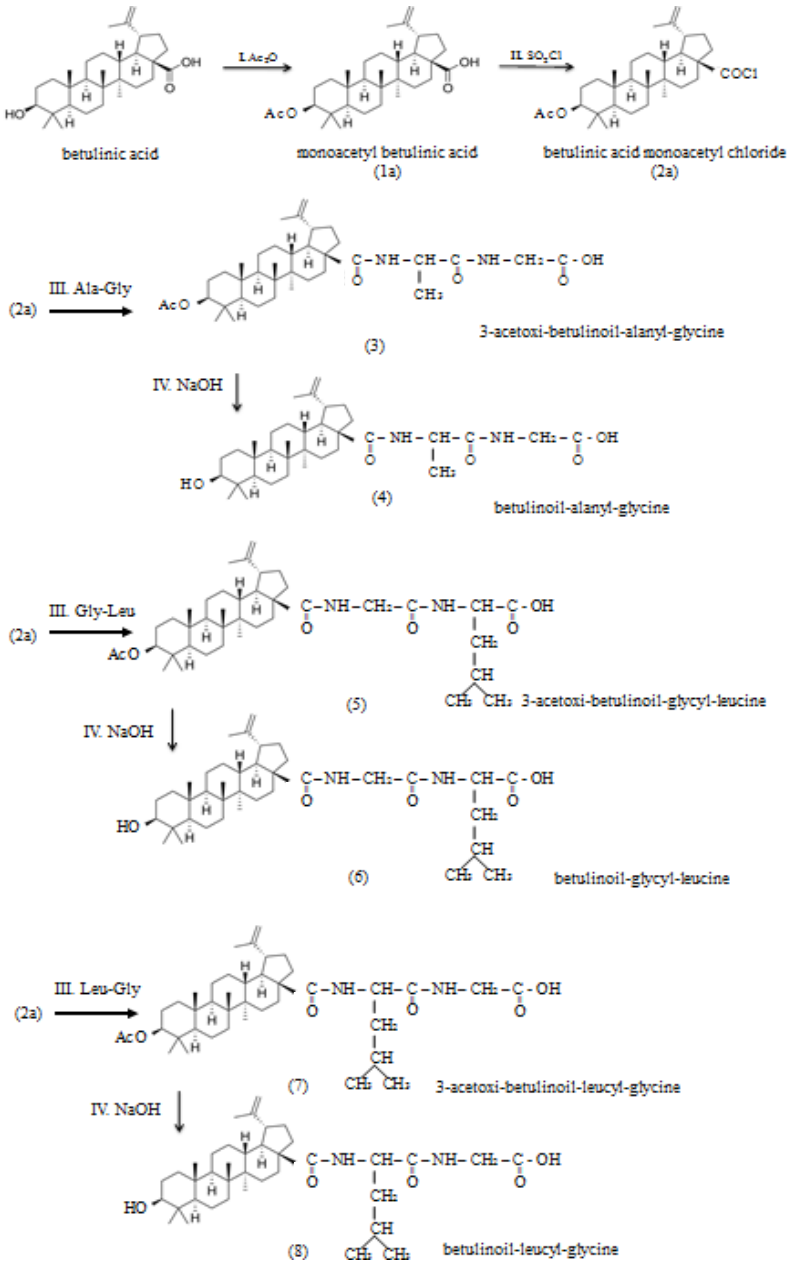


Fig. 7.1. Diagram of synthesis of betulinic derivatives

The compounds (4), (6) and (8) were characterized by two analytical methods, mass spectrometry, (CG-MS) by electronic impact (EI) and spectroscopy in infrared field, (IR). In the figure below, (Fig. 7.1.5) we illustrated the mass spectrum for the betulinol-glycyl-leucine compound, (6) which was tested from the point of view of bilogical effect.

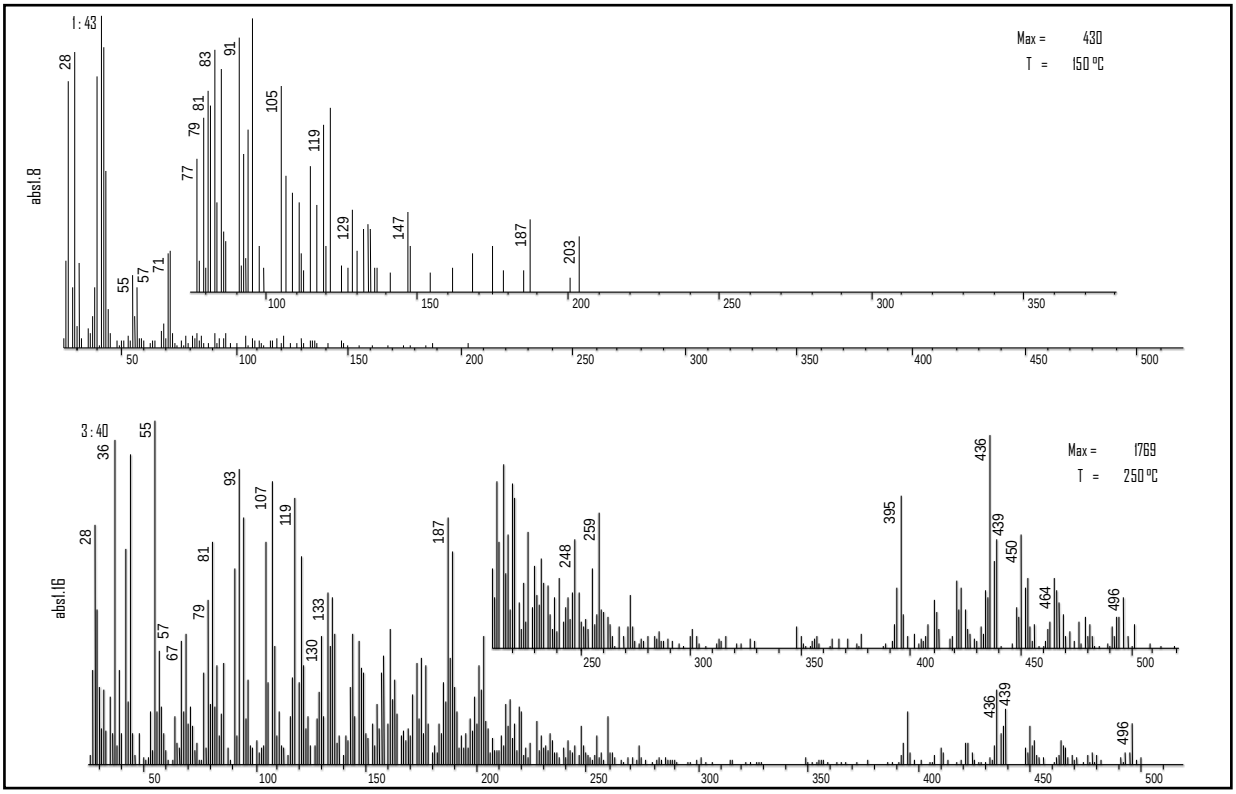


Fig. 7.1.5. Mass spectrum (EI) of betulinol-glycyl-leucine compound, (Bet-Gly-Leu)

7.2. Synthesis of substitute derivatives of ursolic acid

By condensations of ursolic acid with alanyl-glycine, glycyl-leucine and leucyl-glycine we obtained the appropriate dipeptide derivatives, by the diagram from Fig. 7.2. The synthesis of ursolic derivatives implied the performance of a series of reactions carried out in four successive stages, among which the first two, respectively the obtaining of intermediary products, (1a) and (2a), are common as method of work with the obtaining of the three ursolic derivatives, (4), (6) and (8).

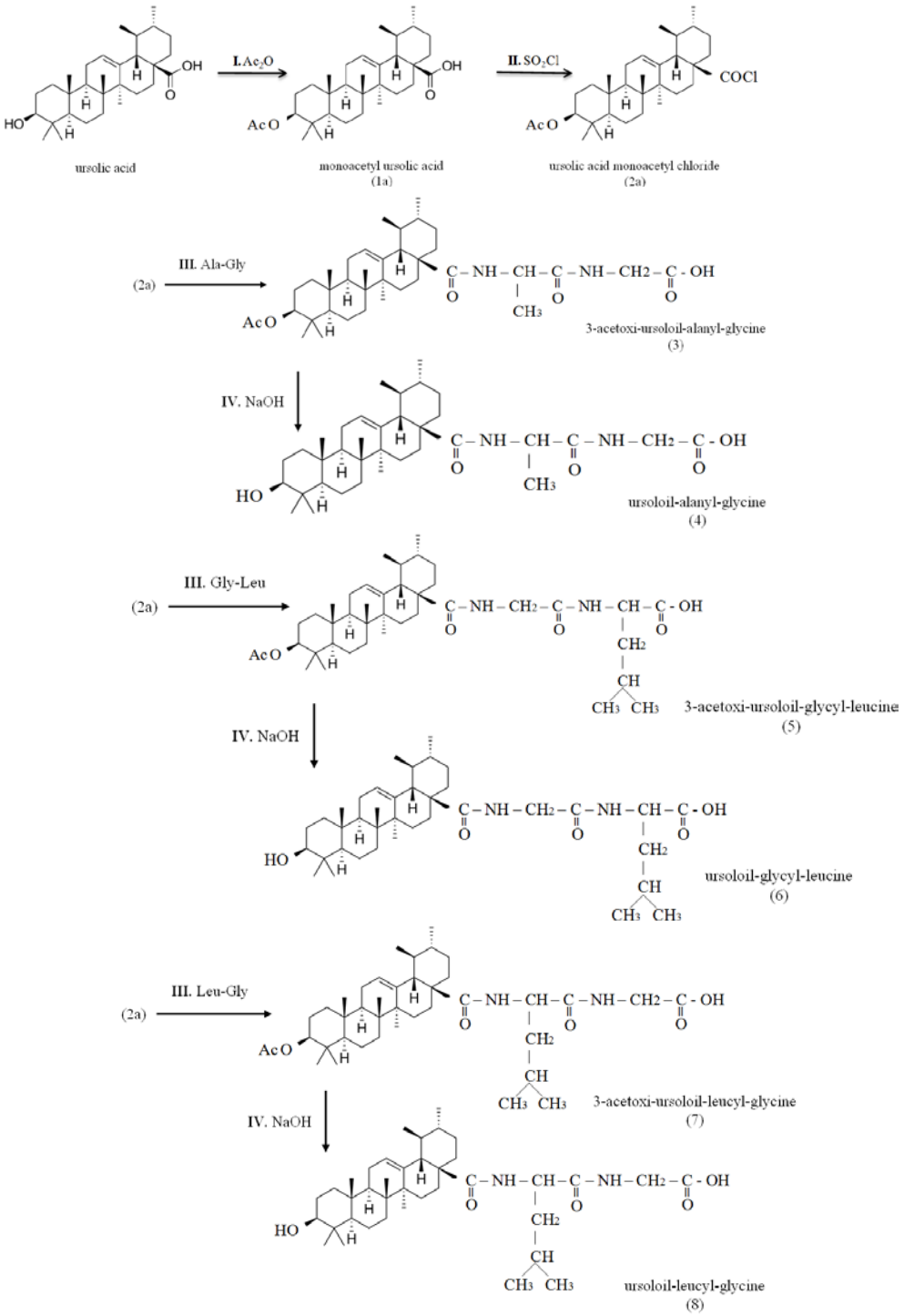


Fig. 2. Diagram of synthesis of ursolic derivatives

The compounds (4), (6) and (8) were characterized by two analytical methods, mass spectrometry, (CG-MS) by electronic impact (EI) and spectroscopy in infrared field, (IR). In the figure below, (Fig. 7.1.5) we illustrated the mass spectrum for the ursoloil-glycyl-leucine compound, (6) which was tested from the point of view of bilogical effect.

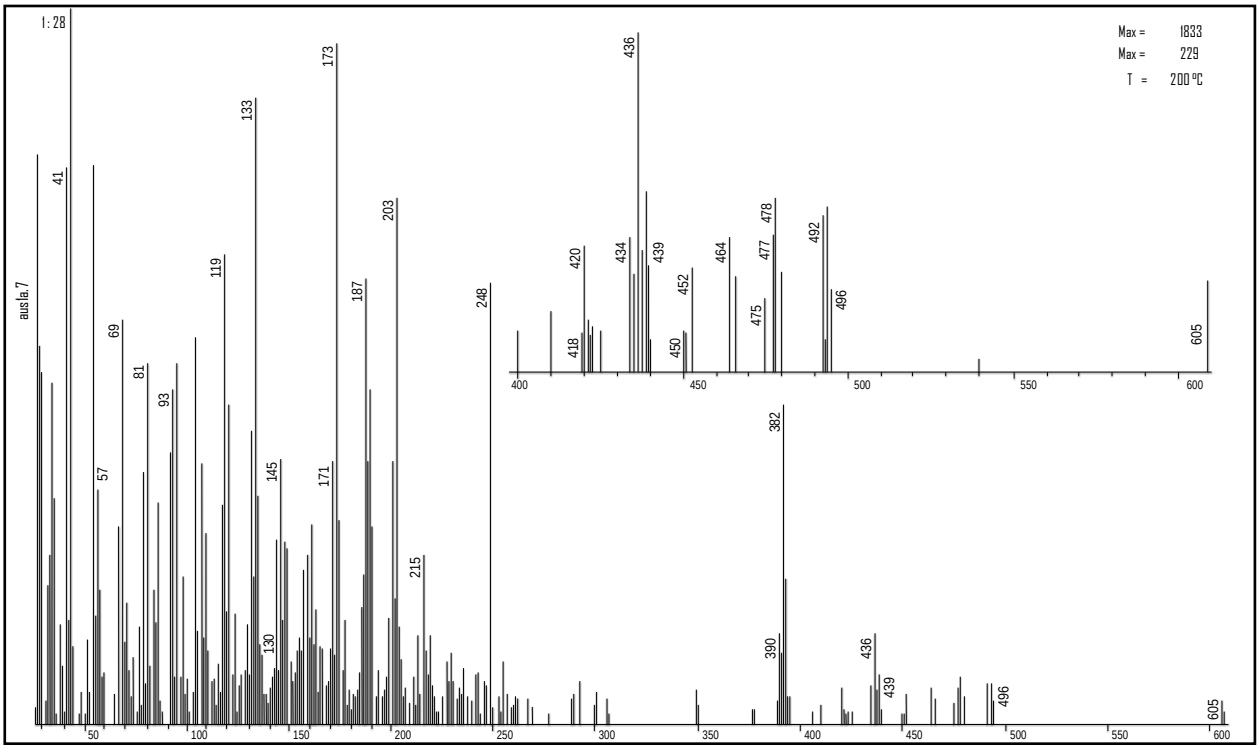


Fig. 7.2.6. Mass spectrum of ursoloil-glycyl-leucine compound, (Urs-Gly-Leu)

Chapter 8. BIOLOGICAL TESTING OF DERIVATIZATION PRODUCTS IN VIEW OF DETERMINATION OF THEIR BIOLOGICAL ACTIVITY

The measurement of antiproliferative effects was performed for the compounds betulinol-glycyl-leucine, (6), (Fig. 7.1) and ursoloil-glycyl-leucine, (6), (Fig. 7.2), and the bilogical activity was tested on several carcinogenic cellular lines, HeLa, MCF-7, A431 and A2780, (Fig. 8.3.2). For the laboratory tests we used concentrations of the compounds of 10 and 30 µM/l.

The data analyses led to the conclusion that for the first compound there are no dramatic differences between the activity of betulinic acid and the activity of its substitute derivative, especially in case of cellular lines, MCF-7 and HeLa, and for A431 we can see a vague tendency of reduction of the antiproliferative character for the lower concentration, 10µM. In case of ursolic derivative, we could not see any significant differences between the activity of ursolic acid and the activity of its substitute derivative, especially in case of cellular lines, A431 and HeLa. For MCF-7 we can see a vague tendency of increase in the antiproliferative character for the lower concentration, 10µM.

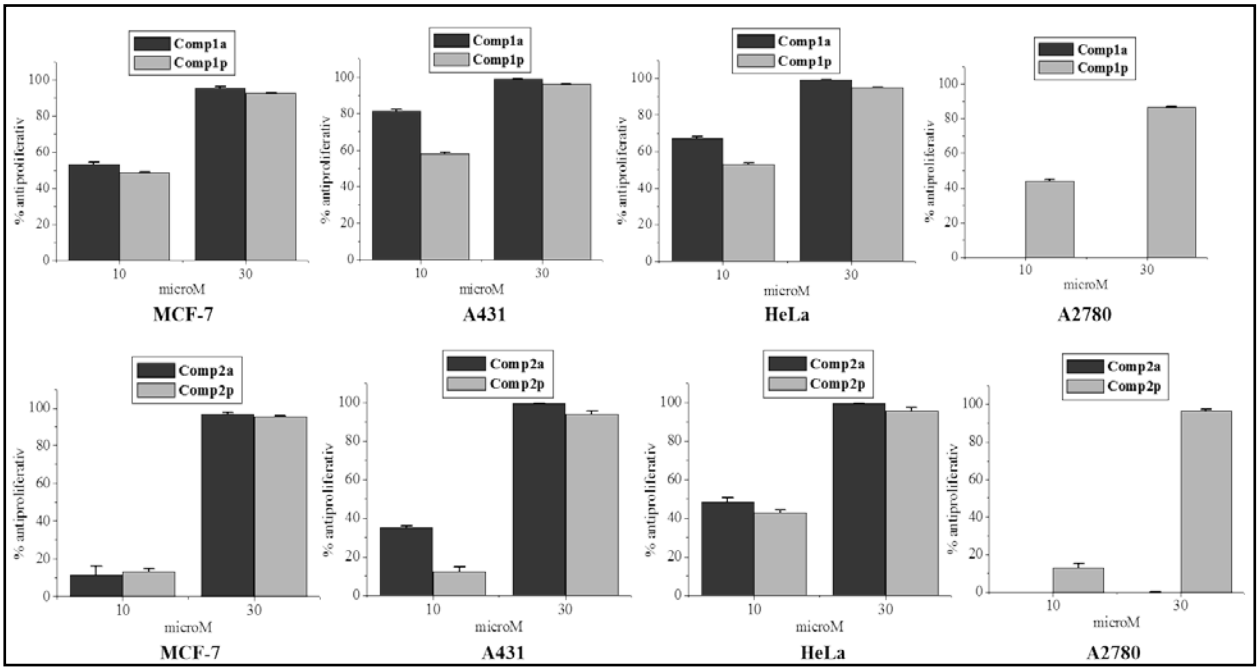


Fig. 8.2.3. Percentage of antiproliferation effect of betulinic acid and ursolic acid and derivatives conjugated with glycyl-leucine for four cellular lines

CONCLUSIONS

1. In view of choosing the optimal drying procedure of the fresh vegetal material we carried out a comparative study regarding the use of techniques of natural and artificial drying by convection and in microwave field for fresh vegetal materials;
2. We performed the extraction of betulinic acid, respectively ursolic acid from a number of three species of plants present in our spontaneous flora and used empirically, Betula pendula, (White Birch), Calluna vulgaris (L) Hull, (black grass) and Bruckenthalia Spiculifolia, (Mountain gooseberry);
3. We proposed a model based on dimensional analysis, starting from the phenomenology of the extraction process and it was checked by experimental way;
4. We performed the derivatization of betulinic and ursolic acids in view of obtaining new biologically active products, by their linking on chemical path to compounds from dipeptide class: glycyl-leucine, alanyl-glycine, respectively leucyl-glycine;
5. We performed the testing of compounds betulinol-glycyl-leucine and ursoloil-glycyl-leucine on several types of carcinogenic / tumoral cells, MCF7, A431, A2780 and HeLa, for the purpose of highlighting the biological activity of synthesized compounds and of their therapeutic effects.

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